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Dedication

Edmund Lawrence Dubois: 1923–1985

Edmund Lawrence Dubois (pronounced “Doo-Boyz”) was born on June 28, 1923, to a middle-class Jewish family in Newark, New Jersey. He was the only child of a general surgeon, and his mother worked in his father's office. Ed, as he was known, graduated from high school in Newark in 1939. He then attended Johns Hopkins University, graduating with a bachelor's degree in 1943. While serving in the Army, he stayed in Baltimore to attend Johns Hopkins Medical School, where he also did his internship under A. McGehee Harvey, the legendary Chief of Service who was a lupus pioneer in his own right. Ed's tendency to know and train under the best and the brightest continued with a residency in medicine at the University of Utah under Max Wintrobe (author of the *Hematology* textbook) and at Parkland Hospital in Dallas under Tinsley Harrison (author of *Harrison's Medicine* textbook). He also completed an autopsy pathology fellowship at Los Angeles County General Hospital in 1948.

Ed decided to stay in Southern California and went into private practice at his father's office in July 1950. In 1951, he met—and, in 1952, married—Nancy Kully, the beautiful daughter of Barney Kully, a local ear, nose, and throat specialist. To keep professionally busy, he volunteered his time at the Los Angeles County General Hospital. The “General,” as it was known, was the largest hospital in the United States at the time, with over 3,000 beds. Dr. Paul Starr, then Chairman of the Department of Medicine, asked him to start a clinic consisting of eight patients who had a newly diagnosed disorder that was characterized by a positive result on a recently described laboratory test known as the “LE cell prep.” Within 10 years, Ed had the largest lupus practice in the world, caring for 500 patients at the General Tuesday morning Lupus Clinic and another 500 patients in his private practice.

By the mid-1980s, more than one half of the rheumatologists in Southern California could say that Ed Dubois had taught them nearly everything they knew about lupus. His first publications on lupus appeared in the *Journal of the American Medical Association* (four of them!) and the *American Journal of Medicine* (two papers) in 1951 and 1952. They described autoimmune hemolytic anemia as a manifestation of systemic lupus erythematosus (SLE), showed that steroids ameliorate the disease, and described the general clinical and laboratory features of patients who had positive LE cell preps.

As the General became more closely affiliated with the University of Southern California, university resources allowed the establishment of lupus research laboratories. Ed Dubois' keen clinical instincts and his demands for perfection among those who worked with him permitted him to publish seminal works that established him as the first, or among the first, to propose insights that we now take for granted. These include: use of nitrogen mustard for serious SLE (1954); use of Atabrine for cutaneous and mild systemic lupus (1954); high-dose steroid protocol for managing central nervous system disease (1956); analysis of why hydralazine induced LE cells (1957); the first description of avascular necrosis in lupus (1960); the first description of steroid-induced peptic ulcers (1960); the first description of gangrene from lupus vasculitis (1962); anticonvulsant drug-induced lupus (1963); establishment of one of the first NZB/NZW mouse research laboratories in the United States (1963), detailed analysis of an accrued, incredible series of 520 patients with lupus (1964); one of the first probes into familial SLE (1964); use of cyclophosphamide in SLE (1967); the first large series of procainamide-induced lupus (1968); absence of erosions in lupus synovitis (1970); phenothiazine-induced lupus (1972); the first report of lupus with myelofibrosis (1973); the first large analysis of causes of death, containing 212 patients (1974); HLA typing of patients with lupus (1974); ibuprofen for SLE (1975); and the incidence of septic arthritis in lupus (1975).

In 1966, Ed wrote the first edition of his monograph, *Lupus Erythematosus: A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Variants.* Dedicated “to the patients from whom we have learned,” this remarkable, largely single-authored textbook was enormously successful and now—with this volume—is in its seventh edition. More than any other publication, this book has shaped how rheumatologists approach and treat this disease. Although he authored 175 papers, abstracts, book chapters, and received numerous international honors while traveling, Ed was most proud of being the founding medical director of The American Lupus Society and President of the Southern California Rheumatism Society.

Ed Dubois was a tireless workaholic. He would rise at 5 am and write for an hour or two before going to work. A humanist of the first order, one half of his time was spent giving free medical care. Ed was known to be exacting and did not suffer fools easily. Although he seemed to be a man of few words, his gentle kind-heartedness was always evident. Ed's probing intellect was apparent within moments of meeting him, and he was always relaxed, modest, and approachable. Ed could be a wonderful teacher when confronted with a student physician who had an inquiring mind and a capacity to work hard. Although he had well-known Hollywood luminaries as private patients, he was never snobbish or conceited, and he felt much more at home seeing indigent patients at the General's lupus clinic.

More than anything else, Ed Dubois was a private man who was devoted to his family. He was happily married and had four children, all of whom now have successful careers. His first grandchild (of six) was born shortly before he died. He was an expert yachtsman who relaxed best on his boat. (When he was terminally ill, he bought a new boat that he named Dubious.) His other consuming passion was photography. Able to be privately tutored by the likes of Ansel Adams, his office was filled with creative and wonderful pictures showing his love of life.

While still a youthful 54 years of age in 1977, Ed complained of low back and knee pain, which turned out to be a compression fracture from multiple myeloma. He privately confided to me that excessive exposure to radiation during his training and in various research laboratories was responsible for this. A fighter to the end, Ed lived with myeloma for 8 years, which must be close to a record. He saw a full schedule of patients 2 weeks before he died, in February 1985, from pneumonia complicated by renal shut-down. I was fortunate to meet Ed Dubois as a medical student at the University of Southern California and as a resident at Cedars-Sinai Medical Center. Among the greatest honors in my life was when he asked me to help in his office part-time in 1977, while I was still a fellow in training, and to edit his book along with him in 1982. I will always value his friendship.

Daniel J. Wallace MD

Acknowledgment: I gratefully acknowledge the assistance of Mrs. Nancy (Edmund) Dubois in preparing this dedication.

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The Clinical Presentation of Systemic Lupus Erythematosus , The Musculoskeletal System , Clinical Aspects of Vasculitis and Selected Cutaneovascular Manifestations of Systemic Lupus Erythematosus , Manifestations Involving the Eye, Ear, and Larynx, Gastrointestinal and Hepatic Manifestations , Serum and Plasma Protein Abnormalities and Other Clinical Laboratory Determinations in Systemic Lupus Erythematosus , Differential Diagnosis and Disease Associations , Principles of Therapy and Local Measures , Antimalarial Therapies , Nonpharmacologic and Complementary Therapeutic Modalities , Additional Therapies Used in the Management of Lupus , Adjunctive Measures and Issues: Allergies, Antibiotics, Vaccines, and Disability , Biologics and Stem Cell Therapies for Lupus , A Patient's Guide to Lupus Erythematosus . Lupus Resources .

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Nonsteroidals and Salicylates for Systemic Lupus Erythematosus

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The Genetics of Human Lupus

Preface

Forty years have elapsed since Ed Dubois wrote the first edition of his monograph in 1966. He wrote 80% of the text himself, using notecards and a typewriter between 5 and 6 a.m. over a 2-year period. Ed would indeed be proud to see the explosion of information that has transformed what at the time was the arcane discipline of lupology. The 7th edition of *Dubois' Lupus Erythematosus* reflects the changes and developments in the field over the last 5 years. Lupus experts are now working together to form consortiums and registries which allow for clinical trials to be performed rapidly and efficiently; provide banks of shared sera, cells, and genetic materials; and permit new clinical indices and ascertainment methodologies to be developed and tested more rigorously. In keeping with the improved quality of studies and in order to focus on more evidence-based findings, we no longer cite every paper published on the subject of lupus but emphasize trends, treatment consensuses and practical clinical information along with succinct summaries of basic science insights. Ironically, the last time a drug was approved for lupus was also in 1966. Almost 20 biologics are now in various stages of clinical development for SLE, and this has created a palpable sense of excitement.

Chapters on cytokines and interferons, pathogenesis of atherosclerosis, immune tolerance, clinical indices, mixed connective tissue disease, reproductive immunology issues, fibromyalgia, women's issues, and biomarkers are included for the first time. We welcome 20 new authors and co-authors and thank our senior experts who graciously agreed to pass the baton on to young, promising investigators.

Dr. Wallace wishes to thank his family, Jody Stanley, and Nancy Winter for their hard work and support. Dr. Hahn thanks her mentors who started her off in lupus studies, Lawrence E. Shulman, MD, PhD and the late Mary Betty Stevens, MD; she also thanks her husband, Theodore John Hahn, MD, who further inspires her, and her daughters, Alysanne Yvonne Hahn and April Diane Hahn Lange, who make it all worthwhile.

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Chapter 1

Historical Background of Discoid and Systemic Lupus Erythematosus

Thomas G. Benedek

The Prescientific Period

Lupus (wolf) was an ancient Roman family name, and there was a St. Lupus who lived in central France in about 600 A.D. (1). How the name of this large carnivore came to have a disease association is obscure. The earliest known medical use of “lupus” appeared in a 10th century biography of St. Martin, who had lived in 4th-century Gaul. The Bishop of Liege was healed at St. Martin's shrine in Tours:

> He was seriously afflicted and almost brought to the point of death by the disease called lupus. … The location of the disease … was not to be seen, nonetheless, a sort of thin red line remained as a mark of the scar (2).

Toward the end of the 12th century, Rogerius Frugardi, a Salernitan surgeon, introduced the term *noli me tangere* (touch me not) to designate a facial ulcer. He also stated, “Sometimes lupus arises in the thighs and the lower legs [and is] distinguished from cancer” (3). This distinction was clarified somewhat by his student, Roland of Parma: “In the early stages it [cancer] is called sclirosis [hardening] or negrosis [blackening]. After it begins to rot it is called cancrena [gangrene ?]; finally it is called carcinoma [cancer].” This progressive lesion also is named according to its location: on the face he used Roger's term, *noli me tangere*, on the trunk, *cingulum* [girdle]. “However, in the lower body, as in the feet, thighs and hips, it is called *lupula* [little she-wolf] and it is incurable … in this place” (4).

“Lupus” remained associated with ulcerated lesions of the legs until the 16th century, after which it was considered primarily a facial lesion. Most authors considered it to be a distinctive disease rather than a phase of an evolving ailment, but no one described lupus in sufficient detail for a modern diagnosis to be inferred. Various ailments likely bore the name. Paracelsus (1493–1541)

> The art of medicine resides in recognizing the site wherein lies the cure, such as cancer, lupus, gout, plague, fever, hydrops, polyuria, menses, worms, etc. (5).

Differentiation from Tuberculosis

Discussion of whether this skin disease is merely a manifestation of tuberculosis, which itself was just being defined, began early in the 19th century. According to the dermatologist Thomas Bateman (1778–1821), his mentor, Robert Willan (1757–1812) had defined lupus:

> … to comprise, together with the “noli me tangere” affecting the nose and lips, other slow tubercular affections, especially about the face, commonly ending in ragged ulcerations of the cheeks, forehead, eyelids, and lips, and sometimes occurring in other parts of the body, where they gradually destroy the skin and muscular parts to a considerable depth (6). In 1853 Sir James Paget (1814–1899), calling the lesion a “rodent ulcer,” identified *noli me tangere* as a neoplasm (7).

In 1845 Ferdinand von Hebra (1816–1880, Vienna) proposed a classification of skin diseases based on abnormalities of specific components of the skin. Under “hyperactivity of sebaceous glands” he described “seborrhea congestiva,” which a few years later was renamed *lupus erythemateux*. Here is his description:

> One sees at the beginning of this illness—mainly on the face, on the cheeks and the nose in a distribution not dissimilar to a butterfly—on an erythematous but not infiltrated base the sebum filled openings of the sebaceous glands as white flat dots (8).

Laurent T. Biett (1761–1840, Paris) may in 1833 have described the same disease as “erytheme centrifuge,” but this was first published in 1851 (Fig. 1-1) by his student, Pierre L. Cazenave (1795–1877, Paris):

> It is a very rare occurrence, and appears most frequently in young people, especially in females, whose health is otherwise excellent. It attacks the face chiefly. It generally appears in the form of round red patches, slightly elevated, and about the size of a 30 sous piece: these patches generally begin by a small red spot, slightly papular, which gradually increases in circumference, and sometimes spreads over the greater part of the face. The edges of the patches are prominent, and the centre, which retains its natural color, is depressed … (9)

At a meeting in 1851, Cazenave also described a case and after giving credit to Biett for having described a variety of

lupus as “erytheme centrifuge,” introduced the term *lupus erythemateaux* (10).

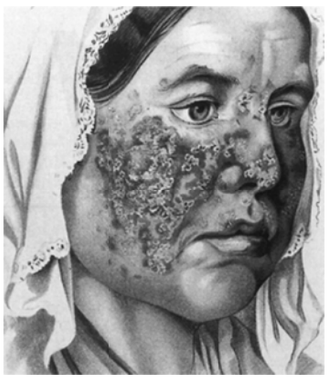

Figure 1-1. (See color plate.) Disseminated lupus erythematosus (LE) showing “livid red slightly raised lesions with flat, depressed, paler centers resembling scars; or covered by an adherent thin scale which feels greasy.” (From

Kaposi M. Neue Beiträge zur Kenntnis des Lupus erythematosus. *Arch Dermatol Syph* 1872;4:36–78.

)

In the 1856 edition of his textbook Cazenave wrote extensively about lupus erythematosus (LE). He mentions the potential occurrence of “fever and even pain,” but his presentation is entirely dermatologic. Cazenave herein is the first to note alopecia as a symptom. He does not mention the “butterfly rash,” but states that the eruption “is very common on the face and the nose.” He emphasizes that lesions heal with scarring, but do not ulcerate—an important distinction from lupus vulgaris (LV)—and states that these patients are not necessarily scrofulous (11) (Fig. 1-2).

The distinction from scrofulous (i.e., tuberculous) was important because lupus was considered a tuberculous disease, “tuberculous” having a different meaning in this prebacteriologic time. According to Erasmus Wilson (1809–1884; 1862, London):

> Destruction, then, we may take as the leading character of lupus. A further inquiry into the nature of lupus is served, however, to show that this destructive disease was preceded by a circumscribed thickening and prominence of the skin, commonly termed a tubercle, hence, lupus is considered as a tuberculous affection of the skin. … Now, the destructive action implied by the term lupus was, in the first instance, intended to be restricted to that form of tubercle which commonly issues in destructive ulceration, but as cutaneous diseases came to be more carefully observed, it was perceived that there existed a kind of tubercle which did not of a necessity ulcerate, which was chronic and lasting in its nature, and which … left behind it a deep pit or a strongly marked cicatrix. … This form of cutaneous disease … has been distinguished by Cazenave under the name of lupus erythematosus (12).

Figure 1-2. (See color plate.) The first modern illustration of cutaneous lupus, labeled “Lupus erythemateux,” in Cazenave (11) in 1856. (From Wallace DJ, Lyon I. Pierre Cazenave and the first detailed modern description of lupus erythematosus. *Semin Arthritis Rheum* 1999;28:305–313. Reproduced with permission.)

Unfortunately, Wilson then confuses this finding with a syphilitic lesion. However, the fact that the medieval description of “lupus” depended mainly on ulceration makes it likely that the nonulcerative lupus was first recognized in the 1830s.

Moriz (Kohn) Kaposi (1837–1902, Vienna) with his publications of 1869 (13) and especially 1872 called attention to LE. He confirmed Wilson's observation that LE occurs more frequently in women and considered that it also is more likely to be severe in them (14). Kaposi believed that tuberculosis and LE may occur in the same patient, but was convinced that LE is not a manifestation of tuberculosis. He became quite annoyed at the confusion:

> … the disease called lupus erythematosus does not have the least in common with lupus vulgaris, and it is not enough to criticise even leading surgeons for confusing these two entirely different processes that have not the least in common, and even less to justify many dermatologic specialists who assume transitional or mixed forms of lupus erythematosus and lupus vulgaris, as has occurred recently (15).

From 1866 to 1871, Kaposi made the diagnosis of LE in 22 patients, while he diagnosed LV (cutaneous tuberculosis) in 279. He introduced the term “discoid” for lesions that

expand from single foci and “discrete and aggregate” for lesions that enlarge by the merger of multiple pinhead size foci (14). Subsequently he altered the latter term to “disseminate and aggregate” (15). Confusion resulted from Kaposi's intention for “disseminate” to refer to cases where the lesions were not limited to the head, while it so happened that systemic symptoms (Fig. 1-3) were observed only in patients with disseminate skin lesions:

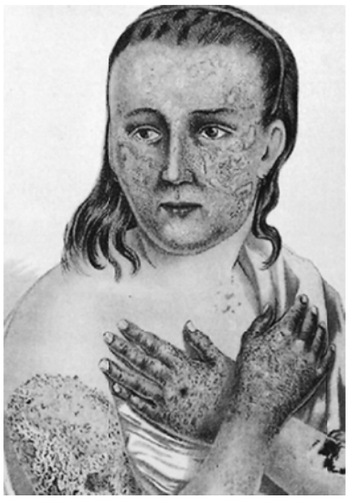

Figure 1-3. (See color plate.) Disseminated LE involving scalp, face, trunk, and extremities. (From Kaposi M. Neue Beiträge zur Kenntnis des Lupus erythematosus. *Arch Dermatol Syph* 1872;4:36–78.)

> Lupus erythematosus may occur and progress with manifestations of a disseminated or universal acute or subacute febrile eruption, and may then frequently involve the entire body with intense local and general symptoms, indeed to endanger and destroy life (14).

Table 1-1: Distinctive Features of Lupus Vulgaris and Lupus Erythematosus

Feature	Lupus Vulgaris	Lupus Erythematosus
“Apple jelly” growth	Characteristic	Little or none
Tendency to ulcerate	Yes	No
Symmetrical lesions	No	As a rule
Childhood occurrence	Yes	No
Sex ratio	Almost equal	Far commoner in women
Related to chilblains	No	Yes
Fatal	Very seldom	Sometimes

Of the 11 cases Kaposi described in 1872, four had pneumonia, three had arthralgias, and three had major adenopathy. Of the three who came to autopsy, two had pneumonia, one of whom also had amyloidosis, and one had tuberculosis. No renal disease was described. He was uncertain whether the relationship between the cutaneous and other findings was more than coincidental. The first American publication on LE, by W.H. Geddings in 1869, described the cutaneous findings of the first case in Kaposi's 1872 publication (16).

Many investigators recognized that the relative prevalence of LE and LV was influenced by socioeconomic factors, LV being particularly associated with poverty. Thus, Jonathan Hutchinson (1828–1913, London) cited Wilson as having seen an equal number of cases of these two diseases among 10,000 dermatologic patients from among the “wealthier classes,” and attributed this to referral bias (17). One decade later, H.R. Crocker (1845–1909), another English dermatologist, found (LV) to be twice as prevalent as LE among 10,000 dermatologic clinic cases (1.27% versus 0.63%), while the opposite was true among 5,000 private patients (0.98% versus 1.80%) (18).

Hutchinson in 1888 made the principal distinctions between the major members of the lupus family (Table 1-1). Hutchinson concluded, “The features which distinguish these two diseases … are useful rather for the purposes of clinical diagnosis and arrangement than as implying essential differences. … The two are closely allied and … are in a general way induced by a similar kind of causative influence. … In the lupus family vulgaris and erythematosus stand as brother and sister, having many essential resemblances and many marked but superficial differences.” In his descriptions of the symmetry of LE lesions he substituted “the bat's wing form” for Hebra's “butterfly.” Six years after the discovery of the tubercle bacillus, Hutchinson conceded that no one had detected them in cases of LE, “but this, no doubt is only a question of time” (17). Hutchinson was in a shrinking minority of advocates of a direct tuberculous etiology of LE. In view of the inability to recover tubercle bacilli from LE lesions, but with no persuasive alternative etiologic hypothesis, the most acceptable compromise was that LE “is a chronic inflammatory process produced by toxic substances of tuberculous origin” (19). Goeckerman (20) in 1921 analyzed Mayo Clinic

data and found tuberculosis equally prevalent among cases of discoid lupus erythematosus (DLE) and those with other dermatoses. In 1933, Harry Keil, a dermatologist in New York, in reviewing autopsy reports of cases of systemic LE, found that only 20% showed evidence of active or remote tuberculosis. He also concluded that the occurrence of the two diseases is coincidental and, in view of the prevalence of tuberculosis, not surprising (21).

Recognition of Systemic Lupus Erythematosus

Between 1872 and the first of William Osler's (1849–1919) three long articles describing a disease he initially called “erythema exudativum multiforme” (EEM), a few cases of LE were described in which some extracutaneous symptoms were present (22 ,23). In 1895, Osler described a disease:

> … of unknown etiology with polymorphic skin lesions—hyperaemia, oedema, and hemorrhage—arthritis occasionally, and a variable number of visceral manifestations, of which the most important are gastro-intestinal crises, endocarditis, pericarditis, acute nephritis, and hemorrhage from the mucous surfaces. Recurrence is a special feature of the disease, and attacks may come on month after month, or even throughout a long period of years. … The attacks may not be characterized by skin manifestations; the visceral symptoms alone may be present, and to the outward view the patient may have no indication whatever of erythema exudativum (24).

The relevance of Osler's contributions to the understanding of LE have been misinterpreted. In 1900, he acknowledged that the cases he was assembling were not uniform: “While I feel that in bringing together a somewhat motley series of cases I may only have contributed to make the ‘confusion worse confounded,’ on the other hand there is, I think, a positive advantage in recognizing the affinities and the strong points of similarity in affections usually grouped as separate diseases” (25). Therefore, he withdrew the term EEM in favor of the less specific “erythema group.” At no time did he use “LE.”

In the last paper (1904), Osler summarized his 29 cases, and they differ markedly in gender and age from typical LE: 18 were male and 12 were between the ages of 3 and 12; 19 had purpura and “colic.” All had some sort of cutaneous findings. The most frequent extracutaneous symptoms were arthralgia in 17 and “nephritis” in 14. None of the seven fatal cases came to autopsy. Osler made no etiologic hypotheses, but in regard to pathogenesis he stated: “The essential process is a vascular change with exudate, blood, serum, alone or combined” (26).

Osler's three articles drew praise for calling attention to the association of cutaneous and visceral symptoms (27 ,28). The modern diagnosis of most of these patients undoubtedly would be Schönlein-Henoch purpura, a possibility about which Osler equivocated (25). Keil, a dermatologist in New York, in 1937 became the first to point out that Osler's 29 cases included two descriptions of typical acute (systemic) LE (29 ,30).

Various modifications of “lupus erythematosus” came to be preferred over Osler's “erythema group.” In 1908, Kraus and Bohac (31) in Prague introduced “acute LE” to indicate the presence of both cutaneous and visceral symptoms. “Chronic LE” became a synonym for DLE. “Acute disseminated LE” was used for “cases which start acutely [i.e., with systemic symptoms], assume a disseminated [cutaneous] form and run acutely throughout” (32). That cutaneous lesions are not a prerequisite for a diagnosis of SLE was rediscovered in 1936 (33), and emphasized in 1942 (34). While various authors used cumbersome descriptive terms, “LE” never was discarded. Brunsting (35) in 1952 in Rochester, Minnesota, introduced “disseminated (systemic) LE,” and Harvey et al. (36) in 1954 in Baltimore finally popularized the contemporary “systemic lupus erythematosus” (SLE) (Table 1-2).

Once visceral symptoms began to be associated with cutaneous LE, the question of whether they are causally related had to be resolved. Consequently there is a gap between the first description of many findings and the recognition that they are a component of LE. Kaposi had mentioned the occurrence of fever and pneumonia in 1872, but Kraus and Bohac (31) concluded in 1908 and 1909 from their eight cases that pneumonia may be a component of LE and that fever is not necessarily a result of infection.

In 1911, Emanuel Libman (1872–1946, New York) hospitalized a 10-year-old girl who had been ill for 10 weeks with polyarthralgia, followed by precordial pain, dyspnea, and oliguria. “There was an erythematous eruption of butterfly pattern, which resembled acute lupus erythematosus disseminatus.” Blood cultures were sterile. Hematuria and a precordial rub developed during a febrile 8-week course. The autopsy revealed “endocarditis of a peculiar type, particularly because of the unusual manner of spread of the endocardial lesions along the posterior wall of the left ventricle,” and also glomerulonephritis. This case was first reported in 1924 as the fourth of four cases of nonbacterial valvular and mural endocarditis treated by Libman and autopsied by Benjamin Sacks (37). Cases 1 and 2 had already been reported in 1923 (38). Two of the four had the butterfly facial eruption and three had nephritis. Libman and Sacks pointed out “the similarity of certain of the symptoms to those observed in the erythema group of Osler,” but declined to make a definite diagnosis of SLE in any case.

Dermatologists, upon reviewing published cases in 1936, came to the confusing conclusion that the “Libman-Sacks syndrome is a subvariety of the Osler erythema group,” but probably not LE (39). An internist and a pathologist finally stated unequivocally in 1940 that this form of endocarditis is a manifestation of SLE, irrespective of the presence of characteristic skin lesions (40). Libman-Sacks endocarditis has become a less common pathologic finding since the introduction of corticosteroid therapy—59% of cases of SLE reported during 1924 to 1951 versus 36% of cases during 1953 to 1976 (41).

At least two of the patients in whom Osler found signs of nephritis did have LE (26 ,30). Sequeira and Balean (42) in

1902 in London found proteinuria in five of ten cases of disseminated (cutaneous) LE, of whom the one fatal case had a pathologic diagnosis of nephritis. Similar single cases were published in the next few years. Keith and Rowntree (43) in 1922 in Rochester, Minnesota, pointed out that nephritis is “a common complication of disseminated LE.” In a pathologic study of 23 cases of SLE, Baehr et al. (44) in 1935 in New York differentiated a type of nephritis in 13 (56%), which they considered to be peculiar to LE. “The commonest and most characteristic glomerular alteration was a peculiar hyaline thickening of the capillary walls. … The thickened wall appears rigid, as if made of heavy wire. We have, therefore, called it the ‘wire loop lesion.’ … It is quite different from the hyaline degeneration seen in glomeruli of arteriosclerotic kidneys or of chronic glomerulonephritis. It apparently represents a toxic degenerative process” (44). Nevertheless, renal failure was not considered a principal cause of death in LE, probably because early death usually resulted from infection. The importance of renal involvement was recognized by Harvey et al., who found that in two thirds of their autopsied cases “SLE alone was responsible for varying degrees of renal damage.” They pointed out “the inability to correlate the degree of renal involvement disclosed clinically and the extent of renal damage at postmortem examination” (36).

Table 1-2: First Descriptions as Components of Systemic Lupus Erythematosus

Component	Author, Year	Site	Reference
Clinical			
Butterfly rash	von Hebra, 1845	Vienna	8
Panniculitis	Kaposi, 1869	Vienna	13
Arthralgia	Kaposi, 1872		14
Adenopathy	Kaposi, 1872		14
Arthritis	Philippson, 1892	Hamburg	23
Nephritis	Osler, 1895	Baltimore	24
Purpura	Osler, 1895		24
Psychosis	Bowen, 1896	New York	48
Pneumonia	Kraus & Bohac, 1908	Prague	31
Raynaud's phenomenon	MacLeod, 1908	London	46
Photosensitivity	Pulay, 1921	Vienna	125
Endocarditis	Libman & Sacks, 1923	New York	38
Retinopathy	Pillat, 1935	Vienna	55
Peritonitis	Friedberg et al., 1936	New York	33
Encephalopathy	Daly, 1945	Minneapolis	49
Myelopathy	Piper, 1953	Madison, WI	52
Laboratory			
BFP reaction	Reinhart, 1909	Hamburg	72
Leukopenia	Goeckerman, 1923	Rochester, MN	60
Anemia	Keefer & Felty, 1924	Baltimore	59
Hematoxylin bodies	Gross, 1932	New York	124
Thrombocytopenia	Lyon, 1933	Philadelphia	62
“Wire loop” glomeruli	Baehr et al., 1935	New York	44
“Onion skin” splenic lesion	Kaiser, 1942	Baltimore	45
Hypergammaglobulinemia	Coburn & Moore, 1943	Baltimore	76
LE cell	Hargraves, 1948	Rochester, MN	79
Lupus anticoagulant	Conley & Hartman, 1952	Baltimore	158
Antinuclear antibody	Miescher & Fauconnet, 1954	Geneva, Switz.	90
“Lupoid” hepatitis	Mackay, 1956	Melbourne, Australia	68

BFP, biologically false positive; LE, lupus erythematosus.

Libman and Sacks (37), incidentally to their description of the cardiac lesion, also described a splenic abnormality: “The greater part of each malpighian body [lymph follicle] was occupied by a number of arterioles, each of which was surrounded by a broad zone of hyaline-like connective tissue. The arteriolar lumen in each instance was diminished in caliber.” Kaiser (45) in 1942 in Baltimore studied this “onion skin” periarterial splenic fibrosis in detail and found it in 83% of cases of SLE, but in only 3% of other diseases. “Its discovery post mortem should at least raise the suspicion of that diagnosis … [and] its coincidence with the other well recognized lesions of the connective tissue such as verrucous endocarditis and the ‘wire loop’ glomerular changes can serve to strengthen the post mortem diagnosis of disseminated lupus erythematosus” (45).

Abdominal symptoms were recognized as peritonitis, which tended to be associated with pleurisy, but there were no primarily gastrointestinal (GI) findings (33).

In regard to cerebral involvement, Osler considered as “peculiarly obscure” the delirium in one case (no. 1), and in case no. 15 recurrent episodes of hemiplegia and aphasia, which he speculated “were associated with changes in the brain of essentially the same nature which subsequently occurred … in the skin. They remind one somewhat of the attacks of recurrent aphasia with paralysis in cases of Raynaud's disease” (24). Whether these patients actually had SLE is equivocal, but this allegation has repeatedly been made. Peripheral Raynaud's phenomenon was associated with SLE by MacLeod (46) in 1908 in London. Until the 1940s abnormalities of cerebral function were generally attributed to fever or uremia. Even though seizures are the most frequent cerebral manifestation of SLE, this symptom was alluded to only incidentally before 1951, when it was also pointed out that seizures may precede diagnosable SLE for years (47). Harvey et al. (36) attributed seizures to SLE in 11% of their patients. According to Bowen (1896), “I have often met with cases of extreme melancholia in the subjects of this disease [LE] and in a number of instances the mind has become really affected” (48). Toxic delirium was described (49), but the range of psychotic manifestations that may occur was not comprehensively reviewed until 1960 (50). Cerebral vasculitis to which the various manifestations might be attributed was described by Jarcho (51) in 1936 in Baltimore and Daly (49) in 1945 in Minneapolis. Paraplegia because of spinal vasculitis was identified in 1953 (52).

Attention to ocular symptoms antedates modern descriptions of cerebral symptoms. Retinal vasculitis was demonstrated pathologically in the 1930s (53 ,54). The first more extensive investigation (1935, Vienna) concluded that the ophthalmoscopic findings of some cases of DLE and SLE are further evidence of a tuberculous disease (55). Maumenee (56) in 1940 in Baltimore introduced the term “cotton-wool spots” for the retinal lesions as a synonym for the older term “cytoid bodies.” However, they “should not be regarded as pathognomonic of acute lupus erythematosus.” More recent investigations have shown that cotton-wool spots are ischemic lesions in the retina, while cytoid bodies are microscopic neural lesions; they coexist, but are not identical (57). Prior to 1970 cotton-wool spots were described in about 10% of cases of SLE, but less frequently more recently (58).

The absence of anemia in early descriptions of SLE can be attributed to the rudimentary state of hematologic methods. However, it is surprising that “secondary anemia often with a normal leukocyte count” was not described until 1924 (59). A case with leukopenia was cited by Goeckerman (60) in 1923. However, Rose and Pillsbury (61) in 1939 in Philadelphia were the first to consider this “the principal feature of the blood picture.” Purpura had been described by Kaposi (14) and by Osler (24), but the thrombocytopenia with which it occurs was not recognized until the 1930s (62 ,63). Conversely, the infrequency of purpura among platelet deficient patients was pointed out in 1951 (64).

Lupoid Hepatitis

In the mid-1950s a syndrome was recognized manifested by progressive liver disease usually associated with arthralgia and/or fever and a markedly elevated serum γ-globulin concentration that occurred predominantly in young women. Bearn et al. (New York) among 26 cases of liver disease (23 female) had eight (mean age 17) who had both fever and arthritis, a mean serum globulin content of 7 gm% and hyperbilirubinemia. The possibility that these were instances of SLE was not entertained. (65) In 1955, Zimmerman et al. (Chicago) evaluated patients with SLE for evidence of liver disease. The serum globulin concentration exceeded 3 gm% in 22 of the 25 cases. The investigators concluded that the numerous abnormal hepatic function test results in most cases reflected globulin abnormalities related to SLE rather than liver disease. (66) Coincidentally physicians in Melbourne, Australia demonstrated the LE cell phenomenon in patients with chronic hepatitis that they presumed to be viral. (67) In 1956, Ian R. Mackay (Melbourne) introduced the term “lupoid hepatitis” for this association (68). Bartholomew et al. (Mayo Clinic) found the serum γ-globulin concentration in seven cases consistently greater than 3.1 gm% (69) In various early descriptions the histopathology of the liver ranged from minimal changes to postnecrotic cirrhosis. In 1956, the first instance of lupoid hepatitis in a case of possibly drug-related SLE was also reported; a sulphonamide was implicated. (70) Mackay et al. (1959) proposed that the etiology may be “mediated via abnormal immunological responsiveness with auto-destruction of host tissues.” (71)

Serologic Aspects

The Wassermann test for syphilis was devised in 1906 and rapidly gained wide use. It soon was discovered that some, mainly tropical, diseases frequently gave positive results in nonsyphilitic individuals. The first such cases of SLE were reported from Germany in 1909 and 1910 (72 ,73). The latter author suggested that this result is evidence that LE is not a manifestation of tuberculosis, since falsely positive reactions had not been reported in the latter disease. The incidence of biologic false-positive (BFP) reactions has ranged from less than 3% to 44% of cases of LE (74 ,75). The variation may in part be a result of insufficient distinction having been made between DLE and SLE, since BFP rarely occurs in the former. Coburn and Moore (76) in 1943 in New York demonstrated hypergammaglobulinemia in SLE and related the BFP reaction to this. With the discovery of the specific *Treponema pallidum* immobilization (TPI) test for syphilis in 1949, complement fixation tests of the Wassermann type gradually were abandoned (77). Haserick and Long (78) in 1952 found that BFP reactions may precede clinical signs of SLE by years. Testing for BFP reactors was abandoned within a few years after the discovery of the LE cell.

In 1943, Malcolm M. Hargraves, a hematologist at the Mayo Clinic, found “peculiar rather structureless globular

bodies taking purple stain" in the marrow aspirate of a child with an undiagnosed disease; 2 1/2 years later he made a similar observation. Symptoms in a third case with this finding, in 1946, suggested that this patient had SLE. Two important observations were made in addition to the association of this unusual cell with the diagnosis of SLE: (a) more of these cells were found when the specimen was not fixed immediately; and (b) two similar cells needed to be differentiated. These findings were first reported in January 1948 (79). The "tart cell" (named after a patient, not the pastry) is not disease specific. Its distinguishing feature "is that the secondary nucleus has retained definite chromatin structure."

The "LE cell" … is the end result … either of phagocytosis of free nuclear material … or an actual autolysis of one or more lobes of the nucleus. … The "LE" cell is practically always a mature neutrophilic polymorphonuclear leukocyte in contradistinction to the "tart" cell which is most often a histiocyte (80).

John R. Haserick at the Cleveland Clinic suggested already in 1948 that "the greatest value of the 'LE' cell lies in its possible presence in suspected cases of acute disseminated lupus erythematosus in which the classic dermatologic manifestations are lacking" (81). Hargraves (82) in 1949 demonstrated LE cells in the buffy coat of centrifuged specimens of peripheral blood of patients in whose marrow LE cells had been detected. Then Haserick did the converse, inducing LE cells by incubating non-LE marrow with serum from LE patients. Thus, LE cells are formed by a factor in the blood of LE patients (83). Then in 1950 he showed that this factor is a γ-globulin (84). In the same year Klemperer et al. in New York discovered "hematoxylin bodies" (85). These appeared to be identical with the phagocytosed substance within LE cells, in various tissues obtained at autopsy of cases of SLE. This strengthened the hypothesis that the LE cell reaction is related to the pathogenesis of the disease (85). LE cells also were demonstrated in vivo in the content of artificially raised blisters (86). Peripheral blood replaced bone marrow as the source of LE cells, and of several techniques the one described by Zimmer and Hargraves (87) in 1952 was generally adopted.

Reliance on the LE cell to diagnose SLE began to diminish after a few years. Kievits et al. (88) in 1956 demonstrated LE cells in 16% of cases of rheumatoid arthritis, increasing doubt about the specificity of the reaction, and Rothfield et al. (89) in 1961 showed that LE cells cannot be detected in about a quarter of cases of SLE, proving poor sensitivity. In 1954, investigators in Switzerland had found that isolated cell nuclei can absorb the serum factor that induces LE cell formation. They therefore postulated that the factor is an antibody against a component of the nucleus (90). In 1957, Friou et al. (91) at Yale devised a technique to demonstrate the antibody semiquantitatively by indirect immunofluorescence microscopy. The reactive substance was identified in 1959 as a DNA-histone nucleoprotein (92) and Beck (93) in 1961 in London showed that at least three fluorescent staining patterns could be distinguished. In the next decade, refined laboratory methods permitted the discovery of numerous antibodies, some of which could be correlated clinically with subsets of SLE and other diseases. The discovery of the LE cell had initiated the discipline of immunopathology.

In 1957, three laboratories almost simultaneously demonstrated a factor in the serum of some cases of SLE that reacts specifically with DNA (94 ,95). Tan et al. (96) in 1966 in New York detected anti-DNA antibodies in SLE sera. Koffler et al. (97) in 1969 in New York found that the detection of native (double-stranded) deoxyribonucleic acid (dsDNA) is more specific for SLE, but less sensitive than antibody to denatured (single-stranded) DNA. Schur et al. (98) in 1971, using more sensitive techniques, confirmed the specificity of the reaction with dsDNA, but obtained positive reactions in only one half of SLE sera. Tan and Kunkel (99) in 1966 in New York detected a cytoplasmic (RNP) antigen in SLE serum that they designated Sm. It was the first antibody to a nonhistone nuclear antigen and highly specific for SLE, although found in only one third of cases.

The next discoveries about the antibody systems related to SLE were gained from the development of techniques to extract uncomplexed histones from nuclei and recombining them with DNA, free of other components (100). Histones are small basic proteins associated with nucleic acids in cell nuclei. Some extracted recombined antigens, depending on the precise histone structure, can be used to detect antihistone antibodies. The important findings were that antihistone antibodies occur more frequently in drug-induced than in idiopathic SLE, and that lupus-inducing drugs vary in their ability to induce these antibodies, procaine amide doing so most consistently (101 ,102).

The role of antinuclear antibodies (ANAs) in SLE became uncertain when clinically typical cases in which ANAs could not be detected began to be described (103). These cases comprise fewer than 5% of cases of SLE, and most have antibody reactive against the cytoplasmic RNA antigen Ro (104).

The introduction in 1963 of a convenient pathologic technique complemented the ever-increasing number of serologic tests. The "lupus band test" determines by immunofluorescence microscopy of skin biopsies whether immunoglobulins are deposited at the dermo-epidermal junction (105). In DLE it is positive in lesional but not in uninvolved skin. It also is positive in the "normal" skin of at least half of cases of SLE (106). It has proven not to be a highly specific finding, since it occurs in about 15% of cases of rheumatoid arthritis (107) and in various bullous dermatoses (108).

Epidemiology

In contrast to DLE, until the 1950s SLE was considered a rare disease. At the Johns Hopkins Hospital as of 1936 five cases were found among 7,500 autopsies (50). Twelve cases were diagnosed at the University of Pennsylvania Hospital during 1932 to 1938 (61). The large referral clientele of the Mayo Clinic included 154 cases from 1918(?) through 1937 and 132 cases during 1938 to 1947 (75). At Columbia-Presbyterian Hospital in New York, 44 cases were recognized

during 1937 to 1952 (109). SLE was diagnosed in 11 cases at the Los Angeles County Hospital during 1946 to 1949, but in 44 cases during the next 2 years. Dubois (110) attributed the increase to use of the LE cell test and better diagnostic acumen. On the other hand, the incidence of hospitalization for SLE in a Swedish city remained about 1 per 100,000 in 1938 to 1939 and 1948 to 1949, but increased to 4.8 per 100,000 during 1954 to 1955. These authors considered this increase genuine, although unexplained (111). The first survey of a circumscribed population in which case findings included outpatient records was conducted in New York in 1951 to 1960. SLE was found to have a higher incidence in the African-American than the white population, and the prevalence showed a greater increase than the incidence (112).

Various factors influencing the prevalence and survivorship with SLE were discussed by Merrell and Shulman (113) in 1955 in Baltimore. They also introduced the calculation of survival probability by life table analysis. Of the cases Harvey et al. (36) diagnosed during 1949 to 1953, 52% survived for 4 years. The extent to which the subsequent prolongation of survival should be attributed to the introduction in 1950 (114) of corticosteroid therapy has remained unsettled (115). Certainly, earlier diagnosis and recognition of less severe cases has contributed factitiously to lengthening the mean life expectancy. Urman and Rothfield (116) found that in a series of SLE patients treated principally with corticosteroids during 1957 to 1967, 10-year survival was 63%, while in a similarly treated series during 1968 to 1975 it increased to 84%. Haserick (117) in 1953 recognized that nephropathy benefits less from corticosteroids than do other manifestations. The 10-year survival of the patient cohort begun by Dubois' group (118), which was treated during 1950 to 1971 with multiple agents, was 87%, but only 65% in those with renal involvement.

SLE and “Collagen Disease”

Development of the modern pathogenetic concept of SLE required the rejection of two principles: that of Giovanni B. Morgagni (1682–1771, Padua) who had concluded in 1761 that every disease resides primarily in a certain organ, and that of Paul Ehrlich (1854–1915, Frankfurt) in 1901, who concluded that an organism cannot react against any of its own constituents. The former was first contradicted by the German pathologist, Fritz Klinge (119) (1892–1974), who showed in 1928 to 1934 that the morbid process in rheumatic fever is not limited to the synovium and heart, but affects connective tissue diffusely, and that such a process is also present in rheumatoid arthritis. Klemperer et al. (120) in 1941 in New York, in their study of the pathology of SLE, reported the following:

> The apparent heterogeneous involvement of various organs in this disease had no logic until it became apparent that the widespread lesions were identical in that they were mere local expressions of a morbid process affecting the entire collagenous tissue system. The most prominent of these alterations is fibrinoid degeneration—a descriptive morphologic term [E. Neumann, 1880] (120) indicating certain well-defined optical and tinctorial alterations in the collagenous fibers and ground substance (83).

This is the origin in 1942 of the term collagen disease, which initially was limited to SLE and scleroderma (120).

Ehrlich's doctrine was first questioned by Wilhelm Gennerich (122), a German dermatologist, who in 1921 speculated about the etiology of SLE:

> Lymphocytic [leukocytic ?] ferments are liberated by the disintegration of lymph nodes. They act on the organism as denatured protein and in sufficient quantity cause anaphylaxis. Furthermore, the liberated ferments exert their biologic effect, which seemingly consists of sensitizing the vascular endothelium and destroying certain components of connective tissue cells, especially, predisposed components of the skin and eventually also of all parenchymatous organs, if an abundant accumulation (acute LE) of the ferments develops in the blood.

This hypothesis gained acceptance in the 1940s because of the research of Arnold Rich (1893–1968, Baltimore), who advocated that the primary lesions of SLE-affected endothelium and collagen occur by anaphylaxis (123). The initiator of such hypersensitivity, however, remained obscure. Gross (124) in 1932 in New York had described microscopic “granular hematoxylin-stained bodies” in the hearts of cases of Libman-Sacks endocarditis. In 1950, Klemperer et al. (85) detected these abnormalities “in 32 of 35 cases of this disease, often widely distributed throughout the body.” Whether they were (or contained) the pathogen could not be ascertained.

Photosensitivity

The main symptom that the popular press has associated with LE is aggravation of the disease by exposure to bright sunlight. This was first described by a Viennese dermatologist in 1921 in regard to a fair-complected woman on whom DLE developed following intensive sun exposure. After several months, when the lesions had diminished, she received one ultraviolet irradiation to the back. On the next day there was a marked proliferation of lesions in the irradiated area (125). Rasch (126) in 1926 in Copenhagen stated that he had seen many such cases since 1907, with the lesions typically limited to the uncovered skin. He concluded that LE (i.e., SLE) “is very decidedly aggravated by light, in fact caused by it.”

Rose and Pillsbury (62) in 1939 in Philadelphia take precedence in the description of exacerbations of SLE following exposure to sun or therapeutic ultraviolet light, and also photosensitivity long preceding the development of recognized symptoms of this disease. Reports of the prevalence of photosensitivity have varied considerably: e.g., 11% of 105 cases (36), 32.7% of 520 cases (127).

Drug-Induced and Aggravated Lupus Erythematosus

Sulfonamides (initially sulfanilamide) began to be used to treat DLE in 1938 (128) and a few years later also SLE, with some benefit being described. In 1945, florid SLE developed in a young soldier who was being treated with sulfadiazine for presumed pyelonephritis (129). Gold (130) in 1951 in London hypothesized that the aggravation of LE by sulfonamide treatment, as had recently been reported, is because of the sensitization of patients by prior exposure to these drugs.

Gold compounds also acquired a reputation for exacerbating preexisting SLE. Their use in treating DLE began and ended long before their use in rheumatoid arthritis, the initial rationale also having been the presumed tuberculous etiology of the disease (131). A review in 1927 concluded that “in the treatment of lupus erythematosus we possess a systemic remedy of real efficacy. When one considers how refractory and unresponsive to therapeutic endeavor lupus erythematosus has been, … the results now achieved are all the more gratifying” (132). As recently as 1956, a therapeutic comparison of gold sodium thiosulfate and chloroquine, the latter first advocated for DLE in 1954 (133), showed similar efficacy (134).

Despite the lack of any effective treatment for SLE, as of 1937: “The general opinion that this method of treatment [gold] is contraindicated for acute and subacute disseminated lupus erythematosus is well founded on sad experience. … The capillaries seem unduly sensitive not only to gold therapy but also to a wide variety of therapeutic agents. … This is understandable in the case of therapy with gold preparations, since it affects the structures (capillaries) attacked by lupus erythematosus itself” (135). It still was deemed necessary in 1949 to warn that “gold is especially dangerous in the acute phases and probably should never be used” (136). This danger never was well documented.

Chronologically, the first commonly used drugs that were implicated to possibly induce rather than aggravate SLE were hydralazine (1954) (137), hydantoins (1957) (138), and procainamide (1962) (139). In the case of hydralazine the development of symptoms clearly was related to the chronic use of large doses to control hypertension. The first reported manifestation was arthritis, and additional symptoms more definitely suggestive of SLE developed if hydralazine was not discontinued. Comens and Schroeder (140) in 1956 found that, although LE cells were not found consistently in patients whose symptoms suggested SLE, these cells could also be demonstrated in some asymptomatic patients who were taking hydralazine. A Mayo Clinic study (1965) compared 50 cases of “hydralazine syndrome” with 100 hypertensive patients who were receiving another therapy. The authors concluded from the prehydralazine histories that “antedating manifestations possibly suggesting lupus diathesis” were nearly six times as frequent in the hydralazine cases as in the controls. Hence, this drug probably uncovers an “underlying lupus diathesis” (141).

Diphenylhydantoin and mesantoin were the first, but not the only, anticonvulsants to be related to the induction of SLE (138 ,142). Since seizures may be an early manifestation of this disease, the possibility that certain drugs “uncover” SLE gained support from these observations.

By far the most unequivocal inducing agent has been the antiarrhythmic drug procainamide. Dubois (143) in 1969 compared 33 well-documented cases against his cohort of 520 cases of idiopathic SLE. This supported the impression that the drug-induced disease tends to exhibit fewer and milder symptoms, particularly lacking GI, renal, and neurologic involvement. In a prospective study, Blomgren et al. (144) in 1969 in Rochester, New York, showed that ANA developed within 6 months in one half of patients who were placed on procainamide, “making it unlikely that the drug simply unveils a latent predisposition to idiopathic lupus erythematosus.”

Diagnostic Criteria

Valid, agreed-upon diagnoses are essential for epidemiologic and therapeutic research. The method for developing disease specific diagnostic criteria was pioneered in 1944 on rheumatic fever (145). When the technique was applied to LE in 1971, the extraordinarily large number of 74 clinical and laboratory items were considered and refined into 14 diagnostic criteria, one of which was the presence of DLE (146). The revision of 1982 placed greater reliance on serologic findings and the number of criteria was reduced to 11. The presence of at least four criteria was required in both schemes. Using the revised set, false-negative diagnoses decreased without a change in the small percentage of false-positive diagnoses (147).

The Relationship of DLE to SLE

Agreement with Kaposi's belief that DLE and SLE are expressions of the same disease has waxed and waned. For example, MacLeod (148) in 1913 in London concluded: “Lupus erythematosus of the acute disseminated type has from time to time been found to occur in association with more or less general toxaemia. … The circumscribed cases have probably a different etiology from those of the acute disseminated type.” Reliance on skin lesions to diagnose SLE was abandoned reluctantly. According to a 1952 textbook, “Diagnosis may be impossible until the appearance of the characteristic rash” (149). Of the pre-1938 cases of SLE diagnosed at the Mayo Clinic, the onset was considered to be with DLE in 47%. This decreased to 17% of those seen in the next decade, perhaps because of greater experience (75). Keil (135) in 1937 pointed out the lack of correlation between the severity of cutaneous and internal manifestations, and considered it probable that the two are variants of the same disorder. However, Baehr et al. (150)

still held in 1951 that “disseminate lupus erythematosus bears no relationship whatever … to the benign indolent skin lesion known to dermatologists as discoid lupus.” Among Dubois' 520 cases of SLE (1950–1963), 10.8% initially had discoid lesions, thrice as many as had a “butterfly rash” (127). According to a more recent multicenter study, 13% of 353 cases of SLE “at some time during the course of their illness” manifested discoid lesions (151). Conversely, none of 120 cases of DLE developed systemic findings during a 5-year follow-up (152). The two opinions were moderated by Burch and Rowell (153) in 1968 in Leeds, England, who theorized that there is a different polygenic predisposition for the development of DLE and SLE: “When a genuine transition from DLE to SLE occurs, the affected patient is genetically predisposed to both diseases.” The question remains open.

Four Subsets of Lupus Erythematosus

Antiphospholipid Syndrome

In 1941 Pangborn (154) in Albany, New York, discovered that the substance in the beef heart extract that was used in the complement fixation test for syphilis was a phospholipid. Keil (155) had recently surmised that the “false positive” reactions in cases of SLE are not merely coincidental. However, evidence of a mechanism to explain “biologic false positive reactivity did not accrue until 1983, when a sensitive method to test for anti-cardiolipin [anti-phospholipid] antibodies was devised” (156).

In 1948, Conley et al. (157) in Baltimore demonstrated an endogenous circulating anticoagulant in nonhemophilic bleeding patients. Four years later two cases of SLE with hemorrhaging attributed to such an anticoagulant were briefly described (158). Lee and Sanders (159) in 1955 in New York found that this substance is not a rarity in SLE, but that it usually does not cause bleeding. This observation was followed in 1963 by the surprising discovery that the anticoagulant may be associated not only with bleeding, but also with thromboses (160). In 1975 spontaneous abortion during the course of SLE was first associated with the lupus anticoagulant (161), and this relationship was subsequently confirmed prospectively (162). In 1988 anticardiolipin antibodies associated with syphilis were differentiated from those related to SLE (163).

Lupus Erythematosus Profundus

“Lupus erythematosus profundus” was coined by Samuel Irgang (164) in 1940 in New York to differentiate from DLE cases with nodular lesions in the deeper portions of the skin, but little epidermal involvement. Such a case had been described by Kaposi (13) in 1869, and this manifestation has been called Kaposi-Irgang syndrome in the dermatologic literature (165). The first American report is attributed to Fordyce (166) in 1924 in New York. Before the 1940s this variant probably was usually misdiagnosed as sarcoid (167). Winkelmann (168) in 1970 suggested that “LE panniculitis” would be a more accurate term, thereby endorsing the pathologic interpretation of Fountain (169) in 1968 in London.

Subacute Cutaneous Lupus Erythematosus

“Subacute cutaneous LE,” described by Sontheimer et al. (170) in 1979 in Dallas, appears to be clinically intermediate between DLE and SLE. The lesions may be preceded by those of DLE and coincide with these at some time in about 20% of cases. They differ from discoid lesions in being annular or resembling psoriasis, lacking follicular plugging, and are less likely to heal with scarring. Patients are more frequently light sensitive than those with either DLE or SLE. About half fulfill the diagnostic criteria for SLE. Most cases are ANA-positive, but resemble “ANA-negative SLE” in being anti-Ro positive (170). Occurrence as a drug-induced phenomenon was first described in 1985, associated with hydrochlorothiazide (171).

Neonatal Lupus Erythematosus

The innocuous transplacental transfer of the LE factor (anti-DNA antibody) was demonstrated in 1954 (172). In the same year a case of transient DLE was described in an infant whose mother subsequently developed SLE (173). Since then, neonatal DLE has usually been found to resolve within the first year. In 1957 a woman with SLE delivered a boy who had complete heart block and died on the second day. His myocardium was found to contain hematoxylin bodies (174). By 1977, sufficient cases of neonatal complete heart block had been described that this became recognized as the most characteristic sign of neonatal LE, occurring in about half of these infants (175 ,176 ,177). Second most frequent are cutaneous lesions. SS-A (anti-Ro) antibody was pointed out in 1981 to be the most consistent serologic finding in both neonatal DLE and SLE (178 ,179).

How Did SLE Come to be Transferred from the Realm of Dermatology to Internal Medicine?

With few exceptions, such as the clinical observations of Osler and of Libman, the delineation and treatment of LE remained in the domain of dermatologists until the 1940s. There are two complementary explanations for this: (a) SLE was recognized by its cutaneous findings and thereby was linked, albeit equivocally, to DLE, a cutaneous disease. SLE was diagnosed much less frequently than DLE and its visceral manifestations were considered secondary to the cutaneous. The 1939 edition of Sutton and Sutton's

Diseases of the Skin contained 13 pages on DLE and SLE (180), while the 1944 edition of Comroe's *Arthritis and Allied Conditions* had three (181). It only became accepted in the 1940s that SLE may occur without skin lesions (35), one factor that moved the disease toward the internist. (b) A medical specialty, when it is circumscribed by more than the patient's age or gender, results from particular technical and/or therapeutic expertise. Although the beginnings of rheumatology may be placed in the late 1920s, its pioneers had neither technical nor therapeutic superiority over other internists. This changed abruptly with the almost simultaneous discovery of two diagnostic methods: the rheumatoid factor and the LE cell in 1948, and cortisone therapy in 1949, followed in 1950 by the establishment of the Institute of Arthritis and Metabolic Diseases in the National Institutes of Health. The LE cell test increased the diagnosis of SLE, and corticosteroids conveyed not only that these patients could be helped, but that the treatment required specialized knowledge, thereby enhancing the status of rheumatology (182).

Interest in LE has shifted from clinical description to immunologic research, with the still frustrated goal of elucidating the etiology, whether it be single or multiple. The continued intensification of scientific interest is reflected in the listing of articles in the *Index Medicus*. These have been increasing steadily, from eight columns in 1960 to 21 in 1982, 25 in 1987, 31 in 1992, and 47 in 1997 to thousands of references in 2006!

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Chapter 2
The Definition and Classification of Systemic Lupus Erythematosus

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Definition of Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystem disease that is caused by antibody production and complement-fixing immune complex deposition that result in tissue damage. As potentially many different antibodies can be produced in SLE patients, the different organ-specific targets of these antibodies can cause a wide spectrum of clinical presentations, which are characterized by remissions and exacerbations. The pathogenic immune responses probably result from environmental triggers acting in the setting of certain susceptibility genes. Ultraviolet light and certain drugs are the only known environmental triggers identified to date.

SLE Classification Criteria

In 1971, the American Rheumatism Association (ARA) published preliminary criteria for the classification of SLE. These criteria were developed for clinical trials and population studies rather than for diagnostic purposes (1). The criteria were based on information from 52 rheumatologists in clinics and hospitals in the United States and Canada; each physician provided 74 items of information on five of their own patients in each of the following categories in which they had classified these patients using their own clinical judgment: unequivocal SLE, probable SLE, classic RA, and medical patients with nonrheumatic diseases. The committee examined the sensitivity and specificity of each item alone and of 140 selected combinations of two or more items. Only 57 of 74 items were included in the final analysis for there was scarcity of information in some of the requested items.

Based on computer analysis of the data, 57 items were reduced to 14 manifestations, which included 21 items. The ARA committee proposed that a person can be said to have SLE if any four or more of the following manifestations are present, either serially or simultaneously, during any period of observation:

- Facial erythema (i.e., butterfly rash): Diffuse erythema, flat or raised, over the malar eminence(s) and/or bridge of the nose; may be unilateral.
- Discoid lupus: Erythematous-raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions; may be present anywhere on the body.
- Raynaud's phenomenon: Requires a two-phase color reaction, by patient's history or physician's observation.
- Alopecia: Rapid loss of a large amount of scalp hair, by patient's history or physician's observation.
- Photosensitivity: Unusual skin reaction from exposure to sunlight, by patient's history or physician's observation.
- Oral or nasopharyngeal ulceration.
- Arthritis without deformity: One or more peripheral joints involved with any of the following in the absence of deformity: (a) pain on motion, (b) tenderness, (c) effusion or periarticular soft-tissue swelling. (Peripheral joints include feet, ankles, knees, hips, shoulders, elbows, wrists, metacarpophalangeal, proximal interphalangeal, and terminal interphalangeal and temporomandibular joints.)
- LE cells: Two or more classical LE cells seen on one or more occasions, or one cell seen on two or more occasions, using an accepted, published method.
- Chronic false-positive serologic test for syphilis (STS): Known to be present for at least 6 months and confirmed by *Treponema pallidum* immobilization (TPI) or Reiter's tests.
- Profuse proteinuria: Greater than 3.5 g/d.
- Urinary cellular casts: May be red cell, hemoglobin, granular, tubular, or mixed.
- One or both of the following: (a) pleuritis, good history of pleuritic pain; or rub heard by a physician; or radiographic evidence of both pleural thickening and fluid; and/or (b) pericarditis, documented by electrocardiogram (ECG) or rub.
- One or both of the following: (a) psychosis, and/or (b) convulsions, by patient's history or physician's observation in the absence of uremia and offending drugs.
- One or more of the following: (a) hemolytic anemia; (b) leukopenia, white blood cell count of less than 4000/mL on two or more occasions; and/or (c) thrombocytopenia, platelet count less than 100,000/mL.

These criteria were chosen because of their high sensitivity and specificity compared to the gold standard, physicians' clinical judgment of the diagnosis; the committee noted 90% sensitivity and 99% specificity against rheumatoid arthritis and 98% specificity against a miscellany of nonrheumatic diseases (1). In a retrospective pilot study of 500 male veterans with scleroderma, only 10 patients satisfied the SLE criteria at the time of diagnosis (1).

These criteria were subsequently tested in other centers; in these various studies, sensitivities varied between 57.2% to 98.0% (2 ,3 ,4 ,5 ,6). The studies with the lowest sensitivities involved patients who were seen either initially or at only one particular point in time (4 ,7); these investigators noted that a higher proportion of their patients eventually demonstrated four or more criteria over time. Lom-Orta et al. (8) studied 31 patients who were thought to have SLE but who did not fulfill the ARA criteria; 21 of them fulfilled the criteria within a few years.

Numerous suggestions were made for improvement of the classification criteria, including the inclusion of antinuclear antibody (ANA) and other autoantibodies (9 ,10 ,11) and the use of a weighted scoring system in which certain criteria are given more weight than others based on clinical importance (12). An ARA subcommittee was created to evaluate these considerations; their study led to the publication of revised criteria in 1982 (13). Thirty potential criteria were studied, including numerous serologic tests and histologic descriptions of skin and kidney, as well as each of the original 1971 criteria. These variables were compared in SLE patients and matched controls. Eighteen investigators representing major clinics contributed patient report forms; these forms indicated the presence or absence of each variable at the time of examination or at any time in the past. Abnormalities that could be attributed to comorbid conditions or concurrent medications were not reported (14). Each investigator was instructed to report prospective data on 10 consecutive patients and the next age-, race-, and sex-matched patient with a nontraumatic, nondegenerative, connective tissue disease seen at that clinic. This generated data from 177 patients with SLE and 162 control patients from 18 institutions. Cluster and other multivariate analysis techniques were used in studying the variables; numerous potential criteria sets were analyzed.

The resulting 1982 revised criteria are a simplified and updated version of the 1971 preliminary criteria that incorporated newer immunologic criteria and aggregates of some organ system manifestations into single criteria. It consists of 11 items, compared with 14 in the initial criteria. As with the initial criteria, patients must fulfill four or more criteria; no single criterion is absolutely essential. ANA, anti-DNA, and anti-Sm antibodies were included in the revised 1982 set; ANA was felt to be the most important addition to the criteria set, because this serologic marker was present at some point during the course of disease in 176 of the 177 patients. Despite the nonspecificity (the marker was present in 51% of the controls studied), the subcommittee felt its almost universal positivity made it a necessary criterion. Because of the scarcity of skin and kidney biopsies, they were excluded from the final revised criteria set. Raynaud's phenomenon and alopecia were also eliminated as a result of low combined sensitivity/specificity scores. Renal criteria were consolidated. In the preliminary criteria set, cellular casts and proteinuria were separate criteria; in the revised set, there is only a single renal criterion, which is satisfied if a patient has cellular casts and/or proteinuria. In addition, the revised criteria reduced the amount of proteinuria that is needed for fulfillment, from greater than 3.5 g/d in the preliminary set to more than 0.5 g/d (or >3 + if quantitation is not performed) in the revised set.

Using the patient database on which they were based, the revised criteria were 96% sensitive and specific, compared with 78% and 87%, respectively, for the 1971 criteria (13). The subcommittee further tested the revised criteria against an ARA database of 590 patients with SLE, scleroderma, or dermatomyositis/polymyositis. Using the revised criteria against this database population, sensitivity in SLE patients was 83%, and specificity against the combined scleroderma and dermatomyositis/polymyositis patients was 89%. Using the preliminary criteria, sensitivity for SLE was only 78% and specificity only 87% (14).

In a subsequent comparison of the relative sensitivities of the 1971 and 1982 criteria, Levin et al. (15) studied 156 SLE patients at the University of Connecticut. Eighty-eight percent met the preliminary criteria, whereas 83% met the revised criteria when arthritis was strictly defined (i.e., nonerosive arthritis). Ninety-one percent met the revised criteria when arthritis was more liberally defined (i.e., nondeforming arthritis). These differences were not statistically significant. Their analysis also noted that of the three serologic tests added in the revised criteria (i.e., ANA, anti-Sm, and anti-DNA antibodies), ANA accounted for the increased sensitivity of the revised criteria. Levin et al. noted that both the preliminary and the revised criteria were inappropriate for diagnostic purposes, in that over 50% of their patients fulfilled neither set of criteria when tested at the time of diagnosis. These patients subsequently fulfilled both sets of criteria at the same rate (77.5% fulfilled preliminary criteria and 78.5% revised criteria 5 years after diagnosis, and 84.5% and 83.0% for preliminary and revised criteria, respectively, at 7 years).

Passas et al. (16) compared specificity of the preliminary and revised criteria in 207 University of Connecticut patients with non-SLE rheumatic diseases that are important in the differential diagnosis of SLE. The specificity was 98% for the preliminary criteria and 99% for the revised criteria. The preliminary and revised criteria also were tested on 285 Japanese SLE patients and 272 control patients with non-SLE connective tissue diseases (17). The preliminary criteria had a sensitivity of 78% and a specificity of 98%, compared with a sensitivity of 89% and specificity of 96% for the revised criteria. Davis and Stein (18) applied the preliminary and revised criteria to 18 Zimbabwean patients with SLE reported up to 1989; they noted a sensitivity of

83% for the preliminary and 94% for the revised criteria. When serologic criteria were excluded, the sensitivity of the revised criteria was only 78%. They concluded that in many areas of Zimbabwe, where serologic tests are not readily available, the preliminary criteria may be more valuable than the revised criteria in the classification of patients with SLE, because the preliminary criteria rely more on clinical rather than serologic variables.

Table 2-1: The 1997 Revised Criteria for the Classification of Systemic Lupus Erythematosus (SLE)

Criterion	Definition
1. Malar rash	Fixed malar erythema, flat or raised
2. Discoid rash	Erythematous-raised patches with keratic scaling and follicular plugging; atrophic scarring may occur in older
3. Photosensitivity	Skin rash as an unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulcers, usually painless, observed by physician
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a. Pleuritis (convincing history of pleuritic pain or rub heard by physician or evidence of pleural effusion) OR b. Pericarditis (documented by ECG, rub, or evidence of pericardial effusion)
7. Renal disorder	a. Persistent proteinuria (>0.5 g/d or >3+) OR b. Cellular casts of any type
8. Neurologic disorder	a. Seizures (in the absence of other causes) OR b. Psychosis (in the absence of other causes)
9. Hematologic disorder	a. Hemolytic anemia OR b. Leukopenia (<4,000/mL on two or more occasions) OR c. Lymphopenia (<1,500/mL on two or more occasions) OR d. Thrombocytopenia (<100,000/mL in the absence of offending drugs)
10. Immunologic disorder	a. Anti-double-stranded DNA OR b. Anti-Sm OR c. Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody (ANA) by immunofluorescence or an equivalent assay at any time and in the absence of drugs known to be associated with “drug-induced lupus syndrome”

For identifying patients in clinical studies, a person shall be said to have SLE if any four or more of the 11 criteria are present, either serially or simultaneously, during any interval of observation.
Ig, immunoglobulin.
From Hochberg MG. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus (letter). *Arthritis Rheum* 1997;40:1725.

Because the presence of antiphospholipid antibodies and the antiphospholipid syndrome (APS) was increasingly recognized in SLE patients, the Diagnostic and Therapeutic Criteria Committee of the ACR updated the 1982 revised criteria for

SLE in 1997 (Table 2-1) (19). Immunologic criteria were modified by removing the LE cell preparation and the item “false-positive test for syphilis” was expanded to “positive finding of IgG or IgM anticardiolipin antibodies or a lupus anticoagulant.” No formal validation studies of these modifications have been performed.

The 1997 revised criteria have not been free from criticism. Classification of neurological and psychiatric manifestations of SLE were expanded to include 19 different syndromes in 1999 (20), whereas all versions of classification criteria for SLE have included only psychosis and seizures. Although the current classification criteria include the presence of certain isotypes of anticardiolipin antibodies and the lupus anticoagulant, it does not include IgA anticardiolipin antibodies, other assessments of antiphospholipid antibodies or any of the clinical features of APS as outlined in the preliminary classification criteria for definite APS, which were published in 1999 (21) including small-vessel thrombosis and prematurity as a result of severe preeclampsia, severe placental insufficiency, venous and arterial thrombosis, recurrent miscarriage, and fetal death.

The heterogeneity of SLE extends to the type of clinical manifestations at disease onset and to the time it may take for these manifestations to evolve and make the diagnosis of SLE possible. As the understanding of the pathogenesis of SLE is improving and more advanced tests are available, it is more evident that there is a growing need for a more organized and more easily translatable classification. Current ACR classification for SLE has allowed the universality of SLE clinical research and has also served as a guide in clinical practice, but the performance of ACR classification needs to be improved for it has inherent limitations, including bias toward more severe disease and longer duration of disease, equal weighing of features that vary in clinical significance, lacking accurate definitions for certain conditions, and not including important and serious forms of lupus that may occur in patients who do not meet the full criteria.

The Systemic Lupus International Collaborating Clinics (SLICC) has proposed to revise the current SLE criteria in order to develop a relatively simple classification system that captures the key considerations used in determining clinical diagnoses of SLE, cutaneous lupus, and APS. Thirty-two international centers are included in the SLICC registry to collect clinical and laboratory data in 600 patients with SLE, APS, and cutaneous lupus cases and disease controls. The study will include data sets of only those cases and controls for which there is agreement on the diagnosis by a “consensus panel” of rheumatologists, dermatologists, and nephrologists. A wide array of multiple variables will be evaluated to individually estimate the sensitivity and specificity of each criterion. Preliminary data from the initial 70 patients recruited for the SLICC database have been evaluated (22); they were categorized based on the diagnosis given to them at the enrollment site: 56 SLE, two undifferentiated connective tissue disease (UCTD), five rheumatoid arthritis, one primary APS, three secondary APS, four vasculitis, and two dermatomyosites. Thirteen SLICC members were asked to enter their diagnosis without regard to the number of ACR criteria for each case. The agreement in diagnosing SLE among members was fair. Perfect consensus was reached for only 33 (47%) patients: 24 SLE and nine non-SLE patients. The difference between consensus rates among SLE patients with three or four ACR criteria did not reach a significant level and there was a large overlap between SLE and UCTD diagnosis. These results suggest that ACR criteria for SLE do not closely reflect clinical diagnoses as assigned by lupus experts. This ongoing cooperative effort will hopefully result in redefining the confusing criteria and validation of new classification criteria.

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Chapter 3
Environmental Aspects of Lupus

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The relative rarity, heterogeneity, and multifactorial etiologies of autoimmune diseases have limited our understanding of their pathogeneses. Although mechanisms for the development of autoimmune diseases have not been fully elucidated, evidence from a number of lines of investigation suggests that these increasingly recognized disorders result from environmental exposures in genetically susceptible individuals (1 ,2 ,3 ,4 ,5). Despite the significant progress that has been made in understanding a number of genetic risk factors for lupus and other autoimmune diseases (see Chapter 6), relatively little information is now available regarding the role of specific environmental agents in the development of these disorders. Nonetheless, there has been a substantial increase in research pertaining to environmental influences on lupus over the past decade.

Environmental risk factors may be considered to be all those susceptibility factors that are not inherited. Exposures that have been suspected of being involved in the pathogenesis of autoimmunity may be divided into two general categories: noninfectious and infectious agents. Here, we focus on the evidence for the role of selected noninfectious and infectious environmental agents in the pathogenesis of systemic lupus erythematosus (SLE) as classified by the American College of Rheumatology (6) as well as lupus-like disorders that do not meet current SLE classification criteria (together referred to as “lupus” in this chapter). Drug associated lupus will not be discussed here as it is covered in Chapter 44 .

Difficulties in the Assessment of Environmental Risk Factors

There are many reasons why so little information is available about the environmental risk factors for lupus and other autoimmune diseases (Table 3-1). First, SLE, like most other autoimmune conditions, is a relatively rare condition and heterogeneous in its phenotypes, genotypes, and likely pathogeneses. Therefore, only a subset of any population of lupus patients is likely to share common gene-environment interactions that would lead to the syndrome under study. Thus, low effect sizes, which often result in odds ratios less than 2.0 for many exposure risks, dictate the need for studying relatively large populations to have adequate statistical power to identify environmental risk factors.

The relative lack of validated exposure biomarkers that allow for confirmation of a given exposure, and can pinpoint exposure to a specific time period, is another difficulty. This is a critical deficiency given the potential long latency (time from agent exposure to disease onset) of some causal factors and the increasing problems of recall bias the longer the period from exposure. The fact that there are few practical clinical assessment tools that have been validated for the identification and quantitation of exposures is another deficiency in the field.

As is the case for some cancers (7), there may be certain exposures that initiate autoimmunity (inducers) and quite different ones that promote (promoters) and sustain (sustainers) the pathologic states we recognize as autoimmune diseases. This possibility is suggested by investigations showing that autoantibodies precede the development of clinical disease by months to years (8 ,9 ,10). Therefore, in some cases, a change induced by one exposure may be necessary before a subsequent exposure can have its effect. This may result in variable latencies for certain causal factors depending on the prevalence of the required secondary exposure and dependent host factors. Alternatively, mixtures of exposures, including possible combinations of infectious and noninfectious agents, perhaps occurring during critical periods when persons may be more susceptible to them (i.e., in utero, in childhood, during puberty, pregnancy, lactation or other stressful conditions) may be necessary in order to overcome tolerance. It is also possible that certain environmental exposures, as is the case for certain genes (see Chapter 6), may be protective for the development of a disease.

In certain circumstances it can be relatively clear when a given exposure is inducing an illness in an individual patient. The definition of an environmental disease in selected persons can be accomplished by identifying a new clinical disorder that develops soon after a novel exposure, resolves when the exposure is removed (dechallenge) and then recurs after reintroduction of the same exposure (rechallenge) (11). This approach is most easily applied in the case of exposures to defined chemical entities that undergo rapid metabolism and elimination such as many drugs, foods, and topical or inhaled toxicants. Unfortunately, a number of xenobiotics (compounds not naturally found in the body) cannot easily be removed from an organism after exposure, and therefore, for these agents this approach is not usually helpful. Exposures in this latter category include inhaled silica, vaccines, fat-soluble oils, and collagen or silicone implants. It is also likely that certain causative agents

induce a positive reinforcement cycle in the pathogenesis of autoimmune disease, so that removing them after some critical point is reached may not result in disease improvement.

Table 3-1: Difficulties in Assessing the Role of the Environment in the Pathogenesis of Lupus

Relatively rare syndromes
Heterogeneity of disease phenotypes, genotypes, and likely pathogeneses
Possible requirement for gene-environment or environment-environment interactions
Lack of validated biomarkers for many exposures
Few practical clinical assessment tools to accurately identify and quantitate exposures
Possibly different agents are needed to initiate autoimmunity (inducers) and promote (promoters) and sustain (sustainers) autoimmune disease
Variability in latencies (time from exposure to disease onset) for different causal factors
Difficult to “remove” many environmental exposures so that a “challenge-dechallenge” study is not feasible
Little formal training in environmental medicine
Lack of comprehensive national registries, databases, and specimen repositories
Little information on the long-term human effects of tens of thousands of xenobiotics registered for commercial use

Other difficulties are that there is little formal training in environmental medicine and there are few resources generally dedicated to this area. Finally, the lack of comprehensive national registries, databases, and specimen repositories means that almost every environmental study in the United States needs to be initiated anew and cannot build on prior efforts in the field.

More than 80,000 chemicals are registered for use in commerce in the United States, and an estimated 2,000 new ones are introduced annually to be included in our foods, personal care products, drugs, household cleaners, and a host of industrial processes (http://www.ntp-server.niehs.nih.gov/). This does not include the many poorly documented xenobiotics present in imported products that are increasing yearly as a result of our ever more global economy. Although relatively few of these are thought to result in significant adverse effects on human health, the long-term impacts of most of these compounds are unknown. It will be increasingly necessary to be able to determine the effect of complex environmental exposures on the development and course of autoimmune diseases.

Evidence Supporting Environmental Aspects of Lupus

Despite the many difficulties listed above that confront investigators, evidence for the role of environmental agents in the pathogenesis of lupus has been accumulating from a variety of approaches for nearly half a century (Table 3-2). Although some of these methods are indirect or may be applicable only to single patients, taken together these complementary findings from many different investigations strongly support the contention that lupus, like most autoimmune diseases, does have an important environmental component (2).

Table 3-2: Lines of Evidence Supporting the Role of the Environment in the Pathogenesis of Lupus

Less than 50% disease concordance in monozygotic twins
Dechallenge (disease resolution or improvement after removal of the suspect agent)
Rechallenge (disease recurrence or worsening after re-exposure to the suspect agent)
Changes in the incidence or prevalence of lupus over time
Different incidence or prevalence rates in the same ethnic group in different locations
Strong biologic plausibility from laboratory studies and animal models
Epidemiologic associations with particular exposures

One line of evidence for the role of the environment in lupus and other autoimmune illnesses is that there is generally less than 50% disease concordance in monozygotic twins who have an identical genetic makeup (2 ,12). Disease concordance for lupus in a series of 45 monozygotic twin pairs was only 24% (13). While this low level of concordance has been suggested to be the result of stochastic or other events, the consistently low disease concordance in genetically identical persons among all the autoimmune disorders studied, and the other lines of evidence suggesting the role of environmental factors, argue against this. These findings also hint that even if all the genetic risk factors for a given autoimmune disease could be fully identified, this would not allow for prediction of disease with any greater accuracy than the flip of a coin, without the incorporation of environmental or other etiologic factors.

As mentioned above, in certain individuals environmental agents may be directly associated with a lupus syndrome by the development of disease in an appropriate time frame after exposure to the suspect agent (challenge), resolution, or dramatic improvement of the condition after removal of the agent (dechallenge) and then redevelopment of the disorder upon re-exposure to the agent (rechallenge).

Significant increases or decreases in the incidence or prevalence of disease over time imply a nongenetic etiology given the relatively slow rate of genetic change in a population. Although data are limited in this area—and studies are possibly confounded as a result of improvements in the ability to diagnose some conditions over time—it appears that lupus, as is the case for some other autoimmune illnesses, may be increasingly prevalent (14 ,15 ,16 ,17), while rheumatoid arthritis may be decreasing in frequency in some populations (15).

Studies of genetically similar populations living under different conditions or in different geographic locations may also be illuminating in understanding possible environmental

or lifestyle effects on autoimmunity. In this regard, the incidence of lupus appears to differ in persons of African or Chinese ancestry in different locales (18 ,19). These differences may be the result of referral, ascertainment, or other biases, however, and more study in this area is needed to understand the true reasons for such differences. Strong biologic plausibility from laboratory studies and animal models (see Chapter 18) is another line of evidence supporting the role of noninfectious and infectious agents in the pathogenesis of lupus.

Finally, epidemiologic associations between particular exposures and lupus, despite their high costs and limitations, are perhaps the most important investigations that have recently widened our perspectives on the role of environmental agents in the development of lupus. Epidemiologic studies linking specific exposures are limited and too often consist of relatively small, underpowered investigations resulting in low effect sizes and wide confidence limits. Yet much of the data presented below have come from such studies, which, when replicated by different study designs in various populations, provide greater confidence in the findings. Larger, well-designed, multicentered, and sometimes international studies, using appropriate controls and collecting adequate information to minimize confounding, are needed in the future to more fully define the specific environmental risk factors for the lupus syndromes and their component phenotypes.

Noninfectious Agents Associated with Lupus

Exposures to a wide variety of compounds with different structures, chemical properties, metabolic pathways, and modes of action have been reported to be associated with lupus. The evidence supporting these proposed associations range from case reports to epidemiologic and laboratory studies, and most of the proposed associations are speculative at this time. We will focus on describing those agents for which more information is available and the strength of the association seems strongest. Table 3-3 lists epidemiologic studies for these agents.

Silica

Crystalline silica or quartz is a very abundant material in sand, rock, and soil. Occupational exposure to respirable silica dust (particles <5 μm) has been associated with the chronic inflammatory and fibrotic lung disease called silicosis (20) as well as a number of autoimmune disorders (21 ,22 ,23). It is possible that the development of silicosis is further modulated by immunogenetic risk factors (24 ,25). The dusty trades, including pottery and china manufacturing, quarry work, masonry, and mining are the traditional occupations that involve significant respiratory silica exposure, although others, including scouring powder manufacturers, some dental workers, and professional housecleaners, can also come into extensive contact with silica dust during their jobs.

Initial case reports and series dating back to the early 20th century focused on the role of silica in rheumatoid arthritis and scleroderma. These early studies encouraged case series and later epidemiologic investigations of the role of silica in SLE (22). Evidence for the association of occupational silica with lupus comes from case series (26 ,27 ,28), animal investigations (29 ,30) and epidemiologic studies (31 ,32 ,33). After therapeutic drugs (see Chapter 44), silica is one of the best-studied environmental agents associated with lupus, and has perhaps the greatest scientific support as an environmental risk factor.

Three epidemiologic investigations have demonstrated significant risks for the development of lupus after occupational silica exposure (31 ,32 ,33). These studies—which utilized different designs and were conducted in different countries and populations—have revealed consistent associations across gender, racial, and educational subgroups, and have shown evidence of a dose-response effect (Table 3-3). These findings increase confidence that potential confounders associated with each approach have been overcome and the association of occupational silica exposure with lupus is real. The limited data, however, do not allow for a comprehensive and precise comparison of clinical features among subjects who have developed lupus after silica exposure to those who have developed lupus without significant silica exposure. Nonetheless, Conrad et al. (34) have described an increase in the prevalence of the 16/6 idiotype, which is a major cross-reactive idiotype of anti-DNA autoantibodies that have been reported in SLE and patients treated with procainamide (35), among uranium miners exposed to quartz dust who developed lupus. All the miners with the 16/6 idiotype had antibodies to double-stranded deoxyribonucleic acid (dsDNA). Furthermore, two of the minors who were 16/6 positive had progression of their disease compared to none who were 16/6 idiotype negative, suggesting this may be a risk factor for progression to SLE. It is also possible that the autoantibody distributions differ in those who develop lupus after significant silica exposure compared to those without such exposure (36).

In addition to the human studies above, a number of laboratory investigations have been undertaken in vitro and in vivo in an attempt to understand pathogenetic mechanisms for the role of silica in inducing autoimmune disease. Silica has been shown to have an adjuvant effect in a variety of animal models and disease states. Respirable silica particles are phagocytized by alveolar macrophages, leading to cellular activation and the release of soluble mediators such as chemokines, proinflammatory cytokines, including tumor necrosis factor-α and interleukin-1-β, transforming growth factor-β, lysosomal enzymes, and reactive oxygen species (20). These soluble mediators act to recruit and activate additional inflammatory cells that may lead to increased antigen processing and accelerated antibody production. Other processes include activation of cell signaling pathways, including the mitogen-activating protein kinase (MAPK) pathways, and phosphorylation and activation of specific transcription factors (e.g., NF-κB). Furthermore, these effects

are not limited to the lung. Migration of silica-containing macrophages to the lymph nodes and increased systemic immunoglobulin production have also been shown to occur (37 ,38).

Table 3-3: Epidemiologic Studies of Noninfectious Agents and Risk of Lupus

Exposure	Setting, Design; (N)*	Association**	Reference
Silica	Sweden, registry linkage silicosis patients, discharge diagnoses; (1052)	RR 23.8 (10.3–47.0)	(31)
	Michigan, registry linkage silicosis patients, medical record diagnosis of SLE; (463)	OR 11.4 (0.2–63.2)	(32)
	North and South Carolina, case-control, structured interview; (265,355)	Medium exposure: OR 1.7 (1.0–3.2) High exposure: OR 3.8 (1.2–1.6)	(33)
Hair dyes	Georgia, case-control, structured interview reuse before diagnosis; (44,88)	OR 7.2 (1.9–26.9)	(47)
	Baltimore, case-control questionnaire, use before diagnosis; (218,186)	Friends as controls: OR 0.9 (0.6–1.5) Relatives as controls: OR 1.3 (0.8–2.1)	(101)
	Pennsylvania, case-control interview, use 1–3 or 3–6 yrs before diagnosis; (195,143); cohort-control study, questionnaire use before diagnosis; (85,106,391)	Friends as controls: OR 1.1 (0.6–2.0) Medical controls: OR 1.3 (0.7–2.5) RR 0.96 (0.6–1.5)	(102) (103)
	United Kingdom, case-control interview, use before diagnosis; (150,300)	OR 1.2 (0.8–2.0)	(104)
	North and South Carolina, case-control interview, use before diagnosis; (265,355)	OR 1.5 (1.0–2.2)	(105)
Hormones	United States, case-control questionnaire reprior hysterectomy or tubal ligation; (109,109)	OR 0.55 (0.31–0.99)	(106)
	Japan, case-control questionnaire remenstrual irregularities; (52,104)	RR 3.79 (1.43–10.0)	(73)
	Japan, new onset, case-control questionnaires age of menarche >15 years: (282,292)	OR 3.82 (1.66–8.81)	(72)
Pesticides	North and South Carolina, case-control interview, use before diagnosis; (265,355)	Mixing: OR 7.4 (1.4–40) Applying: OR 0.77 (0.34–1.8)	(23)
Smoking	United States and Europe, meta-analysis of seven case control and two cohort studies on smoking	Current: OR 1.5 (1.1, 2.1) Former: OR 0.98 (0.75, 1.3)	(61)
Solvents	North and South Carolina, case-control interview, use before diagnosis; (265,355)	Possible exposure: OR 1.0 (0.57–1.9) Likely exposure: OR 1.0 (0.6–1.6)	(105)

*For case control studies, N, (number of cases, number of controls)
**OR, odds ratio or RR, risk ratio and (95% confidence interval)

In murine models, silica has been shown to increase the levels of antigen-specific antibodies in the lung following intranasal or intratracheal instillation (39) and in the blood following intravenous administration (40). In the New Zealand mixed lupus mouse strain, silica exposure accelerated disease expression in that it increased autoantibody production, immune complex deposition, proteinuria, and glomerulonephritis (29). This series of studies provide potential mechanisms through which silica exposure may affect the development of autoantibodies and autoimmune disease. autoantibodies from the exposed mice recognized specific epitopes on apoptotic macrophages and changes in CD4 T cell counts and other T cell populations were also seen (30 ,41).

In vitro studies also provide evidence that silica's properties as an adjuvant may be relevant in inducing autoimmunity. Incubation of silica and silicate with isolated human T cells cause polyclonal lymphocyte activation—which in vivo could lead to a breakdown of tolerance via nonspecific stimulation of autoreactive T cell clones (42) and might also lead to apoptosis (43)—which could result in human cells releasing, concentrating, and altering autoantigens and thereby focusing an autoimmune attack (44). Silica is also present in particulates in air pollution. Studies of particulate air pollutants indicate that silica particles stimulate a Th1 type response, with increased production of interferon-γ and increased levels of antigen-specific IgG, although the effects may vary depending on the particle type and mouse strain (45 ,46). This skewing toward a Th1 response and release of reactive oxygen species promotes the continued secretion of pro-inflammatory cytokines resulting in chronic macrophage activation. Indeed, one

hallmark of silica-induced lung injury is its self-perpetuating nature. In alveolar macrophages, the intracellular release of proteolytic enzymes in response to phagocytosis of silica particles leads to cell death. Silica is released from the dying cells, and is re-ingested by other macrophages, creating a cyclical process of inflammation and necrotic cell death.

Hair Dyes

Since the first epidemiologic study suggesting a strong association of permanent hair dyes and related hair products with SLE (47), an additional five studies have assessed this issue (Table 3-3). The interest in these exposures is based in part on similarities of some of the constituents of these products (arylamines) to medications associated with drug-induced lupus (such as hydralazine and procainamide). None of the subsequent studies, however, many of which were larger than the initial report, have shown the same strong association—four have been negative and only one has found a weak association. Of interest, a significant threefold risk was seen among hair dye users who had the *N*-acetyl transferase (NAT) allele*10 and the NAT2 slow acetylation genotype (48), suggesting a possible gene-environment interaction for this exposure. In summary, it appears that there is not a strong association between hair dye use and the development of lupus, although there may be a weak association and it may be that certain individuals with slow acetylation genotypes may be at increased risk. Additional study is needed in larger cohorts to definitively answer this question.

Pesticides

Pesticides are a varied group of agents that may be classified based on function (herbicides, insecticides, fungicides, fumigants, etc.) or by chemical composition (triazenes, organophosphates, organochlorines, etc.). The pesticides most commonly used in the United States are herbicides (atrazine, glyphosate, acetochlor, and 2,4-D) fumigants (metam sodium and methylbromide) and the insecticide malathion (49). Interest in the possible role of pesticides in the development of autoimmunity has come from animal studies suggesting their role in immunosuppression and hypersensitivity (50) and their capacity to act as endocrine disrupters (51).

Few studies have evaluated pesticides as risk factors for the development of SLE. There were no significant differences in blood levels of DDE (dichlorodiphenyldichloroethylene, the persistent metabolite of DDT, dichlorodiphenyltrichloroethane) and several organophosphate pesticide metabolites in a small case-control study in Nogales, Texas (52). However, in a larger case-control study of self-reported pesticide use in the Carolinas, there was a strong but imprecise association with mixing pesticides, but not applying them (23) (Table 3-3). A community based study in Saskatchewan demonstrated an association of low titer antinuclear autoantibodies (≥1:40) with farming tasks, including the use of pesticides, and a twofold increase in those with a history of exposure to insecticides and herbicides, but not fungicides or algicides (53). Additional study is needed in larger cohorts, utilizing bioassays when possible, to address further this question.

Sobel et al. studied three estrogen-disrupting organochlorine pesticides (o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT), methoxychlor, and chlordecone) in ovariectomized female (NZB × NZM)F1 mice (54). Disease expression (time to renal damage) and anti-dsDNA autoantibody production was accelerated, particularly with chlordecone exposure. These effects were not related to the estrogenic effect of the pesticides as measured by uterine weight, suggesting that pesticides may influence autoimmune disease through mechanisms that are not related to estrogen or endocrine disruption.

Tobacco Use

In addition to several types of cancer and cardiovascular disease, cigarette smoking has been associated with the development of rheumatoid arthritis and other autoimmune diseases (55). Given that smoking is a common habit worldwide and a potentially modifiable risk factor, it is an important exposure to define as a possible risk factor for SLE. The mechanisms by which tobacco smoke may alter the immune system remain unclear. Smoking is anti-inflammatory in some respects, resulting in impaired secretion of pro-inflammatory cytokines and decreased activity of natural killer cells, but other immunomodulating effects may also occur—including rebound sympathetic bias and associated Th2 inflammation—since the effects of smoking differ considerably among the different autoimmune diseases (56). Of possible relevance is that cigarette smoke from certain sources can contain high levels of cadmium and other toxins, likely derived from pesticide applications and local soil conditions (57). An interesting issue with respect to tobacco use is the potential interaction between smoking and other exposures. For example, in a population-based case-control study of silica exposure and SLE, the effects of silica were significantly higher among smokers than among nonsmokers (58). A similar interaction between silica and smoking was seen in a recent case-control study of rheumatoid arthritis (59). It is also possible that the complex mix of chemicals in tobacco smoke may have different effects in different genetic backgrounds, given the interaction of smoking with the human leukocyte antigen (HLA) DRB1 shared epitope in increasing risk for seropositive rheumatoid arthritis (60).

In the meta-analysis recently reported by Costenbader et al., a weak association between current smoking and the development of SLE was found (61). This meta-analysis reviewed nine studies (seven case-control and two cohort studies) published between 1990 and 2003. Six of the nine investigations did not show a significant association between smoking and SLE, and among the three studies showing a significant association, one is an outlier with a much higher odds ratio, which accounts for much of the

association seen in the meta-analysis. In summary, whether cigarette smoking is a risk factor for SLE is controversial and further studies should address this issue, yet, for a variety of reasons, all persons should be encouraged to avoid smoking and second-hand smoke.

Solvents

Solvents are a heterogeneous group of liquid compounds used as degreasers and cleansers in many industries, especially in metal cleaning and dry-cleaning. They include chemicals such as alcohols, glycols, aromatic hydrocarbons (benzene, toluene, xylene), and chlorinated products (carbon tetrachloride and trichloroethylene). Exposure assessment is difficult because of the rapid metabolism of most solvents and the wide variety of solvents used even the same occupations in the same industry. Interest in solvents as a risk factor for lupus comes from data in systemic sclerosis and mixed connective tissue disease consistently showing a moderate (OR 1.5–3.0) association with solvents (22), and recent studies in medical research laboratory (MRL) +/+ mice demonstrating an increase in antinuclear autoantibodies and immunoglobulins, via $CD4^+$ T cell stimulation, by adding trichloroethylene or its metabolites to drinking water (62 ,63). Effects were also seen with specific metabolites of trichloroethylene, but this was blocked when the cytochrome P450 CYP2E1 pathway through which trichloroethylene is metabolized was blocked by the addition of diallyl sulfide (64).

Several studies have evaluated areas contaminated by solvents for a possible association with lupus. Kilburn and Warshaw (65) assessed 362 subjects exposed to trichloroethylene, trichloroethane, inorganic chromium, and other chemicals in water obtained from wells in an industrially contaminated aquifer in Tucson, Arizona for SLE criteria, and compared them to an Arizona control group, to published series, and to laboratory controls. The number of subjects with SLE symptoms was 2.3 times higher in the study group compared to referent women and men, and anti-nuclear autoantibody titers greater than 1:80 were approximately 2.3 times higher in women but equally frequent in men as in laboratory controls. Their conclusions have been challenged (65a) Wallace DJ, Quisinorio FPJr, *The elusive search for geographic clusters of systemic lupus erythematosus,* Arthrites rheum 1995,38: 11564–1567. Another study reported a higher prevalence of SLE compared to published series in a small African-American community in Georgia that experienced long-standing exposures to industrial emissions (66). The only case-control study of solvents in SLE did not find a significant association (23), however, implying that additional focused study is needed in this area to define the association more fully.

Sex Hormones

The predominance of lupus in females, the frequent increase in disease activity with pregnancy, decreased activity in postmenopausal women and the many known immunomodulatory effects of sex hormones in humans and animals are the basis for the interest in the role of sex hormones in the development of the disease (67 ,68 ,69). Some studies have reported an increased risk of SLE among women using hormone replacement therapy (70), but this finding has not been confirmed by other studies (71). If greater estrogen exposure is related to lupus, then one might predict that those with an earlier menarche would be at increased risk for disease. Surprisingly, this has not been the case in the several studies evaluating this issue (Table 3-3). In fact the opposite was found in one study in which a later age of menarche was a risk factor (72). In other studies, no significant associations were seen or a later age at menarche was associated with lupus (71 ,73). Of note, menstrual irregularities were a risk factor in one study (73). Many unanswered questions remain in this area and the issue of possible endocrine dysfunction prior to clinical presentation is one of the many unresolved issues that would benefit from directed investigation.

Infectious Agents

Based upon animal models and more limited human data, infectious agents appear to be able to induce autoimmune disease in some cases and, paradoxically, protect from autoimmune disease in other circumstances (74). The protective aspect has given rise to the "hygiene hypothesis," which is the theory that improved standards of hygiene in the industrialized world have resulted in decreased childhood infections and may account for the noted increases in the incidence of allergies and autoimmunity over recent decades (75 ,76). Based upon case reports, animal models and other studies, a variety of viruses, bacteria, and other infectious agents have been proposed to be associated with SLE (77). Fewer studies, however, have assessed this possibility using more rigorous epidemiologic approaches and we will focus our review on those investigations (Table 3-4).

Epstein-Barr Virus

Investigations showing elevations in titers of anti-Epstein-Barr virus (EBV) antibodies in SLE patients compared to controls (78 ,79), case reports of temporal association of disease onset with EBV infection (80 ,81), animal models in which lupus can be induced by EBV antigens (82) and possible molecular mimicry between Sm B/B' autoantigen and Epstein Barr nuclear antigen (EBNA) 1 (83) resulted in a number of studies to evaluate the possible association of EBV with lupus. Recent case-control investigations in children and adults have suggested an increased proportion of anti-EBV seroconverters and those with EBV DNA in SLE (82 ,84 ,85). This association was not seen in other autoimmune diseases or with related viruses. A different case-control study discovered increased EBV-IgA seroprevalence associated with SLE, and that race, gender and the CTLA-4 genotype influences immune responsiveness to EBV (86).

Table 3-4: Epidemiologic Studies of Infectious Agents and Risk of Lupus*

Exposure	Setting, Design; (N)*	Association**	Reference
Epstein-Barr virus	United States children, EBV antibodies or DNA, cohort-control; (117,153)	Serology OR 50 (9.3–1025) DNA OR >10 (2.5-infinity)	(82)
	United States adults, EBV antibodies or DNA, case-control; (196,392)	Serology OR 9.4 (1.45-infinity)	(84)
	Taiwan, EBV DNA, case-control; (87,174)	OR 4.64 (2.50–8.62)	(85)
	North and South Carolina, EBV IgA antibodies, case-control; (230,276)	African Americans OR 5.6 (3–10.6) Whites >50 years OR 4.1 (1.6–10)	(86)
Cytomegalovirus	United Kingdom, anti-CMV antibodies, cohort-control; (97 SLE, 50 RA, 97 controls)	OR 14.5 (6.4–33)	(90)
Herpes zoster virus	Philadelphia, interviews and chart reviews, history of shingles prior to diagnosis, case-control; (195,143) (friend/clinic controls)	Adjusted OR 6.4 (1.4–28.0)	(92)
	North and South Carolina, interviews, history of shingles prior to diagnosis, case-control (population-based controls) (265,355)	Adjusted OR 2.5 (1.1–5.5)	(94)
	Ottawa, questionnaire, history of shingles prior to diagnosis, case-control (93,353), clinic controls	Adjusted OR 2.98, p < 0.003	(93)

*For case control studies, N, (number of cases, number of controls)
**OR, odds ratio and (95% confidence interval when available)

In another study, 50% of the patients with SLE and 100% of the patients in the acute phase of infectious mononucleosis, but none of the EBV-seropositive normal individuals, produced IgG antibodies to an EBNA-2-derived synthetic peptide that cross reacts with SmD1 (87). This suggests that EBV may establish a persistent infection in some SLE patients and elicit antibodies to the viral antigen EBNA-2, which may cross-react with SmD1 and result in these autoantibodies in SLE patients. Of interest, a recent investigation has also suggested that the frequencies of EBV-infected cells are higher in patients with SLE, as is EBV gene expression in these cells, compared to healthy individuals and patients with other autoimmune diseases (88).

Taken together, these and other data are suggestive of higher rates of EBV infection and persistent or altered expression of that infection in lupus. Yet, as is the case for all studies of infectious agents in SLE to date, it remains unclear if there is a causal relationship or whether there may simply be a predisposing immunosuppression in lupus patients to allow for these infections to occur more frequently after the initiation of the disease process. Nonetheless, a recent study has suggested the possibility that early immune responses to the 60 KD Ro antigen, prior to the development of clinical lupus, cross react with EBV nuclear antigen 1 epitopes, and animals immunized with either of these antigens later develop clinical signs of lupus (89). Thus, these findings, if confirmed, may be an example of molecular mimicry in which early responses to EBV antigens lead to antibodies that are cross-reactive with lupus autoantigens and to later clinical disease.

Cytomegalovirus

Cytomegalovirus (CMV) has been of interest as a possible pathogenetic factor in SLE. A group in the United Kingdom demonstrated a significantly higher prevalence of anti-CMV antibody by enzyme immunoassay in SLE compared to controls but not compared to RA patients (Table 3-4) (90). Interestingly, herpes simplex virus-1 (HSV-1) seropositivity was not different among these same groups suggesting a specific association between infection with CMV and SLE. CMV infection has also been suggested to be a factor in the increase of disease activity in several patients with SLE (91).

Herpes Zoster (Varicella-Zoster Virus)

Herpes zoster (shingles) before the clinical diagnosis and therapy was associated with risk of lupus in three case-control studies, with odds ratios between 2.5 and 6.4 (92 ,93 ,94). It is of note that the viruses that have been associated with lupus in epidemiologic studies (EBV, CMV, and Varicella-zoster virus) are all herpetoviruses, which can persist in a latent state for years before reactivation and the development of clinical disease. Factors affecting the re-activation of the infection (e.g., stressor immunosuppression) may also affect the risk of lupus.

Other Agents Suggested as Possible Risk Factors for SLE

A variety of additional noninfectious and infectious agents have been proposed to be possible risk factors for SLE, although the data supporting these assertions has not been as well studied or reproduced as the other examples given in this chapter. These agents include allergens, selected foods, metals, stressful life events, vaccines, ultraviolet radiation, certain bacteria, retroviruses, and parvovirus B19 (Table 3-5). While part of the limitation in understanding the role of some of these agents relates to the lack of adequate study, in other cases, extensive basic and clinical investigations have been undertaken with contradictory findings in subsequent studies.

Table 3-5: Other Exposures Proposed as Possible Risk Factors for Lupus

Exposure	Evidence	Comments (references)
Noninfectious Agents		
Allergens	Case reports, epidemiologic studies	Hives and medication allergies were both found to be significant risk factors in a case-control study (92); asthma also a risk factor (72)
Foods	Case reports, animal models, epidemiologic studies	Monkeys develop lupus-like illness after alfalfa sprouts or L-canavanine seeds with dechallenge and rechallenge in some animals; case reports of lupus after alfalfa and some dietary supplements (18;107); frequent meat intake a risk factor (73); alcohol consumption may be protective (108)
Heavy metals	Multiple lupus-like syndromes described in case reports and animal models	“Pink disease” (acrodynia), elevated autoantibodies and glomerulopathy in humans from mercury toxicity; related syndromes with elements of autoimmunity from cadmium and gold salt toxicity; granulomatous pneumonitis from beryllium exposure; strong support for glomerulopathy, multiple autoantibodies and genetic risk factors in mercury treated animal models (109,110,111)
Stress	Case reports and series	Case series suggest emotional stress or stressful life events were precipitating events prior to diagnosis and disease flares; biologic plausibility (112,113,114,115)
Ultraviolet B radiation	Case reports, case control study, animal models	A type I/II sun-reactive skin type was a risk factor for developing lupus (108)); UV up-regulates interferon inducible products in cutaneous lesions (116); decreased minimal erythemal dose in lupus (117); increased UV is associated with disease activity (118) and mortality (119)
Vaccines	Case reports and a case-control study for SLE; epidemiologic data for other syndromes	Case reports of SLE after multiple vaccines; increased vaccines in SLE vs. controls (OR 2.2); arthritis after rubella virus vaccines; thrombocytopenia after measles vaccines; Guillain-Barré syndrome after swine flu vaccine and tetanus, but controversy remains over others (93,120,121)
Infectious Agents		
Bacteria	Animal models	Bacterial DNA serves as an adjuvant in mouse models and can induce specific reactivities (122)
Endogenous and exogenous retroviruses	Case reports and series, animal and lab studies	Unconfirmed increased antibodies to retroviral transcripts and proteins in humans and animal models (reviewed in (123))
Parvovirus B19	Case reports and case-control study	Case reports described similarities between infection and lupus (124) but a case-control serology study did not find an association (125)

Identifying and Defining Environmentally Associated Lupus

Many of the exposures described in this chapter are common and the research pertaining to their role in lupus has been conducted primarily within the population of SLE patients as defined by current classification criteria. It is likely, however, that a number of cases of lupus developing as a result of environmental exposures do not meet these criteria. Drug associated lupus-like disorders are examples in which most cases in fact will not fulfill SLE criteria (95 ,96). Additionally, there are circumstances in which a novel "lupus-like" or related disease is seen, and a relevant question is whether specific environmental triggers may be associated with such atypical or new syndromes. Examples of this phenomenon include the epidemics of the toxic oil syndrome (97) and eosinophilia myalgia syndrome following L-tryptophan exposure (98). Members of the American College of Rheumatology Environmentally Associated Rheumatic Disorders Study Group have developed consensus on a general approach to the identification of such environmentally associated disorders (11). In this scheme, the overall process, from the identification of the first possible patient who developed a disease after an exposure, to the refinement of classification criteria for the disease, is divided into four stages (Table 3-6).

The first stage of this process is the identification and reporting of a case, or a series of cases, with a condition or syndrome that is suspected of resulting from a given exposure. The consensus proposal is that such cases that may have resulted from an environmental agent should meet certain criteria to assure that certain attribution elements are present (11). At least four of eight possible attribution elements should be present, including at least three of five primary or critical elements. These five primary attribution elements are temporal plausibility (after considering the pharmacokinetics/pharmacodynamics of the suspect agent, the minimum possible induction time and the maximum possible latency, if known); exclusion of other likely causes for the syndrome; dechallenge (if possible); rechallenge (if appropriate), and evidence for biologic plausibility. An additional three secondary elements are previous reports of cases that are similar in many respects (analogy); prior publication of essentially identical cases (specificity); and some evidence for a dose-response effect. In addition to meeting these criteria, however, demographic information, the medical history, laboratory testing, family history, prior infections or other immune-altering exposures, prior diagnoses and support for them, and the type/route/dose/duration of the suspect exposure should all be detailed in the report.

Table 3-6: Proposed Stages for Identifying and Defining Environmentally Associated Autoimmune Disorders*

Stage	Description	Nomenclature (Example)
Stage 1—Proposing the association	Case reports, defined by certain ascertainment criteria, propose the possible association of a specific clinical syndrome with a given exposure	Syndrome following exposure (Lupus following hepatitis B vaccination)
Stage 2—Testing the association	After a number of such cases are reported, surveillance criteria are proposed and epidemiologic and laboratory studies test that hypothesis	Cardinal signs, symptoms, and laboratory findings but without the putative exposure (Eosinophilia Myalgia syndrome)
Stage 3—Defining criteria for the condition	If studies above are positive, then specific preliminary classification and other criteria are defined for that specific environmental disease	Exposure associated disorder (L-tryptophan-associated Eosinophilia Myalgia syndrome)
Stage 4—Refining criteria for the condition	Criteria are reassessed and refined as additional data are obtained about the disease	Exposure induced disorder (Hydralazine-induced lupus-like disorder)

*Modified from Miller FW, Hess EV, Clauw DJ, et al. Approaches for identifying and defining environmentally associated rheumatic disorders. *Arthritis Rheum* 2000;43:243–49.

After a sufficient number of cases are reported in the first stage, the next step is to formally test the possible association of the suspect environmental exposure with the syndrome. This could include epidemiologic studies, using validated criteria or consensus surveillance criteria, if necessary, to assess the strength of the relationship between a given exposure and a given syndrome. The biologic effects of the agent, via in vitro, in vivo, and animal studies should also be determined to gauge the plausibility of, and assess possible modulators of, the development of the syndrome. Other approaches, such

as clinical, laboratory or genetic risk factor studies, could determine in case-control settings if individuals with a given phenotypic syndrome developing after a suspect environmental agent differ from those with similar diseases but without the exposure or differ from subjects similarly exposed who do not develop disease.

The third stage would be developing preliminary criteria for that specific environmentally associated syndrome or disease. This would be undertaken only if several lines of evidence from the second stage support a strong association between the exposure and the syndrome. Classification criteria would be developed to segregate, with reasonable sensitivity and specificity, groups of patients with this environmentally associated disorder from closely related diseases. Such criteria could be developed by expert committee consensus, using Delphi or nominal group techniques (99), mathematical algorithms, or other approaches. Symptom, sign, and laboratory criteria should be expressed in clinically sensible and practical formats with precise definitions of all constituent elements. If adequate data are available, diagnostic, prognostic, and outcome criteria, and disease activity and damage indexes should be considered. The fourth stage repeats the same processes used in the third stage if sufficient new information is collected to warrant a redefinition of the disease or the redefinition of diagnostic, prognostic, or outcome criteria.

This proposed staging approach clearly has limitations, in that the decisions as to when to progress from one stage to the next remain poorly defined and would be dependent on the number of cases reported, the resources available and the wider public health or medical-legal implications of the syndrome. Nevertheless, this approach does provide a practical framework to organize studies and it allows the classification of current environmental agents into groups with different levels of evidence for their association with specific syndromes. In this regard, nearly all environmental agents suspected of being associated with lupus and other autoimmune diseases today remain in stages 1 or 2 using this scheme.

Summary and Future Directions

The multifactorial nature of autoimmune diseases, as well as their rarity and heterogeneity, has inhibited understanding of the mechanisms that initiate, promote, and sustain them. Nonetheless, the information presented in this chapter, taken together with other findings, suggests that autoimmune syndromes arise from a complex and poorly understood interplay of predisposing, as well as possibly protective, genetic, and environmental risk factors. While some progress is being made in defining genetic risk and protective factors, we are in our infancy in the identification of the environmental risk and protective factors for SLE and other autoimmune illnesses because of the many difficulties in the field mentioned above. Although selected environmental agents can clearly be associated with the development of lupus in certain individuals, based on dechallenge and rechallenge methods, support for the role of the environment in most cases is often circumstantial and relies on imperfect clinical, epidemiologic, and laboratory studies. As a result, we usually cannot answer the question so often posed by patients: “Doctor, do you think my exposure to “X” before coming down with lupus had anything to do with the onset of this disease, and if so, should I or my family continue to be exposed?” Many patients are also convinced that they know the environmental trigger that caused their disease. However, because most of us in the industrialized world live in an increasingly complex sea of infectious and noninfectious agents, over which we have little control, none of us really knows the full range of environmental agents we are exposed to on a daily basis.

Beyond the need to identify additional risk factors, there is also a need to understand the mechanisms by which the few documented risk factors for the development of lupus induce their effects. The lack of common structures, shared metabolic pathways, or biochemical actions of suspect environmental agents suggests that mechanisms will vary from exposure to exposure and that the a priori prediction of future risk factors based on their characteristics alone will be difficult. Certainly, there is no lack of hypotheses for how agents may induce lupus. Current conjectures range from the alteration of target tissue autoantigen structure (with supporting data for bystander drugs and heavy metals), to upregulation or altered locations of normally sequestered autoantigens (as occurs with UV radiation), to cytotoxic, inhibitory, or stimulatory effects on components of the immune system (from therapeutic cytokines for example), to molecular mimicry (where antigenic structures are shared between infectious agents and self), and finally to other effects and combinations of the above (100).

Understanding the interactions of those elements that are necessary for disease development offers the promise of preventing or treating autoimmune diseases in novel ways. But before these approaches can be considered, important questions need to be answered. Which specific combinations of genetic and environment risk factors lead to which specific clinical syndromes? After such gene-environment interactions occur, which processes or mechanisms then lead to the observed pathology? Is lupus, as currently understood, actually composed of many subsets or elemental disorders, each of which may be defined by a unique pathogenesis resulting from interactions of the necessary and sufficient risk factors? Can lupus or other autoimmune diseases be better treated, cured, or even prevented through answers to some of the above questions?

Many difficulties have prevented a complete understanding of the environmental risk factors that might trigger lupus and other autoimmune diseases in genetically susceptible individuals. A number of coordinated initiatives may be useful in overcoming these obstacles and making more progress in the future (Table 3-7). Fundamental to all these efforts though are greater attention to and funding for understanding the necessary and sufficient environmental

exposures that initiate, promote, sustain, or even possibly prevent autoimmune disorders. Although such studies are time-consuming and expensive, they should be excellent investments, which will likely be very cost effective, in eventually minimizing new cases of lupus and ultimately improving the public health.

Table 3-7: Possible Approaches to Enhance Identification of Environmental Risk Factors for Lupus and Other Autoimmune Diseases*

Foster national and international collaborations and coordination to integrate existing and developing clinical databases, registries, specimen repositories and other resources
Develop and validate clinically useful standardized environmental exposure assessment tools
Develop and validate standardized biomarkers for environmental exposures
Increase support for well-designed, population-based, and case-control hypothesis-testing studies for suspected environmental agents
Increase support for hypothesis-generating studies to identify new agents and syndromes
Collect systematic descriptive epidemiologic data for autoimmune diseases as a baseline for future comparisons
Increase use of information technology and other novel approaches to enhance communications, coordinate efforts, and facilitate clinical studies
Improve coordination between animal model and epidemiologic studies
Develop novel mathematic, statistical and bio-informatic approaches to enhance epidemiology studies
Define gene-gene, gene-environment, and environment-environment interactions
Elucidate mechanisms by which risk factors result in lupus
Establish an international coordinating committee to oversee and facilitate the above, encourage multidisciplinary research, and prepare for and respond to new epidemics of environmentally induced immune-mediated diseases

Modified from Miller FW. Non-infectious Environmental Agents and Autoimmunity. In: Rose NR, Mackay IR, eds. *The Autoimmune Diseases.* 4th Ed. New York: Elsevier; 2005: in press.

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Chapter 4
The Epidemiology of Systemic Lupus Erythematosus

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Epidemiology can be defined as the study of the frequency and distribution of disease and the determinants (i.e., factors) associated with disease occurrence and outcome in populations. Epidemiologists design and conduct four major types of studies to evaluate chronic disease: (1) descriptive studies to estimate the incidence and prevalence of and the mortality from disease in relation to characteristics of person, place, and time; (2) observational studies, either retrospective or prospective in design, to derive inferences about etiologic factors associated with the occurrence of disease; (3) observational cohort studies to determine the course and prognosis of patients with a disease; and (4) experimental studies to evaluate preventive or therapeutic measures.

Epidemiologic studies of systemic lupus erythematosus (SLE) have focused on: (1) development and validation of criteria for disease classification; (2) estimation of morbidity and mortality rates in different populations at different times; (3) determination of etiologic factors, both host and environmental; (4) estimation of prognosis and survival of patients; and (5) evaluation of treatments in randomized controlled trials. This chapter reviews major findings in the epidemiology of SLE in the first two areas: disease classification and morbidity and mortality rates. The reader is referred to chapters 3 , 6 , and 69 for discussion of association of genetic and environmental etiologic factors with SLE and estimation of prognosis and survival.

Classification Criteria

In 1971, the American Rheumatism Association published preliminary criteria for the classification of SLE (1). Currently, the American College of Rheumatology (ACR) 1982 revised criteria for the classification of SLE (2), as modified in 1997 (3) are used for case definition (Table 4-1). The original 1982 data set was reanalyzed by Edworthy et al. using recursive partitioning to generate two classification trees in an attempt to identify simpler and more explicit rules to classify patients with SLE (4). The resulting simple classification tree requires knowledge of only two variables: immunologic disorder and malar rash. A more complex tree requires knowledge of six variables, including serum complement levels, which are not included within the 1982 revised criteria. The sensitivity, specificity, and accuracy were 96% for the 1982 revised criteria and 92% for the simple classification tree (Table 4-1). Perez-Gutthann et al. (5) compared the sensitivity of the 1982 revised criteria in the traditional format and both classification trees in 198 patients from the Johns Hopkins Lupus Cohort. The revised criteria were significantly more sensitive than the simple classification tree, correctly identifying 184 (93%) compared with 168 (85%) cases, respectively (p = 0.016); the full classification tree correctly identified 186 cases (94%). Thus, these data support the use of the 1982 revised criteria in the traditional format to classify patients with SLE.

Other groups have examined the relative value of individual criterion in selected patient populations using receiver operating characteristic curves (6) and Bayesian theory (7 ,8 ,9). Although weighted criteria have a higher sensitivity compared to the ACR criteria and allow the identification of a larger number of lupus patients (9 ,10), their specificity is lower and their use might result in inclusion in clinical trials of patients that do not have SLE according to experienced rheumatologists (10).

There has been little cross-cultural or ethnic validation of the 1982 ACR criteria. A validation study performed in Japan in 1985 demonstrated a sensitivity of 92% and a specificity of 89% (11). Individual criterion items were detected with different frequencies in the Japanese compared to U.S. SLE patients, demonstrating the need for generalizable, internationally valid criteria.

In 1997, the Diagnostic and Therapeutic Criteria Committee of ACR updated the 1982 criteria (3). Within criterion 10, item (a) the positive LE cell test was deleted and item (d) was changed to positive test for antiphospholipid antibodies including (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test for lupus anticoagulant using a standard method, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization (TPI) or fluorescent treponemal antibody adsorption (FTA) test. The updated version was more sensitive (78% vs. 72%), but slightly less specific (88% vs. 91%) than the 1982 criteria in a cohort of 346 patients with connective tissue disease (12).

As patients with primary antiphospholipid syndrome (APS), as defined by the Sapporo criteria can present three or more of the current SLE criteria, confusion between SLE and primary APS can occur in borderline cases.

Table 4-1: 1982 Revised Criteria for SLE

	Sensitivity	Specificity	Accuracy*
1. Malar rash	57	96	76
2. Discoid rash	18	99	57
3. Photosensitivity	43	96	68
4. Oral ulcers	27	96	60
5. Nonerosive arthritis	86	37	63
6. Pleuritis or pericarditis	56	86	70
7. Renal disorder	51	94	71
8. Seizures or psychosis	20	98	57
9. Hematologic disorder	59	89	73
10. Immunologic disorder	85	93	88
11. Positive antinuclear antibody	99	49	77
>4 criteria	96	96	96
Simple classification tree	92	92	92
Full classification tree	97	95	96

*Accuracy is defined as the average of sensitivity and specificity. For definitions of individual criterion items, see Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–1277.
Data from Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25:1271–1277 and Edworthy SM, Zatarain E, McShane DJ, et al. Analysis of the 1982 ARA lupus criteria data set by recursive partitioning methodology: new insights into the relative merit of individual criteria [see comments]. *J Rheumatol* 1988;15:1493–1498.

None of the methods for classifying patients with SLE was designed for diagnostic purposes. These criteria should be used mainly for the purpose of classifying patients in clinical, epidemiologic, and pathogenetic studies of SLE. Assuring homogeneity of the case population is ideal for epidemiologic studies that address etiology or risk factors. However, classification criteria lack sensitivity for recognizing milder cases of SLE, which becomes a limitation for descriptive studies of morbidity and observational studies of prognosis, because subjects with a multisystem disease that is consistent with SLE will not be included if they fail to fulfill the criteria. Data from the Rochester study suggest that the prevalence of suspected SLE is comparable to that of definite SLE: 64 versus 54 per 100,000 in white females and 33 versus 40 per 100,000 overall, respectively (13). For understanding the social burden of the disease and guiding health care and research policy, all patients should be included.

Criteria for classifying the subsets of neuropsychiatric (NP) syndrome of SLE were developed by an Ad Hoc Neuropsychiatric Workshop Group in 1990 (14). The ad hoc multidisciplinary committee developed standard nomenclature as well as detailed diagnostic and exclusion criteria for 19 NP syndromes in 1997 (Table 4-2). Chapter 37 includes a detailed discussion of these criteria.

The ACR nomenclature and case definitions for neuropsychiatric syndrome of SLE (NP-SLE) have been validated in a cross-sectional, population based study by Ainiala et al (15). The overall prevalence of NP-SLE in four different SLE cohorts varied between 37% to 95% (16 ,17 ,18 ,19). However, attribution of NP events to SLE is a challenge, with as much as 41% of all NP events in SLE patients being attributable to factors other than lupus (17).

Despite the fact that the revised ACR criteria have performed well, certain deficiencies have been recognized (20): (1) emphasis on skin involvement with the inclusion of four criterion items for mucocutaneous features but only one item for many of the other organ systems involved (21); (2) the omission of hypocomplementemia, which is one of the laboratory features characteristic of SLE (4); (3) lack of coverage of the entire spectrum of organ involvement (especially for renal and neurologic criteria); (4) lack of validation of the criterion for antiphospholipid antibodies and the potential confusion between SLE and primary APS; and (5) the criteria are not generalizable to multiple ethnic groups and need international validation. The Systemic Lupus International Collaborating Clinics (SLICC) have suggested that the above limitations justify an attempt to identify new classification criteria for SLE. Among proposed revisions, the SLICC group discussed the inclusion of abdominal serositis to the current serositis criterion, inclusion of additional NP manifestations such as mood disorder, cerebrovascular disease, neuropathy, mononeuritis multiplex, acute confusional state, and myelopathy and the introduction of a clinical APS criteria

(22 ,23 ,24). Nonetheless, the current ACR criteria perform well for their original stated purpose (2 ,3).

Table 4-2: Neuropsychiatric Syndromes in SLE as Defined by the ACR Research Committee

Central	Peripheral
Aseptic meningitis	Guillain-Barré syndrome
Cerebrovascular disease*	Autonomic neuropathy
Demyelinating syndrome	Mononeuropathy*
Headache	Myasthenia gravis
Movement disorder	Cranial neuropathy*
Myelopathy*	Plexopathy
Seizure disorders*	Polyneuropathy
Acute confusional state*	
Anxiety disorder	
Cognitive dysfunction	
Mood disorder*	
Psychosis*	

*Under consideration by SLICC for inclusion in potential revision of ACR classification criteria for SLE.
Adapted from Hanly JG. ACR classification criteria for systemic lupus erythematosus: limitations and revisions to neuropsychiatric variables. *Lupus* 2004;13(11):861–864.

Morbidity Data

Prevalence

The overall prevalence of SLE in the continental United States and Hawaii has been reported to range between 14.6 and 122 cases per 100,000 persons (Table 4-3). Studies have varied over time and place and used different methods of case ascertainment (13 ,25 ,26 ,27 ,28 ,29 ,30). The studies conducted in San Francisco, California, and Rochester, Minnesota, used both inpatient and outpatient records for case identification and published criteria for case validation; the major differences were the sampling frame (members of Kaiser Foundation Health Plan and residents of Olmsted County, respectively) and racial composition of the populations (81% and 99% white, respectively). The sex- and race-specific prevalence estimates for white males and females, however, are comparable, because the 95% confidence intervals for these ratios overlap. Estimates of the overall prevalence in whites were 44 and 40 per 100,000, respectively (Table 4-3). The most current estimate in blacks is that from the San Francisco study, but it is based on only 19 patients, 16 of whom were women. Thus, the confidence intervals are wide, limiting the reliability of this estimate. There is no recent study that has estimated the prevalence of SLE in African-Americans.

Applying the rates obtained from the San Francisco study to the 1990 U.S. population, the National Arthritis Data Workgroup estimated that 239,000 cases of suspected or definite SLE were present in the United States: 4000 white males, 31,000 black males, 163,000 black females, and 41,000 white females (31). Because of limited data on other racial and ethnic groups no estimates for Hispanics and Asians were attempted. Both of these groups have a higher reported prevalence of SLE than whites (*vide infra*). In a recent meta-analysis based on 16 population-based prevalence studies from Europe and North America, Jacobson et al. estimated the weighted mean prevalence rate of SLE at 23.8 per 100,000. By applying these rates to the 1996 U.S. census data the authors estimated that a total of 63,052 persons are afflicted with the disease (32). Most recently the National Arthritis Data Workgroup revised the previous estimates (R. Lawrence, personal communication, September 27, 2005). Applying prevalence rates to 2002 U.S. Census data, we estimate that at least 153,000 persons in the United States between the ages of 15 and 64 years have definite SLE: 11,000 white men, 78,000 white women, 7,000 African American men, 51,000 African American women, 1,000 men of other race, and 5,000 women of other race. Using a prevalence of 80 to 160 per 100,000 persons, the number of cases of suspected and definite SLE ranges between 153,000 and 306,000 cases.

Table 4-3: Prevalence of SLE by Sex/Race Group in the United States*

Authors	Location	Year	WM	WF	BM	BF	Overall
Siegel and Lee (28)	New York	1965	3	17	3	56	14.6
Fessel (26)	San Francisco	1973	7	71	53	283	50.8
Michet et al. (13)	Rochester, MN	1980	19	54	ND	ND	40.0
Maskarinec, et al. (70)	Hawaii	1989	ND	ND	ND	ND	41.8

*Rates per 100,000 persons.
Abbreviations: BF, Black females; BM, black males; ND, no data; WF, white females; WM, white males.

Three studies based on self-reported, physician-diagnosed SLE in the United States using data from telephone screening (25 ,30 ,33) suggest that the prevalence of SLE might be much higher. In one study, the prevalence of SLE by unsubstantiated claim was 1 in 177 (30). The prevalence reported by Hochberg et al. after validating the diagnosis of SLE by medical record review was 124 per 100,000 (25). This higher prevalence was confirmed in the study from Rochester, Minnesota, that reported age- and sex-adjusted prevalence rates of 1.22 in 1000 (29) and by the most recent study of Ward MM (33) who found a prevalence of treated SLE in adult women of 100 per 100,000.

International studies to estimate the prevalence of SLE have been conducted in Sweden (34 ,35 ,36), Finland (37), Iceland (38 ,39), the Arctic region of Norway (40) New Zealand (41 ,42), Malaysia (43), England and Wales (44 ,45 ,46 ,47), China (48 ,49), Japan (50 ,51), the Caribbean island of Curacao (52), Northern Ireland (53), Saudi Arabia (54), Greece (55), and Italy (56) (Table 4-4). Of the studies conducted in countries with predominantly white populations, prevalence estimates varied from a low of 12.5 per 100,000 females in England (47) to a high of 39 per 100,000 of both sexes combined in Sweden (35). This variability may result from differences in methodology of case ascertainment, including use of general practice diagnostic registries (47), hospital discharge records (37 ,42 ,50), outpatient clinic records, surveys of physicians, or combinations thereof (35 ,39 ,44 ,45 ,46) as well as from differences in host and environmental factors among different populations. In comparing studies using similar methodologies for case identification and validation, the prevalence of SLE

is almost identical. Studies from the United States and Sweden that used hospital records for case identification, reported similar prevalence rates of about 40 per 100,000 (13 ,35) while studies from the United Kingdom using multiple case-finding methods found consistently lower prevalence rates of about 25 per 100,000 (44 ,45 ,46). The lowest rate at 12 per 100,000 was determined through use of general practice diagnostic registries (47). True geographic differences in the prevalence of SLE among whites cannot however be excluded and may result from differences in genetic and other host or environmental factors (57).

Table 4-4: Prevalence of SLE: Selected International Studies*

Study	Country	Year	Cases (*n*)	Rate
Meddings and Grennan (42)	New Zealand	1980	16	15
Nived, et al. (34)	Sweden	1982	61	39
Helve (37)	Finland	1978	1323	28
Hochberg (47)	England	1982	20	12**
Nakae, et al. (50)	Japan	1984	NS	21
Gudmundsson and Steinsson (39)	Iceland	1990	86	36
Samanta, et al. (44)	England	1992	50	26
Nossent (52)	Curacao	1992	69	48
Hopkinson, et al. (45)	England	1993	137	25
Anstey, et al. (74)	Australia	1993	22	52
Johnson, et al. (46)	England	1995	242	28
Gourley, et al. (53)	N. Ireland	1993	467	254
Nossent HC et al. (40)	Norway	2001	83	44.9
Alamanos Y (55)	Greece	2003	178	39.5
Benucci M et al. (56)	Italy	2005	23	71

*Rate per 100,000; both sexes combined.
**Females only; as no cases identified among males.
NS, Not stated.

Incidence

The average annual incidence of SLE in the continental United States has been estimated in several studies; incidence rates vary from 1.8 to 7.6 cases per 100,000 persons per year (13 ,26 ,27 ,28 ,29 ,58 ,59) (Table 4-5). International studies from Iceland (39), Sweden (34 ,46 ,60), the United Kingdom (45 ,46), Japan (51), and the Caribbean island of Curacao (52) reported incidence rates for SLE of similar magnitude. A recent study from Brazil reported a higher overall incidence of 8.7 in the tropical region of Natal (61) while a study from northwest Greece reported a lower annual incidence rate of 1.9 (55) (Table 4-6).

Table 4-5: Incidence of SLE by Sex/Race Group in the United States*

Study	Location	Date	WM	WF	BM	BF	Overall
Siegel and Lee (28)	New York	1956–1965	0.3	2.5	1.1	8.1	2.0
Fessel (26)	San Francisco	1965–1973	ND	ND	ND	ND	7.6
Michet, et al. (13)	Rochester, MN	1950–1979	0.9	2.5	ND	ND	1.8
		1970–1979	0.8	3.4	ND	ND	2.2
Hochberg (58)	Baltimore, MD	1970–1977	0.4	3.9	2.5	11.4	4.6
McCarty, et al. (59)	Pittsburgh, PA	1985–1990	0.4	3.5	0.7	9.2	2.4
Uramoto, et al. (29)	Rochester, MN	1950–1979	ND	ND	ND	ND	1.51
		1980–1992	ND	ND	ND	ND	5.56

*Incidence rates per 100,000 persons per year.
BF, Black females; BM, black males; ND, no data; WF, white females; WM, white males.

Despite using different methods for case ascertainment, average annual incidence rates of SLE from 1985 to 1990 in Allegheny County, Pennsylvania (59), are quite similar to those reported in Baltimore during an earlier period (58). Of particular interest are the differences in incidence reported by Kurland et al. (27), Michet et al. (13) and Uramoto et al. (29) for the same population using or identical medical record retrieval system. Michet et al. attributed these differences to changes in diagnostic classification.

Nonetheless, a temporal trend in incidence among whites can be inferred from the Rochester data. Rates nearly tripled from 1.5 in the 1950 to 1979 cohort to 5.56 per 100,000 in the 1980 to 1992 cohort (29). Possible explanations for the increase in incidence is improved recognition of mild disease, increased exposure to hormones such as oral contraceptives and estrogen replacement therapy and greater exposure to ultraviolet light because of depletion in the ozone layer because of global warming. In contrast, in a recent study in Sweden the incidence of SLE over a period of 11 years (1981 to 1991) has been constant (36).

Table 4-6: Incidence of SLE: Selected International Studies

Study	Country	Date	Cases (*n*)	W	M	Overall*
Nived, et al. (34)	Sweden	1982	61	7.6	2.0	4.8
Jonnson, et al. (46)	Sweden	1986	39	—	—	4.0
Iseki, et al. (51)	Japan	1984	566	5.3	0.6	3.0
Gudmundsson and Steinsson (39)	Iceland	1990	76	5.8	0.8	3.3
Nossent (52)	Curacao	1992	94	7.9	1.1	4.6
Hopkinson, et al. (45)	England	1993	23	6.1	1.3	3.7
Johnson, et al. (46)	England	1995	33	6.8	0.5	3.8
Nossent, et al. (40)	Norway	2001	83	4.6	0.6	2.6
Vilar MJ and Sato (61)	Brazil	2002	43	14.1	2.2	8.7
Alamanos (55)	Greece	2003	178	—	—	1.9

*Rate per 100,000; both sexes combined.

Effects of Age and Sex on Morbidity Rates

The variability in prevalence and incidence rates in different published studies may be explained in part by the effect of age, gender, and race. Overall, prevalence and incidence rates are higher in females compared to males, and higher in African Americans, Afro-Caribbeans, and Asians than in Caucasian populations.

Among white females, age-specific incidence rates have varied among studies and peak incidence rates per 100,000 per year occurred in the 15- to 44-year age group (28), the 20- to 39-year age group (59), the 25- to 44-year age group (13), the 35- to 54-year age group (58), the 45- to 64-year age group (35), the 50- to 74-year age group (39), the 50- to 59-year age group (45), and among those aged 18 and 19 years (46). Median age at diagnosis for white females in these studies ranged from 37 to 50 years, emphasizing that SLE is not necessarily a disease of young women.

Age-specific incidence rates in white males are difficult to interpret because of the small numbers of cases. In those studies with adequate data, peak rates occurred in the 50- to 59-year age group (45) and in those aged 65 and older (13 ,28). The later onset of SLE in white males compared with white females was also noted in the Baltimore study (58) and the Leicester, United Kingdom study (44).

Age-specific incidence rates in black females were greatest in the 15- to 44-year age group in New York City (28), the 20- to 39-year age group in Pittsburgh (59), and the 25- to 34-year age group in Baltimore (58) and Birmingham, United Kingdom (46), exceeding 20 per 100,000 per year. Age-specific rates in black males can be reliably estimated only from the Baltimore study and reached a peak in the 45- to 64-year age group of 5 per 100,000 per year (58). Among Afro-Caribbeans on the island of Curacao, peak age-specific incidence rates occurred in the 45- to 64-year age group in women and among those aged 65 and older in men (52).

Age-specific prevalence rates for females in the United States are best estimated by the data of Fessel: approximately 1 and 4 per 1000 for white and black women aged 15 to 64 years, respectively (26). The prevalence of SLE among white women in southern Sweden, 99 per 100,000, is similar to that among white women in the United States (34). Age-standardized prevalence among white women in Iceland was slightly lower, at 62 per 100,000 (39), while that among white women in the United Kingdom was markedly less, at 32 per 100,000 (44) and 36 per 100,000 (46).

Clinical studies have consistently demonstrated a female predominance approaching 90% of SLE cases. This excess is especially noteworthy during the 15- to 64-year age group, wherein ratios of age- and sex-specific incidence rates show a six- to tenfold female excess in whites and blacks (Table 4-7). No such excess was noted in the 14 and younger or the 65 and older age groups in New York City (28), Rochester, Minnesota (13), Sweden (34), or Nottingham, UK (45). A fourfold greater incidence rate in females age 65 and older than in males was found among whites but not blacks in Baltimore (58). These age-related differences in the ratio of sex-specific incidence rates have been thought to relate to hormonal changes that occur during puberty and the childbearing years.

Table 4-7: Female-to-Male Sex Ratio at Age of Onset or Diagnosis in SLE

Age of Onset (*y*)	Female (*n*)	Male (*n*)	F:M Ratio
0–9	39	19	2.0
10–19	220	39	5.6
20–29	369	49	7.5
30–39	298	37	8.0
40–49	183	35	5.2
50–59	98	25	3.9
60+	58	25	2.3
TOTAL	1265	229	5.5

Adapted from Masi and Kaslow (80)

Effect of Race on Morbidity Rates

The relative increased frequency of SLE in people of African origin was examined by Symmons et al. (62) and more recently by Bae et al. (63). A greater incidence and prevalence of SLE has consistently been found in blacks than in U.S. whites (26 ,28 ,58 ,59). Studies in both New York City (28) and San Francisco (26) found three- to fourfold greater prevalence in black females aged 15 to 64 than in white females (Table 4-3) and studies in Baltimore and Pittsburgh found a threefold greater age-adjusted average annual incidence rate in black females compared with white females (58 ,59) (Table 4-5). A study in Birmingham, England, found higher age-adjusted incidence and prevalence rates in Afro-Caribbeans than in whites (46). Age-adjusted incidence rates were 25.8 and 4.3 per 100,000 for Afro-Caribbeans and whites, respectively, and age-adjusted prevalence rates were 112 and 21 per 100,000 for Afro-Caribbeans and whites, respectively. Despite the apparent predilection for women of African origin in Northern Europe, North America and the Caribbean, the frequency of SLE in West African countries where most of their ancestors originated, as estimated from case reports and small series is low (63). This “prevalence gradient” may be related to genetic and environmental factors as well as gene-environment interactions (63 ,64).

In a number of studies, the age distribution of incident cases differed significantly, with a younger median age and earlier peak incidence rates in women of African origin. Mean age in Afro-Caribbean females was of 34.5 years, compared with 41 years in white females, and, the peak incidence rate was in the 30- to 39-year age group compared with the 40- to 49-year age group, respectively (46). These results are almost identical to those from a study in Baltimore, wherein the mean age of black females with SLE was 35.5 years, compared with 41.7 years for white females (58), those from a study in Pittsburgh, wherein the mean age of black females was 35.2 years, compared with 39.8 years for white females (59) and those from another population-based study performed in North Carolina where mean age at diagnosis was 6 years younger among African-American compared to white patients (65). The mean age at diagnosis was 31 years, with a peak age at diagnosis in the 21- to 30-year age group, in 93 Afro-Caribbean patients from Jamaica; no comparison with white patients was available (66). On the island of Curacao, the mean age at diagnosis was 34 years, with a peak age at diagnosis in the 45- to 64-year age group, in 94 Afro-Caribbean patients; again, no comparison with whites was available (52).

Conflicting data exist regarding excess prevalence of SLE among Asians compared with whites (41 ,44 ,45 ,46 ,48 ,49 ,50 ,67 ,68 ,69). In Hawaii, compared to Caucasians, the prevalence odds ratio was 1.3 for Japanese, 1.5 for Filipinos, 2.4 for Chinese, and 0.8 in Hawaiians (70). An earlier study performed by Serdula and Rhoads (67) from 1970 through 1975 reports an estimated age-adjusted prevalence of 5.8 per 100,000 in whites, compared with 17.0 per 100,000 among Asians (Chinese, Filipino, and Japanese). Age-adjusted prevalence rates of SLE in Auckland, New Zealand were 14.6 and 50.6 per 100,000 in whites and Polynesians, respectively (41). Three studies from the United Kingdom (44 ,45 ,46), found higher age-adjusted incidence and prevalence rates of SLE in Asians (Indian, Pakistani, Bangladeshi) than in whites. In Leicester, England, the age-adjusted prevalence of SLE was 64.0 per 100,000 in Asians, compared with 26.1 per 100,000 in whites (44). In Birmingham, England, the age-adjusted incidence and prevalence rates of SLE in Asians were 20.7 and 46.7 per 100,000 compared with 4.3 and 20.7 per 100,000 in whites, respectively (46). Of interest, neither incidence nor prevalence rates differed by country of birth among Asians in this study.

Casting doubt on real differences between Asians, specifically Chinese, and whites are the findings of Fessel (26) and Nai-Zheng (48 ,49). In the San Francisco study, the prevalence of SLE was not increased among Chinese compared with whites (26). Data from China based on population surveys suggest a prevalence of SLE between 40 to 70 per 100,000 (49). Finally, a survey in Taiwan identified only one case of SLE among 1836 residents and no cases among 2000 female students (68). Thus, population-based data in three countries fail to support an excess prevalence of SLE among Chinese compared with Caucasians. Prevalence data from Japan also fail to support the observations in Hawaii of an excess prevalence in Japanese compared with whites (50).

No published data exist about incidence rates in Hispanic Americans, but the LUpus in MInority populations: NAture vs. nurture (LUMINA) study has compared genetic, clinical, and outcome features in Hispanics, African Americans and Caucasians (71 ,72 ,73) demonstrating significant ethnic disparities between these groups with respect to time to accrual of ACR criteria, time to initial damage accrual, and disease severity with African Americans and Hispanics from Texas having more severe disease than Caucasians and Hispanics from Puerto Rico.

The incidence and prevalence of SLE estimated in an Australian Aboriginal population in the Top End Northern

Territory (74) were 11 per 100,000 per year and 52 per 100,000, respectively. The authors suggested these rates were higher than those for Australian whites; however, estimates for the European population in Australia are not available.

An excess incidence and prevalence of SLE among Native American Indians compared with whites was suggested by three studies (75 ,76 ,77). This excess was isolated to only 3 of 75 American Indian tribes (75); a single Pacific Northwest Indian population, the Nootka (76); and three different Indian groups, the Tlingit, Haida, and Tsimshian, living in coastal southeast Alaska (77). Of interest, the prevalence of SLE among Alaskan Inuits is not increased over what would be expected (78 ,79). These isolated observations could represent chance findings; on the other hand, inbreeding and/or environmental factors may explain this clustering. Further studies of American Indian populations could identify additional clusters with excess morbidity from SLE in an effort to test hypotheses regarding risk factors.

Mortality Data

Mortality attributed to SLE in the continental United States has been estimated from community-based (28) as well as national data (80 ,81 ,82 ,83 ,84 ,85) (Table 4-8). These data need to be interpreted with caution as SLE was recorded as an immediate, underlying, or contributory cause of death in only 60 percent of known deaths of patients enrolled in the LUpus in MInority populations: NAture vs. nurture (LUMINA) and Carolina Lupus Study cohorts (86).

Lopez-Acuna et al. (82) identified all deaths attributed to both discoid and systemic lupus erythematosus from National Center for Health Statistics (NCHS) data tapes for the period 1968 to 1978. A total of 11,156 deaths were identified, 2568 (23.0%) of which were attributed to discoid lupus and 8588 (77.0%) to SLE. There were no differences in the distribution of deaths from discoid lupus and SLE by sex/race, region, or year; therefore, the authors combined results for their analysis. There were a total of 6452 deaths in white females, 2573 in black females, 1760 in white males, and 371 in black males, with average age-adjusted mortality rates of 6.0, 17.6, 1.8, and 3.0 per 1 million persons per year, respectively (Table 4-8). Age-specific average annual mortality rates showed a unimodal distribution for all sex/race groups, with maximum rates occurring in the 45- to 54-year age group for blacks and the 65- to 74-year age group for whites (Fig. 4-1).

Table 4-8: Cause-Specific Mortality from SLE by Sex/Race Group in the United States*

Study	Years	WM	WF	BM	BF
Cobb (92)	1959–1961	1.1	4.0	1.8	10.6
Siegel and Lee (28)	1956–1965	1.6	6.6	4.4	20.0
Kaslow and Masi (82)	1972–1976	1.5	5.2	2.2	14.8
Gordon, et al. (81)	1972–1976	1.2	4.5	1.9	13.1
Lopez-Acuna, et al. (84)**	1968–1978	1.8	6.0	3.0	17.6

*Rates per 1 million persons per year.
**Includes deaths attributed to both discoid lupus erythematosus and SLE.
BF, Black females; BM, black males; WF, white females; WM, white males.

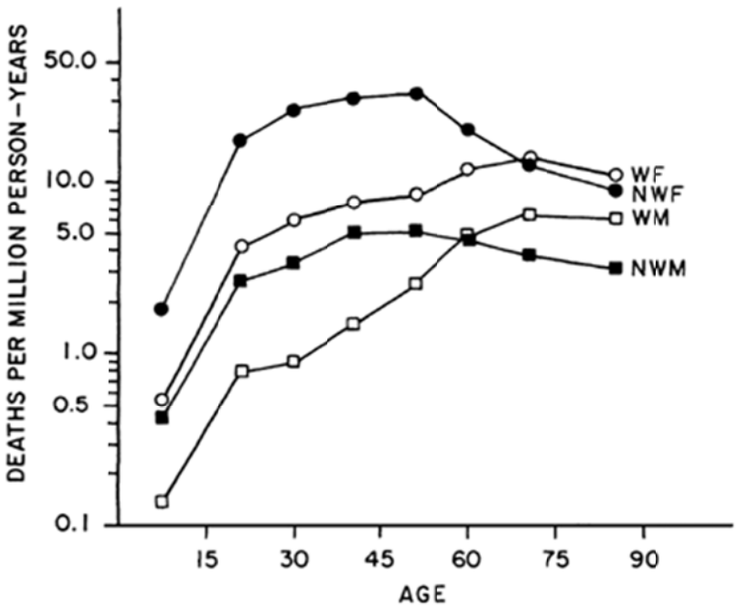

Figure 4-1. Average annual age-specific mortality rates attributed to lupus erythematosus by sex/race group in the United States, 1968 to 1978. (White males □, white females ○, black males ▪ black females •).

Kaslow (83) analyzed a subset of these mortality records and examined deaths attributed to SLE alone from 1968 through 1976 in 12 states with 88% of U.S. residents of Asian descent. Mortality rates were threefold greater among blacks and twofold greater among Asians compared with whites: 8.4, 6.8, and 2.8 per 1 million person-years, respectively. Age- and sex-adjusted mortality rates for

Chinese, Japanese, and Filipinos were 7.5, 6.8, and 5.1 per 1 million persons-years, respectively. Age- and sex-adjusted race-specific mortality rates in Hawaii were greatest among Filipinos and higher in the combined Asian group than for the U.S. mainland population, confirming previous observations (67). A study from Hawaii reported that mortality rates were three times higher in non-Caucasian than Caucasians in 1985 to 1989 (70). It is unclear whether the differences in mortality rates between Asians and whites mirror true differences in incidence rates as seen with blacks (*vide supra*).

Siegel and Lee (28) noted greater mortality and morbidity from SLE among Puerto Ricans compared with whites in New York City. Lopez-Acuna et al. (87) analyzed mortality from SLE in Puerto Rico from 1970 through 1977 as well as a subset of the NCHS data set for five southwestern states with the highest proportion of Hispanics: Arizona, California, Colorado, New Mexico, and Texas. A total of 92 deaths from SLE occurred in Puerto Rico; the average age-adjusted mortality rates of 7.5 and 2.0 deaths per 1 million person-years in females and males, respectively, were not significantly different from those noted among U.S. whites over this period. A correlation between the proportion of Spanish-heritage population and county-specific mortality rates from SLE was noted for females but not males in these five states; the implications of this finding may reflect both ethnic/racial and socioeconomic factors. Indeed in a recent study, Ward demonstrated that socioeconomic status, as measured by education level is associated with mortality due to SLE in whites (84). Relative odds of mortality due to SLE were almost double in white women and white men with <8 years of education compared to those with >16 years of education. In contrast to the findings in whites, and in contrast to many prior studies, higher education levels were not associated with lower mortality due to SLE in African-Americans or Asian/Pacific Islander women most likely as a result of underdiagnosis of SLE in minorities with low education levels. Cause-specific mortality data from Latin American countries have not been reported.

Data on nationwide mortality from SLE have been reported from Finland (37), Iceland (39), England and Wales (88), and the island of Curacao (52). Average mortality rates were 4.7, 2.5, and 17.0 per 1 million person-years in Finland, England and Wales, and Curacao, respectively. Patterns of age-specific mortality rates in Finland as well as England and Wales were similar to those in U.S. whites. The fourfold greater age-adjusted mortality among English females compared with males is similar to that seen in the United States as well. Patterns of age-specific mortality rates in Curacao were similar to those in blacks, with a peak mortality rate in the 45- to 64-year age group and a fourfold greater mortality in women than in men (52).

Temporal trends in mortality rates have been examined in the United States (81 ,82 ,85) and in England and Wales (88). In the United States, there was a significant decline in age-adjusted annual mortality rates between 1968 and 1978 for all sex/race groups (82) (Fig. 4-2). More recently, the Center for Disease Control and Prevention (CDC) reviewed SLE deaths during 1979 to 1998 and reported an overall increase in the annual number of deaths from 879 in 1979 to 1,406 in 1998 (Table 4-9) (85). The crude death rate increased from 3.9 to 5.2 per 1 million population. Of all SLE deaths, 36.4% occurred among persons aged 15 to 44. For each year, crude death rates increased with age, were >5 times higher in women than men, and were >3 times higher among blacks than whites. Among black women, death rates were highest and increased most (69.7%) among those aged 45 to 64 (Fig. 4-3). Of all death from arthritis, SLE accounts for 44% of deaths among persons aged <45 years (85). This report indicates marked age, sex, and race-specific disparities that exist in SLE death rates. Differential ascertainment and reporting of SLE death by race is possible but probably does not account for the magnitude of observed differences. A higher incidence of SLE among black women along with later diagnosis, problems in access to care and less effective and poorer adherence with treatment might account for the racial differences in death rates. Rates for racial/ethnic populations other than white or black were not calculated because numbers were too small for meaningful analysis. A large population-based registry of SLE will be developed by CDC to examine the causes of these marked disparities and the temporal changes in death rates.

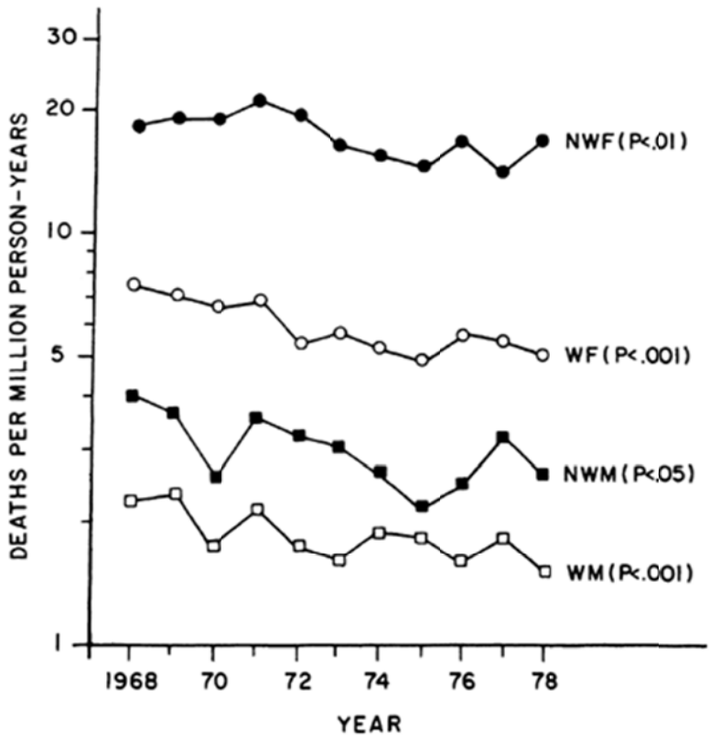

Figure 4-2. Trends in age-adjusted annual mortality rates from lupus erythematosus by sex/race group in the United States, 1968 to 1978. (White males □, white females ○, black males ▪, black females •).

A significant temporal decline in age-adjusted annual mortality rates from SLE also was observed among females in England and Wales from 1974 to 1983 but not among males, probably because of the small numbers of deaths

from SLE among males (88). A similar decline in mortality was observed in the Toronto Cohort, where estimated risk for death was compared for patients entered in the cohort between 1970 to 1977, 1978 to 1986, and 1987 to 1994 (89). The standardized mortality ratios declined from 10.1 in the first group to 3.3 in the last group. The same decline was observed for the first two groups followed over the next time period. The decline in mortality rates observed in these developed countries is probably a result of improved survival in patients with SLE, as reflected by improving 10-year cumulative survival rates approaching or exceeding 90% in some studies (90 ,91) (see Chapter 69).

Table 4-9: Numbers of Deaths from SLE by Age Group, Sex, and Race in the United States, 1979-1998

	Age Group (yrs)				Sex		Race*			
Years	<15	15-44	45-64	≥65	Female	Male	White	Black	Other	Total
1979	15	369	253	242	725	154	610	249	16	879
1980	11	383	313	298	848	157	700	276	23	1,005
1981	15	390	302	339	863	183	747	270	21	1,046
1982	19	407	283	304	840	173	706	276	27	1,013
1983	12	375	333	339	855	204	695	329	20	1,059
1984	13	402	302	362	910	169	743	307	24	1,079
1985	8	383	310	373	889	185	723	313	30	1,074
1986	16	412	289	352	886	183	700	336	24	1,069
1987	5	364	303	374	886	160	718	299	21	1,046
1988	11	399	317	386	933	180	717	359	26	1,113
1989	10	439	317	429	979	216	778	379	38	1,195
1990	11	402	349	418	998	182	801	338	41	1,180
1991	6	406	299	405	942	174	703	376	37	1,116
1992	17	443	308	382	968	182	749	352	49	1,150
1993	11	388	368	415	981	201	779	354	49	1,182
1994	10	440	370	416	1036	200	799	388	49	1,236
1995	11	474	405	434	1119	205	837	437	50	1,324
1996	8	464	404	456	1127	205	857	417	58	1,332
1997	9	501	414	433	1160	197	868	427	62	1,357
1998	8	471	485	442	1214	192	887	469	50	1,406
Total	**226**	**8,312**	**6,724**	**7,599**	**19,159**	**3,702**	**15,117**	**6,951**	**715**	**22,861**

*Totals do not add to 22,861 because of missing data.
From Centers for Disease Control and Prevention. Trends in deaths from systemic lupus erythematosus—United States, 1979–1998. *MMWR Morbid Mortal Wkly Rep* 2002;51(17):371–374.

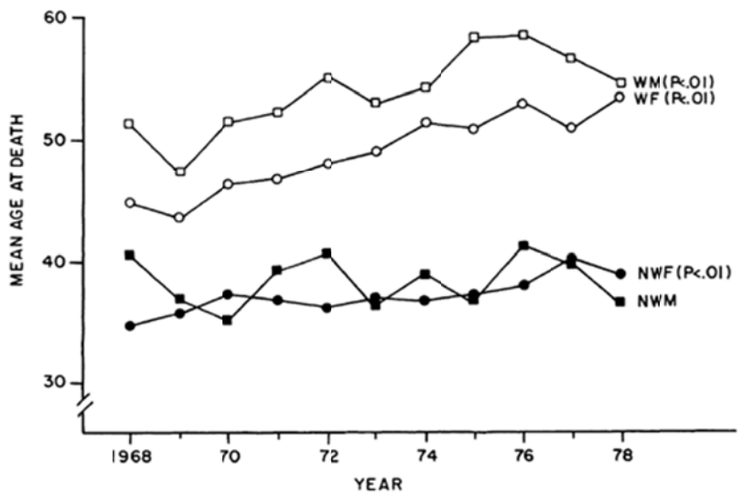

Figure 4-3. Trends in mean age at death from lupus erythematosus by sex/race group in the United States, 1968 to 1978. (White males □, white females ○, black males ▪, black females •).

Summary

Descriptive epidemiologic studies of SLE have been conducted worldwide; the most extensive data are available for the United Kingdom, Scandinavia (especially Sweden), and the United States. In the United States, blacks have a threefold higher incidence and prevalence of SLE, as well as cause-specific mortality rates, compared with whites, whereas in England, Asian Indians and Afro-Caribbeans have higher incidence and prevalence rates than whites. The reasons for this excess, however, remain unknown. Finally, observational epidemiologic studies have demonstrated an increasingly favorable prognosis for patients with SLE, allowing the identification of potentially preventable causes of death and a better understanding of long-term morbidity and impact on overall health status.

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Section II

Pathogenesis of Lupus

Chapter 5

Overview of Pathogenesis of Systemic Lupus Erythematosus

Bevra Hannahs Hahn

The purpose of this brief chapter is to review how systemic lupus erythematosus (SLE) evolves and is sustained. Ideas reflect the author's opinions, which are based largely on the information provided throughout this book. References are not extensive because each topic is addressed in detail in other chapters.

The Phases of SLE: Evolution of Disease in Susceptible Persons

As shown in Fig. 5-1 , individuals who ultimately develop SLE do so in a series of steps. There is a long period of predisposition to autoimmunity, then (in a small proportion of those predisposed) development of autoantibodies, which usually precede clinical symptoms by months to years (1). A proportion of individuals with autoantibodies develop a clinical prodrome with nonspecific symptoms that do not meet criteria for classification as SLE, and then among those a proportion develop full-blown SLE with various symptoms, autoantibodies, and laboratory abnormalities that make the diagnosis clear. Finally, individuals with clinical SLE usually experience over a period of many years intermittent disease flares and improvements (usually not complete remission), and compile organ damage and comorbidities related to chronic inflammation, to therapies, and to aging.

Genetic Predisposition

Genetic predisposition is probably the single most important factor in phase 1 (2 ,3). The risk for SLE is increased approximately 10-20-fold in monozygotic compared to dizygotic twins, and in siblings of patients with SLE compared to the healthy population. However, concordance for SLE in monozygotic twins is considerably less than 100%, suggesting that nongenetic factors play a major role in disease susceptibility. With the possible exception of the very rare homozygous deficiency of C1q (>90% of those individuals develop SLE) (4), there is no single gene polymorphism or abnormality that accounts for SLE in all affected individuals in one family, or in the various populations that develop the disease. Approximately 75% of SLE patients in all ethnic groups have at least one human leukocyte antibody (HLA) gene that increases risk (subsets of DR2, DR3, DR4, or DR8, particularly in Caucasians). However, each of these alleles increases risk a relatively small amount (1.7-fold to 2.5-fold), indicating that multiple susceptibility genes, and/or other factors such as environment are required for disease development. At the time of this writing (June 2006), genome scan studies of different populations from several laboratories have identified at least eight chromosomal regions that contain susceptibility genes; the regions are not the same in all racial/ethnic populations. In addition, individual genes have been associated with or linked to risk for SLE in more than one cohort (but not all) including the Fcγ receptors IIA, IIIA, and RIIB, which in some groups (especially African Americans) predispose more to lupus nephritis than to SLE itself. Other predisposing genes identified by at least two groups include alleles of interleukin 10 (IL-10), monocyte chemotactic protein-1 (MCP-1), mannose binding ligand (MBL), protein tyrosine phosphatase non-receptor type 22 (PTPN22) and interferon-response-factor-5 (IRF5). Finally, some genes are associated with high risk for damage, such as end stage renal disease (FcRIIIA). One gene region (16q12) is linked to several different autoimmune diseases, including SLE. Thus, it is possible that some individuals are predisposed genetically to autoimmunity, and other genes determine whether the disease that develops will be SLE, type 1 diabetes mellitus, Crohn's disease, and so on. There is one report of a gene that confers protection from SLE (5)—a polymorphism for the Toll-like receptor (TLR)5 that reduces the levels of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1-beta (IL-1β), and IL-6 released from cells stimulated by bacterial flagellin. One can speculate that 1. HLA-DR genes predispose to autoimmunity because they determine what peptides can be presented to T lymphocytes to activate help for autoantibody production and T-mediated immune responses. 2. FcγR genes predispose because those that increase risk for SLE bind the Ig in immune complexes and antibodies less well than the other alleles (probably impairing clearance). 3. Deficiency of C1q or predisposing alleles of MBL predispose by impairing clearance of apoptotic

bodies (which bind C1q) that contain self-antigens recognized by individuals with SLE. 4. Alleles of IL-10 that increase B cell maturation predispose by increasing autoantibody production. 5. Alleles of PTPN22 predispose if their product has less negative regulation of T cell function than do other alleles. However, these ideas have not been proven. In addition to identification of genes and gene regions that increase susceptibility to SLE, genes and/or regions have been identified that predispose to certain phenotypes of disease, such as nephritis, vasculitis, arthritis and hemolytic anemia. In addition, genes have been identified that increase the risk for end stage renal disease in people with lupus nephritis. Now that single nucleotide polymorphisms (SNPs) identifying the entire human genome are available (the haplotype map-HapMap- project), we can expect more precise analysis of small chromosomal DNA regions containing suspect genes. For example, a major experiment is in progress in which deoxyribonucleic acid (DNA) from multiple cohorts with large numbers of SLE patients is being analyzed for genomic content using 500,000 single nucleotide polymorphisms (SNPs), which promises to identify more genes that influence disease susceptibility, phenotype and outcome. Hopefully this work will also result in better understanding of the mechanisms of disease. For now, it is safe to say that almost all patients have inherited multiple susceptibility genes, that each gene has a relatively small impact on immune or inflammatory response, and that the sum of these many small differences results in autoimmunity with characteristics of SLE.

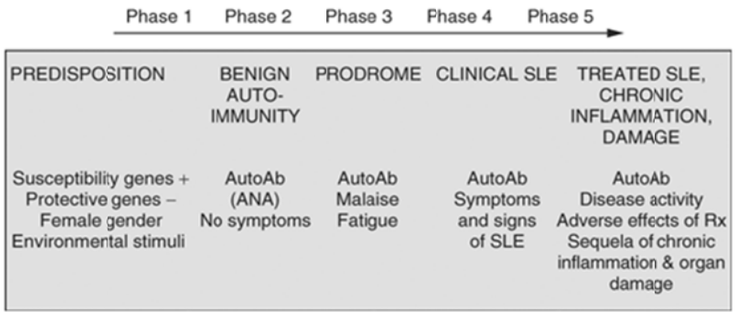

Figure 5-1. The five phases of SLE. Time is shown from left to right. Many individuals inherit susceptibility genes increasing risk for SLE. The progression from health to benign autoimmunity characterized by autoantibodies with no symptoms, to disease and to chronic damage is shown. See discussion in text. AutoAb, autoantibodies, Rx, treatment.

The Influence of Gender

Gender influences on disease susceptibility must be of major importance, since there are nine females for every male with SLE. The most important impact may be hormonal, since sex differences in susceptibility are largest during reproductive years. Estradiol probably prolongs the life of autoreactive B and T lymphocytes (6). Women exposed to oral contraceptives, or to hormone replacement therapy regimens containing estrogenic compounds, have a small but statistically significant increased risk for SLE. Prospective, randomized, blinded, controlled trials showed that administration of one hormone replacement therapy containing conjugated estrogens and a progesterone significantly increased the risk of mild/moderate disease flare in women with established SLE (in contrast, one oral contraceptive tested did not) (7 ,8). There are many experiments in murine SLE showing that alteration of sex hormones that increase estrogen or progesterone-like effects worsen disease. However, other features of femaleness may also be important in predisposing to SLE. For example, most women after pregnancy have circulating stem cells from their fetuses (microchimerism), which might set up a graft-versus-host type immune reaction (9). Women may be predisposed to SLE because their inactive X chromosome is enriched in hypomethylated regions. The CpG in these regions can be bound by TLR9 receptors thus activating innate immune responses and increasing risk for autoimmunity (10 ,11).

The Influence of Environment

Environmental factors that predispose to SLE are undoubtedly important, although many are unknown at this time. Ultraviolet light clearly exacerbates disease in a majority of people with SLE, and in some the clinical onset of disease is preceded by unusually large exposure. Mechanisms might include alteration of the structure of DNA in the dermis to render it more immunogenic, and induction of apoptosis in keratinocytes and other dermal cells, presenting self antigens to the immune system via apoptotic bodies. Infections have long been suspect as inducers and enhancers of SLE. Recent work from several laboratories has linked infection with Ebstein Barr virus (EBV) to SLE (12 ,13 ,14). EBV infection activates B lymphocytes, which might cause a genetically predisposed person to make large quantities of autoantibodies, overwhelming regulatory mechanisms. The Epstein-Barr (virus) nuclear antigen (EBNA) 1 molecule of EBV has molecular mimicry with a sequence in the Ro particle; immunization with the sequence can induce multiple autoantibodies and lupus-like disease in animals (14). Of course, there are many potential toxins in the environment that may initiate and influence immune and inflammatory responses.

Autoantibodies as Disease Effectors

Autoantibodies are the main effectors of disease in SLE. In humans, they are probably necessary for disease, but not sufficient. That is, their deposition must be followed by activation of complement and/or other mediators of inflammation, and a series of events that include chemotaxis for lymphocytes and phagocytic cells, and release of cytokines, chemokines, and proteolytic enzymes, as well as oxidative

damage, must occur for organ inflammation and damage to be severe. There are several experiments in mice showing that autoantibodies and/or immune complexes can deposit in glomeruli without inducing significant renal damage, if the processes listed above do not occur (15 ,16). Recent data (1) suggest that in nearly 85% of SLE patients, autoantibodies precede the first symptom of disease by an average of 2 to 3 years—sometimes as long as 9 years. The autoantibodies appear in a temporal hierarchy, with antinuclear antibody (ANA) first, then anti-DNA and antiphospholipid, and finally anti-Sm and anti-RNP. These observations imply that immunoregulation of potentially pathogenic autoantibodies can occur for a sustained period of time, and only in individuals whose regulation becomes “exhausted” does disease appear (17). Among autoantibodies, some are clearly pathogenic, such as certain subsets of anti-DNA that cause nephritis upon transfer to healthy animals, antibodies to neurons that cause neuronal death, antibodies to Ro which cause damage to the fetal heart conduction system (proven in mice), antibodies to phospholipid that cause fetal loss (proven in mice), and antibodies to platelets and erythrocytes that cause the cells to be phagocytized and destroyed. Additionally, antibodies to DNA/nucleosomes contain amino acid sequences that are T cell determinants (18); these peptides activate helper T cells to further expand autoantibody production. Mechanisms of pathogenicity are discussed in detail in other chapters, and for many autoantibodies are not entirely known.

Development of Clinical Disease

The “later” phases of SLE include the prodrome of fatigue and malaise that many patients describe, which may last months prior to development of classical SLE. That prodrome may resolve, or may evolve into recognizable disease that fits criteria for classification as SLE. At this point, treatment designed to suppress SLE is introduced. Most patients improve and reach a point where they endure mildly active disease in the setting of tolerable side effects of treatments. As relatively low levels of chronic inflammation and oxidative damage continue in tissues, patients age, and adverse effects of treatment accumulate, damage accrues. For example, clinically important atherosclerosis, including myocardial infarcts and strokes, become increasingly common in SLE patients after the first decade of disease. Risk for atherosclerotic disease is five- to 10-fold increased in SLE patients compared to age-matched non-SLE populations. Renal failure, hypertension, obesity, and reduced physical activity combine to add to the morbidity and mortality of the disease.

The Immune Abnormalities of SLE

Two major immune networks contribute to generation of autoreactive T cells and autoantibodies in individuals with SLE—the innate immune network, and the adaptive immune network.

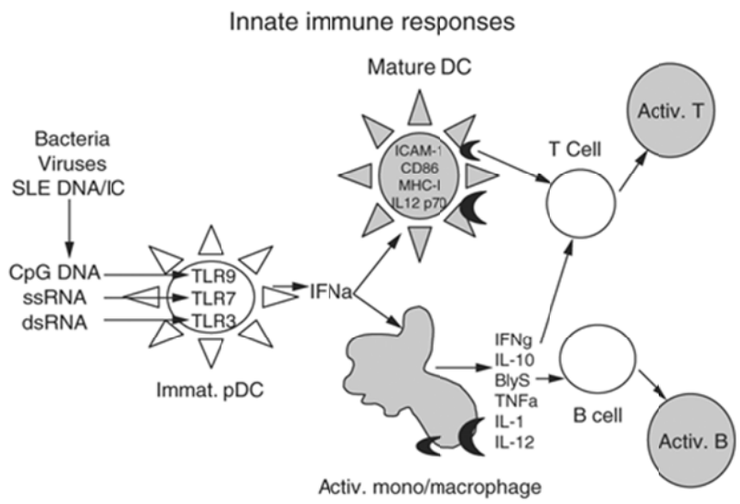

Figure 5-2. The role of innate immunity in promoting development and continued activity of SLE. Danger provided by bacterial, viruses, and some self antigens are recognized primarily by the plasmacytoid dendritic cells (pDC) deposited in tissues as a first defense. Immature, inactive cells are shown as white throughout this figure. Cytosine/guanosine nucleotides in DNA, (characteristic of bacterial DNA but also found in mammalian DNA, enriched in SLE patients), and DNA in immune complexes bind TLR9 in pDC; dsRNA from viruses binds TLR7 and 8; single-stranded RNA from viruses binds TLR3. Each of these bindings can trigger release of IFNα from the pDC, which then acts on monocytes/macrophages and DC to promote expression of the molecules indicated, as well as increases in MHC I and II expression on cell surfaces with conversion to potent antigen-presenting cells. MHC/peptide complexes are shown as crescent-shaped black-filled surface structures. These APC provide first activating signals to T cells. Second signals are provided by CD86 on activated macrophages and DC. Some cytokines released by the monocytes (IL-10, BLyS) promote maturation of B cells and Ig secretion; others promote maturation/activation of T cells (IFN-γ). Thus, in response to environmental dangers and some self molecules (cytosine/guanosine nucleotides in DNA), DC, monocytes/macrophages, T cells and B cells become mature and activated (depicted in light gray). This sets the stage for production of autoreactive T cells and pathogenic autoantibodies that cause and perpetuate SLE. IC, immune complexes, TLR, Toll-like receptor, Immat, immature, pDC, plasmacytoid dendritic cell, IFN, interferon.

Figure 5-2 shows generation of T and B cell activation via innate immunity. External and internal danger signals, including infections and self antigens, activate innate immunity primarily via dendritic cells (DC) located in tissues that sample the environment (lungs, intestines, skin, peripheral lymphoid tissues). Pathogen-associated molecular patterns (PAMPs) shared by many bacteria and viruses are recognized by Toll-like receptors in DC. Quite important to SLE is the fact that TLR9 in DC and B lymphocytes can bind CpG DNA sequences (11 ,19 ,20). Such sequences are fairly common in bacterial DNA, are uncommon in mammalian DNA, but are increased in patients with SLE. Therefore, lupus DC can be activated by CpG nucleotides in tissue or circulation. Furthermore, DNA in DNA/anti-DNA immune complexes binds TLR9, and the anti-DNA binds FcRγRIIA receptors on

DC, thus further activating innate immunity. Other TLR (TLR 3,7,8) recognize viral single-stranded DNA (ssRNA) or double-stranded (dsRNA); RNA/protein complexes characteristic of SLE can also bind TLR7 (21). Binding of any of these TLR, particularly in plasmacytoid DC (pDC), results in release of IFNα and other cytokines from the immature pDC; interferon-α (IFN-α) promotes maturation of DC and of monocytes/macrophages. Both cell types then express peptides in their major histocompatibility complex (MHC) surface molecules, becoming potent antigen-presenting cells (APCs) for activation of T lymphocytes. Additionally, the activated macrophages release multiple cytokines that promote maturation and activation of T lymphocytes (particularly toward the Th1 phenotype) and of B cells, with increase in immunoglobulin production and in switching to pathogenic IgG subclasses. Thus, the stage is set with activated T and B cells (some of which are autoreactive) ready to participate in adaptive immune responses. All of these processes occur in healthy individuals. What is probably different about persons with SLE compared to healthy people is that they have greater quantities of hypomethylated DNA with CpG sequences (22), circulating DNA/anti-DNA complexes, and possibly recent or reactivated infection with EBV (12 ,13 ,14). Additionally, certain polymorphisms in various TLR and in FcγR may predispose these individuals to abnormal innate responses. Thus, it is likely that innate immune responses are upregulated in patients with SLE, promoting antigen processing/presentation and the activated state of T and B lymphocytes participating in and driving adaptive immunity against self.

Figure 5-3 illustrates generation of activated, autoreactive T and B cells by adaptive immunity in SLE. Antigens, both external (foreign) and internal (auto), encounter activated APCs, which internalize them and process them into peptides, subsequently presenting the peptides in surface MHC molecules. B lymphocytes and monocyte/macrophages are the main APC in adaptive immunity, whereas DC are prominent APC in innate immunity. Some non-immune cells, when activated, such as mesangial cells, also express MHC class II on their surfaces and serve as APC for local T cell activation. T lymphocytes with antigen-specific TCR receive first activating signals from MHC/peptide complexes, and then second signals from molecules such as CD86, which are also expressed on activated APC. In the case of helper T cells (mostly $CD4^+$, can include $CD8^+$ in people with SLE), cells are activated to secrete cytokines (IFN-γ, IL-6, IL-10), that help B lymphocytes produce autoantibodies. Some antibodies fix directly to target organs, such as platelet surface molecules or α-actinin in glomeruli (23). Other autoantibodies form pathogenic immune complexes of correct size, charge, conformation, and antigen-reactivity to bind to tissue (platelets, glomeruli, skin, blood vessels are frequent targets). Antibody-dependent cell cytotoxicity, or complement activation with subsequent inflammation can result, leading to clinical disease and to tissue damage. While this process of autoantibody production is quite active in SLE patients, many of the regulatory networks designed to shut off antibody production are defective. This includes decreased phagocytosis of apoptotic cells, and of immune complexes (24). Additionally, generation of regulatory $CD4^+CD25^+$ T cells and suppressive $CD8^+$ T cells is probably defective (17). Defects in function of these cells have been well documented in several models of murine lupus; evidence that they occur in human disease is accumulating. For example, patients with SLE compared to normals produce abnormally small quantities of transforming growth factor beta (TGF-ß) from peripheral blood cells (25). TGF-ß, along with IL-2 (production of which is also reduced in T cells of many SLE patients), is required for generation of $CD4^+CD25^+$ Treg, and some $CD8^+$ inhibitory cells in humans. With regard to $CD8^+$ T cells, one group has shown that such cells from SLE patients cannot suppress proliferation of $CD4^+$ T cells and autoantibody production in the normal manner, particularly when disease is clinically active (26).

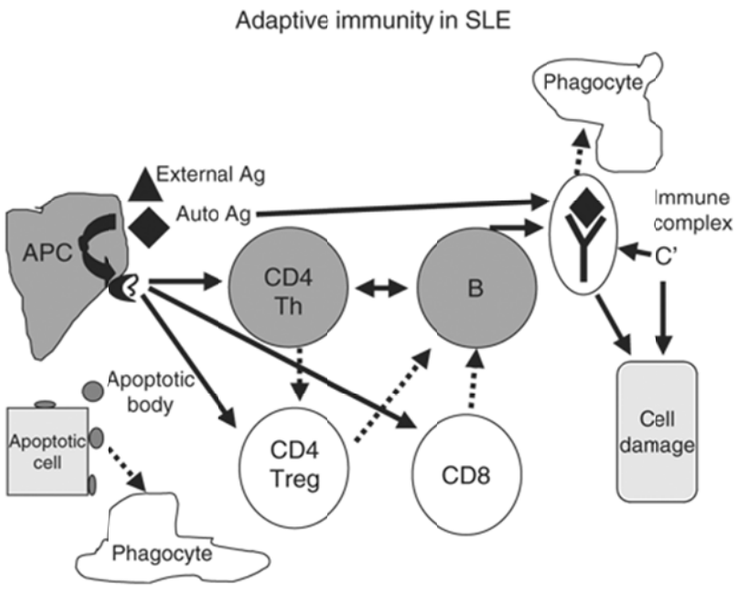

Figure 5-3. Adaptive immunity in SLE. Activated hyperfunctioning cells are filled with gray; underfunctioning cells are filled with white. Solid arrows indicate active, ongoing processes that result in production of autoantibodies and immune complexes. Dashed arrows indicate hypofunctioning processes that contribute to failed regulation of autoantibody/immune complex production. External and autoantigens are taken up by APC and processed into peptides that are presented in MHC molecules on the surface of the APC. These Ag are present in the environment; many self Ag are exposed to the immune system in apoptotic cells and bodies, which are not disposed of by phagocytes in the normal fashion. T cells receive first activating signals from the MHC/peptide complexes; helper type T cells receive second signals from activated APC and are activated to drive B cell production of autoantibodies (circled in bold oval). In contrast, regulatory $CD4^+$ and $CD8^+$ T cells after receiving first signals are either anergic or are driven to apoptosis rather than to activation. Therefore, autoantibody and immune complex production are favored. Regulatory networks, including phagocytosis of the complexes, are impaired so that autoantibody/immune complex formation continues to occur until quantities and properties reach levels that can fix to target cells and activate complement to cause cell damage and clinical disease. APC, antigen-presenting cell, Ag, antigen, MHC, major histocompatibility complex, C', complement, Th, helper T cells, usually $CD4^+$; Treg, regulatory T cell, usually $CD4^+$.

In summary, the immune abnormalities characteristic of SLE include 1. brisk production of autoantibodies and immune complexes that contain pathogenic subsets, 2. enhanced upregulating mechanisms, and 3. defective downregulating mechanisms.

Why Do Some Persons with Autoantibodies Develop SLE While Others Do Not?

If we assume that anyone with sustained autoantibodies has at least a few susceptibility genes predisposing to SLE, we must still address the reasons that SLE develops in only some. Why do some people progress from phase 2 to phases 3 through 5? It is well known that in normal individuals autoreactive T and B lymphocytes are detectable in lymphoid tissues, as well as in circulating peripheral blood cells. Thus, it is not unusual to detect autoantibodies (especially rheumatoid factor and ANA) transiently during infections, or even sustained for long periods of time, sometimes associated with aging. Table 5-1 , lists some ideas about what might distinguish a person destined to develop SLE from a person who" tolerates" autoantibodies and never develops clinical disease.

With regard to genetic predisposition, it seems likely that some individuals develop clinical SLE because they have a "critical mass" of susceptibility genes. Each gene contributes an abnormality that permits enhanced autoreactivity, or impaired immune regulation, or both. After repeated stimulation of immune responses by the usual external stimuli, such as infections, SLE develops. Although no single environmental stimulus has been identified as sufficient and necessary for triggering clinical SLE, several are known to participate (EBV infection, ultraviolet light). An antibody to pneumococcal polysaccharide can become an antibody to DNA after a single mutation (27); it is likely that this process can occur in many antibodies to infectious agents. Furthermore, toxins released by infectious agents (e.g., lipopolysaccharide) can disrupt the blood-brain barrier and allow access of antibodies that react with central nervous system (CNS) antigens, such as glutamate receptors on neurons (27 ,28). Thus, a previously healthy person with this autoantibody develops CNS lupus. Stress, caused by infections or injury, may also contribute to going from healthy but predisposed to full clinical SLE. Proteins released from cells during stress, including many heat shock proteins, can induce autoantibody responses and can activate T cells (29). People who develop SLE may be exposed by predisposition or environmental stressor to higher quantities of autoantigens or to altered forms that are more antigenic. When cells of the lymphoid system undergo apoptosis or necrosis, enzymes are activated that alter composition of some intracellular antigens to more immunogenic forms (30). Apoptotic blebs on cells contain many of the self antigens recognized in SLE—nucleosomes (DNA/histone), Ro, La, Sm (RNA/protein), and phospholipids. Individuals with increased numbers of apoptotic cells, or who cannot clear those cells properly by the usual phagocytic mechanism, might develop SLE (4 ,24). In a person genetically programmed to "hyperrespond" to antigenic stimuli, the development of autoantibody with one specificity may induce reactivity to others—a phenomenon known as "antigen spreading." As immune responses to one antigen mature, the expanding T and B cells develop cross-reactivity or even new reactivity to other autoantigens. The data showing addition of different autoantibodies over time following appearance of ANA in people who later develop SLE supports this idea (1), and immunization of animals with an autoantigen such as Ro or the SmD1 peptide can induce ANA and other autoantibodies over time (14 ,27 ,31).

Intrinsic abnormalities of cells of the immune system contribute to development of SLE. These abnormalities are likely to be genetic in origin, although only a few have been identified. For example, some SLE patients have a mutation in an RI α a gene that results in deficient functions of protein kinase (PKA)-I and PKA-II enzymes important in transmitting intracellular signaling after TCR stimulation: the end result is increased calcium signaling and probably hyperactive T cell responses (32). Several additional abnormalities in responses to TCR-signaling characterize SLE T cells and result in abnormal cell function, including underproduction of IL-2 (33). IL-2 is required to generate regulatory and cytotoxic T cells that dampen autoimmunity. People with SLE are more likely than healthy people to have circulating T cells that recognize self antigens (18), and smaller quantities of antigen are required to trigger T- and B cells activation (34). Higher proportions of B and T cells from SLE patients compared to normals are activated, with expression of surface molecules induced by activation, such as Fas, CD86, CD40L, and ICAM (35 ,36). Such cells are resistant to deletion and anergy (33 ,37)—mechanisms of tolerance that prevent T or B cells from going to full activation after first signals are delivered. These combined abnormalities in T and B lymphocytes could account for a predisposed person developing full-blown SLE.

Although we know many of the characteristics that contribute to an autoantibody being pathogenic—the antigens it binds, its ability to activate complement, its charge, its idiotypes, its contents of T cell determinants, etc., we do not know whether the important differences between inducing disease or not are primarily quantitative, or depend on these qualitative differences, or both. We also do not know whether the qualitative characteristics of pathogenic autoantibodies are shaped primarily by genetics or by the environment in which the B cell produces the autoantibodies. For example, can the quantities of IFN-γ or IL-10 or BLyS in the immediate environment of B and T cells influence mutations in the variable region of the Ig molecule that introduce amino acids (e.g., arginine) that increase binding of the molecule to DNA? If so, does the release of the influential molecule depend on the genetics of the individual, or is it a normal response to a particular stimulus?

Table 5-1: Possible Explanations for Development of SLE in Genetically Predisposed Individuals with Autoantibodies

1. Genes: Critical “dose” of susceptibility genes causing enough immune response abnormalities to permit sustained production of pathogenic autoantibodies and immune complexes, once that response has begun.
2. Environment:
a. An infection that increases autoantibody/immune complex production and/or impairs downregulatory mechanisms (EBV, pneumococcus, etc). Such an infection might also disrupt the blood-brain barrier and permit access of antibodies that cross-react with central nervous system antigens.
b. Increased exposure to ultraviolet light or other environmental toxins
c. Exposure to a drug or biologic that tilts the immune response to autoimmunity (interferons, TNFα inhibitor isoniazid, some anti-hypertensives, etc.)
d. Exposure to increased estrogen/progesterone/prolactin sex hormones
e. Severe stress
3. Antigens:
a. Alteration to more immunogenic forms during cell activation, apoptosis, or necrosis than in normal circumstances
b. Antigens find their way to normal “protected” environments such as the brain.
c. High quantities of apoptotic cells are present and/or the presence of apoptotic bodies/cells is sustained longer than normal. This would allow presentation of nucleosome, Ro, La, phospholipids, and other autoantigens to the immune system.
d. An infection, bacterial or viral, provides foreign antigens that mimic self, and induce autoreactive T and B cells (molecular mimicry)
e. One autoantigen present in high quantities, or in an immunogenic form, triggers antigenic “spreading” within the immune system, so that T and B responses are educated to recognize additional self antigens.
4. Intrinsic abnormalities of cells of the immune system (B and T lymphocytes):
a. Cells are activated by lower concentrations of antigen than normal
b. Cells are more easily activated by normal stimuli than in healthy individuals. They have sustained surface expression of activation markers some of which provide second signals. This may relate to mutations or abnormal functions of receptors and kinases involved in signaling that occurs after T cell antigen receptor (TCR) or B cell antigen receptor (BCR) engagement (first signals), including generation of certain cyclooxygenase-2 (COX-2) molecules.
c. Cells that are activated are relatively resistant to apoptosis and anergy: they persist too long, escaping regulation.
d. Activation of cells in a person with SLE, compared to normals, results in increased numbers of activated cells, as well as increased quantities or qualities of the cytokines and chemokines released (for example, the typical signature of IFN type 1-related gene increase in peripheral blood cells of patients with SLE).
5. Differences in the characteristics of autoantibodies made by someone with SLE compared to someone without:
a. More autoantibody subsets are pathogenic in SLE than in healthy individuals. For example, more autoAb with cationic charge, ability to bind with high avidity to self antigens in tissues—such as α-actinin or laminin in glomeruli or glutamate receptors on neurons in the brain.
b. Idiotypes within the antibodies identify them as pathogenic or activate idiotype/anti-idiotype networks that promote more autoantibody production. For example, IdGN2 is present in glomeruli of patients with proliferative, but not membranous, forms of lupus glomerulonephritis.
c. Autoantibodies in SLE patients are enriched in T cell determinants that activate help for more autoantibody production.
d. Characteristics of immune complexes favor tissue capture rather than elimination.
6. Differences in the composition of target tissue in SLE permit the tissue to become a target of antibodies, which are usually benign.
a. In rats, the structure of myosin in the heart determines whether autoantibodies and autoreactive T cells can induce myocarditis.
b. In people, tissue responses to injury differ in terms of susceptibility to damage. For example, African Americans with any type of glomerular disease are more likely than Caucasians to develop end stage renal disease. This may be because of differences in enzymes (ACE for example), chemokines, cytokines, growth factors released upon initial injury, or to differences in tissue structure.
7. Responses to therapy: There is evidence for some immunosuppressive drugs that persons with certain genetic polymorphisms that influence metabolism of or effects of the drugs respond differently. Similar information should be discovered for most of the medications/biologics used in SLE over the next few years.
a. Azathioprine
b. Cyclophosphamide

It is also possible that the nature of the response of activated immune cells is at least as important in determining disease as the antigens that stimulate the response, and the antibodies that result. What is the importance of the recent observations that peripheral blood cells of SLE patients are enriched in upregulated genes induced by interferons (probably more specifically by type 1 than type 2 IFN)? It seems unlikely that this response merely reflects activation of cells in a person with ongoing inflammation, since individuals with other inflammatory diseases, just as juvenile arthritis, do not show this gene “signature” in their cells (38). There is substantial evidence that in some individuals increased cell and/or serum and/or urine levels of certain cytokines, chemokines, products of activated complement and soluble receptors such as IFN-α, IFN-γ, TNF-α, IL-6, IL-2R, IL-6R, IL-10, intracellular adhesion molecule (ICAM), C4d, and monocyte chemoattractant protein-1 (MCP-1) are associated with active disease or predisposition to SLE (33 ,39). The specificity of these changes for SLE and their utility as biomarkers of disease activity are under intense study. Again, we do not know if these “abnormalities” are controlled genetically or are products of the particular response initiated by disease triggers, or both.

We should also consider the possibility that difference in composition of tissue targeted by autoantibodies play a role in why some persons develop SLE while others do not. There is an example in two mouse strains—one resistant and one susceptible—of differences in two amino acid residues in the α heavy chain of cardiac myosin that influence susceptibility to autoimmune carditis (40). In another example, one strain of mice developed sclerosing glomerular disease after deposition of autoantibody, whereas another strain did not (16). This might relate to different abilities to secrete cytokines that promote scarring, such as TGFß, rather than to differences in composition of glomerular antigens. To date, it is not possible to corroborate the data in human SLE as to whether sequence or other structural differences in tissue target molecules determine susceptibility to disease, but it is likely that differences in the individual's response to autoantibody/immune complex deposition are critical in whether or not tissue injury occurs.

Finally, as there are more patients with SLE surviving to enter phase 5—chronic disease with periods of flare and improvement—the characteristics of each patient in terms of response to therapies will have to be dealt with, both the probability of clinical response and of adverse effects. It is customary to think of patients who are “resistant” to therapy as having particularly severe disease, but it may be that in some differences are not in disease but in genetically determined responses to treatments. For example, cyclophosphamide is metabolized via cytochrome P450 (CYP) enzymes. A recent study (41) suggests homozygosity for certain polymorphisms of CYP enzymes strongly influence both ovarian toxicity and clinical responses of patients with lupus nephritis treated with cyclophosphamide. Azathioprine is metabolized in part by thiopurine methyltransferase (TPMT), which influences tissue and blood concentration of the active thioguanine metabolite. Homozygous deficiency of the enzyme is highly correlated with bone marrow suppression. Some experts have suggested that testing for the presence of TPMT, and for polymorphisms in both coding and promoter regions of its genes, should be used to determine dosing. In some series (42), this approach has prevented toxicities; in others (43), it has not been effective, probably because there is not a clear correlation between the multiple polymorphisms and drug levels, with the exception of homozygous deficiency. Clinical experience tells us that some patients are good responders to azathioprine or to cyclophosphamide or to mycophenolate; others are not. Some patients respond well to only one of these agents; others respond to several. The same is likely to be true of the interventions coming into clinical trials; antibodies to CD20, CTLA4Ig, Edratide, and so on. It will be a great advance in management of SLE when patients can be screened for drug response and risk for adverse effects before exposing them to these potentially toxic agents. In the same regard, it will be a great advance to learn more about preventing the comorbid conditions that result from chronic inflammation and treatment toxicities, such as atherosclerosis, osteoporosis, and infections. Although there are effective preventive strategies for some of these problems, there is much room for improvement.

When there is a more complete understanding of the pathogenesis of SLE, preventive strategies in order to keep individuals “locked” in phase 2, never progressing to clinical disease, can be developed. This is the most exciting aspect of the future with regard to this disease.

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Chapter 6
The Genetics of Human Lupus

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Cumulative genetic epidemiology studies have clearly demonstrated the importance of genetic influences on systemic lupus erythematosus (SLE). Early studies have focused on genes located within the major histocompatibility complex (MHC). Subsequently, both MHC genes and non-MHC genes are shown to participate in the pathogenesis of SLE. Using a candidate gene approach, many immunologically relevant genes, including certain MHC loci, deficiencies of complement components, Fcγ receptors, and cytokines, have been associated with SLE using the case-control, population-based studies. Complementary to association studies of candidate genes, linkage analysis is another tool that tests whether a particular chromosomal region is likely to harbor a disease susceptibility gene by testing whether genetic markers and a disease phenotype show correlated transmission within pedigrees. Eleven whole-genome scans have been performed using family collections containing multiple members affected with SLE, resulting in at least eight susceptibility loci (1q23, 1q31, 1q41–42, 2q37, 4p16, 6p21, 12q24, and 16q12–13) reaching the threshold for significant linkage and confirmation in independent cohorts. Within the linked intervals, fine mapping experiments (6p21 and 16q12) and positional candidate gene studies (1q23, 1q41–42, and 2q37) have further supported the presence of lupus susceptibility gene(s). These linkage results have shown that (1) no single locus is shared in all collections of SLE-multicase families, and (2) susceptibility genes contributing to the development of SLE may vary depending on ethnic and genetic heterogeneity. Recently, candidate genes encoding immunoregulatory molecules have been implicated in susceptibility to SLE including programmed cell death 1 (PDCD-1) and protein tyrosine phosphatase (PTPN22). These gene variants have also been associated with multiple autoimmune diseases, which may help to explain the observed familial clustering of multiple autoimmune diseases. Increased levels of interferon (IFN)-inducible gene expression has been consistently found in peripheral blood cells of many SLE patients. In addition, a few gene variants encoding proteins participating in the type 1 IFN pathway have been associated with SLE, suggesting the important role of type I IFN-inducible genes and pathway in the pathogenesis of SLE. The identification of susceptibility genes and the delineation of genetic mechanisms mediating the disease pathogenesis should provide new targets for more focused therapy in the near future. All these studies will be reviewed in this chapter, with an emphasis on the more recent publications since the last edition.

Genetic Epidemiology Studies

Familial Aggregation of SLE and Other Autoimmune Diseases

The prevalence of SLE in the U.S. population is 15 to 122 cases per 100,000 persons reported among studies varying over time, place, and methods of case identification (1). This variation may be partially attributed to ethnic diversity that the disease is more prevalent in Hispanics and African-Americans. A recent survey of 20,050 adult Americans yields an estimate of 241 self-reported, physician-diagnosed SLE cases per 100,000 persons, projecting 108,300 adult women afflicted with SLE (2). Compared to the population prevalence of SLE, the disease is much more prevalent among family members of SLE patients. A familial prevalence of 10% to 12% has been documented using surveys of several hundreds of SLE patients who reported having at least one first-degree relative with the disease (3 ,4). Using a case-control design of 77 SLE patients and 77 controls (matched for age, gender, and race), Hochberg also reported significant familial aggregation of SLE that 10% of the SLE probands have at least one first-degree relatives affected with disease compared to only 1% in the controls (5). The prevalence of SLE in female first-degree relatives was estimated to be 2.64 per 100 SLE patients and 0.4 per 100 normal controls. A study examining 1,177 Latin American patients affected with SLE has reported familial aggregation of SLE in 9.9% of relatives of the SLE probands (6% first-degree relatives, 2.6% second-degree relatives, and 1.3% third-degree relatives), as well as familial aggregation of rheumatoid arthritis (6.7% of the relatives) and other autoimmune diseases (2.0%) (6). Comparing risk for SLE between relatives of various degrees, their data support a polygenic additive model rather than multiplicative model for SLE inheritance. Familial clustering of autoimmune diseases may be explained by a common set of susceptibility genes shared among in family members controlling clinically distinct autoimmune diseases (7). Examples of a single susceptibility gene shared in more than one autoimmune diseases include cytotoxic T lymphocyte antigen-4 (CTLA-4) gene in both type 1 diabetes and Graves disease (8), NOD2/CARD15 in Crohn

disease, psoriatic arthritis, and Blau syndrome (9 ,10 ,11), PDCD1 in SLE, type 1 diabetes, and rheumatoid arthritis (12 ,13 ,14), and PTPN22 in type 1 diabetes, rheumatoid arthritis, SLE, and/or autoimmune thyroid disease (15).

Twin Studies

Twin studies can offer clues to distinguish between genetic and environmental influences in the pathogenesis of a disease. Because twins usually share the same environment, concordance of disease in monozygotic twins and discordance in dizygotic twins imply genetic contribution to disease susceptibility. The concordance rate in monozygotic twins (24% to 58%) is approximately ten times the rate in dizygotic twins or in siblings (2% to 5%) (16 ,17). The deviation from 100% concordance in monozygotic twins indicates nongermline factors also are required for the development of disease, which may include environmental triggers, the diverse repertoire of immunoglobulin genes and T cell receptor genes, the inactivation of X chromosome, or genetic imprinting (18). In the largest twin study in SLE thus far, the 24% concordance rate in 45 monozygotic versus 2% in 62 dizygotic twins (17), is similar to those found in type 1 diabetes, multiple sclerosis, and rheumatoid arthritis (18 ,19).

Sibling Recurrence Risk

The risk for siblings of SLE patients to develop disease (λs, sibling recurrence risk (20)) has been estimated to be as high as 29 times than that of the general population (6). The value of λs has been used to assess evidence for sibling familial clustering of a disease. The λs of 29 in SLE is in the range of that observed in other autoimmune diseases, for example, 8 in rheumatoid arthritis, 15 in type 1 diabetes, 20 in multiple sclerosis, and 54 in ankylosing spondilitis (19). Comparing risk for SLE between relatives of various degrees, their data support a polygenic additive model rather than multiplicative model for SLE inheritance.

In summary, the importance of genetic influences on SLE has been consistently supported by studies of populations, twin concordance rates, and aggregation of disease in families. In addition, the existence of multiple inbred strains of mice that spontaneously develop lupus-like disease further lends support for a genetic basis for SLE (reviewed in Chapter 7). What were these genes that increase risk for SLE? Many investigators have addressed this question. These studies are reviewed below.

Genetic Studies in SLE

Genetic Terms and Methods for Genetic Studies

The term “complex trait” refers to the lack of direct correlation between a phenotype and a genotype (21). For example, SLE is a complex trait that does not exhibit classic Mendelian recessive or dominant inheritance attributable to a single gene locus. The complexity can arise either because the same genotype can result in different phenotypes (modified by effects of environment, stochastic events, or interactions with other genes) or different genotypes can result in the same phenotype. Since the disease definition is dependent upon fulfillment of 4 of the 11 ACR criteria (22 ,23), SLE represents an array of heterogeneous phenotypes and thus further increases its complexity.

Genetic dissection of complex traits has been approached by two main methods: association and linkage (21). Historically, association studies that use the case-control design have been commonly used to compare unrelated affected and unaffected individuals from a given population. An allele A of a candidate gene is associated with the disease of interest if it is present at a significantly higher frequency in affected patients than unaffected controls. This method is suitable to detect genes with small effect because it is relatively easier to recruit large numbers of unrelated subjects than families with particular family structures. This study design needs to carefully match the subjects and the controls with respect to the age, gender, genetic background, and environmental exposure to avoid false-positive assertions caused by unanticipated differences between the two groups.

The availability of large numbers of mapped DNA markers throughout the whole genome has facilitated linkage analysis. Linkage analysis use families with multiple affected members to assess cosegregation of the test maker allele with a phenotype of interest. A model can be proposed to explain the inheritance pattern of phenotypes and genotypes observed in pedigrees (21). Alternatively, in the absence of a known mode of inheritance of the trait, an allele-sharing method can be used because affected relatives should show excess allele sharing of the test marker if it is near the gene conferring the phenotype. The commonly used markers include microsatellites (di-, tri-, or tetranucleotide repeats varying greatly among individuals in size) and single nucleotide polymorphisms (SNPs). Traditionally, the application of genome-wide scans using approximately 400 microsatellites in complex diseases have led to the identification of many large linked genomic intervals of 20 to 30 cM (1cM is about 1 million base pairs of DNA) that are likely to contain disease susceptibility genes. In the past decade, dramatic advances in high-throughput technology and bio-informatics have led to the determination of the complete human genome as well as the chromosomal location of almost all human genes (24 ,25). The most common DNA variations in the human genome are SNPs, occurring on average once every 200 base pairs. Although biallelic SNPs are not as polymorphic as individual microsatellite, the abundance of SNPs in the human genome makes them well suited to generate high-resolution genetic maps. Recently, whole genome scans using >10,000 SNPs have dramatically decreased the intermarker spacing, improved the information content, and vastly narrowed the linked intervals in comparison to traditional microsatellite based scans (26 ,27).

Once an approximate estimate of the linked interval has been s identified, one can make use of linkage disequilibrium for a more accurate location. Linkage disequilibrium occurs when certain alleles of two physically closely located genes (or DNA markers) appear to be jointly transmitted more frequently than expected from their respective frequency. As the result of linkage disequilibrium, a particular combination of alleles at a set of closely linked loci, termed a haplotype, tends to be transmitted as a block from generation to generation. Thus, marker loci near a susceptibility gene are often observed to be in linkage disequilibrium with the disease, resulting in significant differences in the relative frequencies of marker alleles among affected individuals from those in the general population. Although population-based association studies are powerful, the family-based association design, for example, the transmission disequilibrium test (TDT), has the advantage of avoiding population stratification that may cause spurious associations (21). The TDT method on families uses the preferential transmission of the test allele(s) from heterozygous parents to affected children as evidence for association between the test allele and the disease (28). Currently, a large international consortium, the HapMap Project, is working to identify a large portion of SNPs in different human populations, haplotype blocks derived from these SNPs, and tagSNPs that depict a particular haplotype structure by eliminating redundant SNPs in strong linkage disequilibrium (29). Common SNPs (a minor allele frequency >10%) are of interest because of the common disease/common variant hypothesis (CDCV)(30). Because association analysis is expected to be more powerful for the detection of common disease alleles that confer modest disease risks (31), mapping complex disease loci using whole genome association studies has been gaining popularity (32). This promising approach has been supported by the recent success in the identification of a major gene variant, the Tyr402His of the complement factor H, that accounts for 20% to 50% of the overall risk in developing age-related macular degeneration (33 ,34). The international consortium for SLE genetic study has begun a whole-genome association study using 500,000 SNPs. In the coming years, we anticipate many SLE susceptibility genes will be identified.

Genetic Linkage of Genomic Loci to Human SLE

Linkage analysis studies families containing multiple relatives affected with the same disease. The rationale is that DNA markers located near a susceptibility gene will be transmitted together with the disease gene in affected members of each family because they are likely to share the same susceptibility gene allele at a particular disease locus (21). The identified murine lupus susceptibility loci (the overlapping Sle1/Nba-2/Lbw-7 loci mapped to the distal end of mouse chromosome 1, reviewed in (35)) provided a guide to explore the syntenic human chromosomal region, which led to the initial identification of linkage to human SLE of the chromosome 1q41-q42 region (36). Subsequently, 11 genome-wide scans (37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46) and 8 fine mapping linkage analyses in various cohorts (47 ,48 ,49 ,50 ,51 ,52 ,53 ,54) have shown many genomic locations that may harbor SLE susceptibility genes (Table 6-1). Despite tremendous variations in their ethnic compositions, sample sizes, family structures (affected sibpairs containing nuclear families and pedigrees containing multiple generations), and geographic locations (Table 6-1), eight loci (1q23, 1q31, 1q41–42, 2q37, 4p16–15.2, 6p21–11, 12q24, and 16q12–13) have reached the threshold for significant linkage to SLE (38 ,39 ,40 ,43 ,45 ,46 ,47 ,51) (Table 6-2), and all eight have been confirmed in at least one independent cohort for linkage to SLE (37 ,39 ,40 ,44 ,45 ,46 ,48) (Table 6-2) using Lander and Kruglyak's criteria for interpretation of linkage statistics (55). Of interest, not a single, common locus was identified in all these scans, and the MHC containing region (6p21) is not the strongest locus detected in these scans. Replication of significant linkage of a locus provides the best evidence for the presence of putative susceptibility gene(s), which warrants further fine mapping studies to localize the disease predisposing gene(s).

Four recent genome scans for lupus susceptibility genes have been reported since the last edition, and their major findings include (1) 12q24 is a newly established and confirmed locus that is mainly observed in Hispanic and European American families (46); (2) a nationwide study in Finland (a geographically isolated population) including 35 multiplex SLE families (containing 73 patients and 96 healthy relatives) has provided support for linkage at 14q21–23, the HLA region, 5p13, and 6q25–27 (42); (3) a genome scan of 62 multiplex SLE families (containing 88 affected and 456 total sibling pairs) has provided evidence for linkage to four previously reported (1q23, 2q33, 16q12, and 17q21–23) and four novel (3p24, 10q23–24, 13q32, and 18q23) genomic intervals (37); (4) evidence for linkage of 17p12-q11 to SLE has been reported using 20 Argentine SLE multiplex families (41). A meta-analysis of 9 individual genome scans containing 605 families with 1,355 SLE patients has identified two loci, 6p22.3–6p21.1 and 16p12.3–16q12.2, reaching genome-wide significance (56), which further confirms chromosomes 6 and 16 harboring lupus susceptibility genes.

The extensive heterogeneity of clinical and laboratory manifestations among SLE patients has prompted various approaches to reduce disease heterogeneity, and presumably genetic heterogeneity. One methodology adopted by Dr. Harley and his colleagues, stratifying linkage analysis on a subset of pedigrees in which one SLE patient has a particular clinical manifestation, has yielded more than 10 publications reporting strong evidence for linkage (57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67) (Table 6-3). Because many parallel studies have been conducted, SLE patients who exhibit multiple manifestations used in pedigree stratifications. Stratification on a disease subset can result in a reduction in sample size, which makes the result more vulnerable to random fluctuations, thus highlights the importance of independent confirmation of the findings. Furthermore, may skew linkage

results by being overrepresented in many parallel studies. To this end, linkage at 5p13 (SLER1) to SLE stratifying on families containing one member affected with SLE and self-reported RA has been confirmed (65) (Table 6-3). Similarly, the SLEV1 (17p13), SLEN1 (10q22.3), and SLEN2 (2q34–35) have also been verified (Table 6-3). Alternatively, a principal component approach has used seven major SLE-related phenotypes plus age at SLE onset and race to conduct multivariate analysis of the genome scan data, which identifying suggestive linkage of several SLE-related traits, however the clinical implication of this method seem less clear (68). Since the same (or enlarged) genome scan data
from the OMRF SLE multiplex pedigrees have been used to conduct one principal component analysis (68), 4 affected relative pair linkage analyses (40 ,45 ,46 ,69), and 11 linkage analyses on selected pedigrees in which one affected member exhibiting a specific disease feature (46 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,66 ,67), the possibility of false discoveries as the result of multiple testing needs to be considered. Familiality of thrombocytopenia, discoid rash, neurologic disorder (defined as seizure or psychosis), hemolytic anemia, co-occurring neurologic disorder plus hemolytic anemia, and age at SLE diagnosis has been observed in 159 sibpairs affected with SLE (70). Linkage to familial component phenotypes of SLE may reduce the disease heterogeneity facilitating to the localization of susceptibility genes for a particular clinical manifestation. The identification of many chromosomal regions linked to SLE has paved the way to fine map the genomic intervals of interest for the identification of putative SLE susceptibility genes.

Table 6-1: Comparisons of 11 Whole Genome Scans in Human SLE

Group	Reference	# of Families	Cohort Ethnicity (%)	# SLE Subjects	# Total Subjects	Major Loci[§]	LOD (or P value)
UMN1*	(38)	105	80 Caucasian	220	375	6p11-21	3.90
			8 Hispanic			16q13	3.64
			5 African-American			14q21-23	2.81
			3 Asian			20p12	2.62
			4 Other				
UMN2*	(39)	82	78 Caucasian	179	280	7p22	2.87
			6 Hispanic			7q21	2.40
			15 African-American			10p13	2.24
			1 Other			7q36	2.06
UMN1+2*	(39)	187	79 Caucasian	399	655	6p11-21	4.19
			7 Hispanic			16q13	3.85
			10 African-American			2p15	2.06
			2 Asian			7q36	2.06
			2 Other				
OMRF1**	(45)	94	58 Caucasian	220	533	All:	
			33 African-American			1q23	3.45
			9 Other			13q32	2.50
						20q13	2.49
						1q31	2.04
						Caucasian:	2.21
						14q11	2.18
						4p15	2.15
						11q25	2.09
						2q32	2.05
						19q13	2.04
						6q26-27	2.01
						12p12-11	3.50
						African-American: 1q41 11q14-23	2.10
OMRF2**	(40)	126	61 Caucasian	295	744	All:	
			32 African-American			4p16-15.2	3.44
			7 Other			1q22-24	2.75
						Caucasian:	2.04
						12q24	2.36
						African-American:	2.08
						6p12-q14 9p24-21 6p24-23	2.06
OMRF3**	(46)	37	100 Hispanic	91	ndf	12q24 16q1	22.38
USCL†	(44)	80	46 Caucasian 54 Hispanic	188	434	1q43	2.41
UCLA‡	(37)	62	44 Caucasian	160	356	All:	
			27 Hispanic			18q22-23	(0.0003)
			11 African-American			17q11	(0.002)
			18 Asian			17q21-23	(0.002)
						10q23-24	(0.003)
						16q12-13	(0.004)
						13q32	(0.006)
						1q23	(0.01)
						Caucasian:	(0.0002)
						10q24	(0.005)
						18q22-23	(0.0002)
						Non-Caucasian:	(0.0006)
						17q11 17q21 17q23	(0.002)
Finland	(42)	35	100 Caucasian	73	169	6q25-27	2.47
						14q21-23	2.20
						6p21	2.17
						5p13	2.03
Sweden& Iceland	(43)	19 (11+8)	100 Caucasian	44 (28+16)	150 (54+96)	All:	
						2q37	4.24
						Iceland:	3.20
						4p15-13	2.58
						19p13	2.06
						19q13.1	2.06
						2q37	2.18
						Sweden: 2q37 2q12	2.13
Argentine, Italy & other Europe	(41)	70 (20+12+38)	100 Caucasian	154 (41+25+88)	397(89+67+241)	All:	
						4p13	2.65
						4q13	2.22
						Argentine:	3.49
						17q11	3.16
						19q13	2.64
						17p12	2.02
						1p35	2.45
						Italy:	2.41
						3q29	2.04
						1q31	2.87
						5p15	2.69
						Europe: 22q11 11q23 2q37	2.44

*The first and second cohort studied at University of Minnesota are independent.
**The first cohort was extended and used for the second (but not the third) genome scan conducted at Oklahoma Medical Research Foundation
†A study conducted at University of Southern California
‡ A study conducted at University of California, Los Angeles.
§ Only loci with a LOD score >2 (Z scores were converted to LOD scores using LOD = Z2/2ln10) or the strongest locus of the whole genome scan using the complete cohort are listed here. LOD = logarithm of the odds (the log base 10 of the likelihood ratio under the hypotheses of linkage and nonlinkage)
{ParaMarks} Not described.

Table 6-2: Eight Significantly SLE-Linked Regions

Groups	Reference	1q23	1q31	1q41-42	2q37	4p16-15.2	6p21-11	12q24	16q12-13
11 whole genome scans									
UMN1	(38)			+			+		+
UMN2	(39)								
UMN1 + 2	(39)			+			+		+
OMRF1	(45)	+	+	+*					
OMRF2	(40)	+				+		+**	
OMRF3	(46)							+	+
USC	(44)	+							
UCLA	(37)	+							+
Finland	(42)						+		
Sweden & Iceland	(43)		+†		+				
Argentine, Italy & other Europe	(41)		+‡		+				
8 finemapping scans									
UCLA1	(47)			+					
UCLA2	(52)	+							+
OMRF	(48)			+					
UMN1	(49)			+					
Sweden & Iceland1	(50)				+				
European & Mixed American	(51)	+	+						
Sweden & Iceland2	(53)						+		
UMN2	(54)								+

*In African-American cohort
**In European American cohort
†In Sweden cohort
‡In Italy cohort

Compared to other autoimmune diseases such as type 1 diabetes mellitus and multiple sclerosis, currently available results from independent linkage analyses in human SLE have been remarkably consistent. Of interest, 1q23–42 is syntenic to the identified susceptibility loci on murine chromosome 1 (71). Several putative murine susceptibility genes located in the distal end of the mouse chromosome 1 have been identified including Ifi202 (an interferon [IFN]-inducing gene for the Nba2 locus derived from the NZB strain), Cr2 (complement receptor 2 gene for the Sle1c locus), and the Slam/Cd2 gene cluster (for the Sle1b locus derived from NZW strain) (72 ,73 ,74). Although none of these murine candidate susceptibility genes have been reported to be associated with human SLE, their gene products may participate in molecular pathways common in the pathogenesis of both murine and human SLE.

Within the 8 genomic segments (1q23, 1q31, 1q41–42, 2q35–37, 4p16–15.2, 6p21–11, 12q24, and 16q12–13) that strongly linked to human SLE, 10 positional candidate susceptibility genes have also been associated with human SLE (Table 6-4). Because evidence implicating these genes in susceptibility to SLE includes both linkage and association that are two independent and complementary approaches, these genes (or gene complexes) (MHC haplotypes, CRP, FCGR2A, FCGR3A, FCGR2B, PARP, TLR5, PDCD1) will be reviewed as followed.

Table 6-3: Loci Linked to SLE Identified Using Stratification of Pedigrees in Which One Affected Member Exhibited a Specific Clinical Manifestation

Locus Name	Chromosome Location	Stratifying Manifestation	Reference
SLEB3	4p16	Neuropsychiatric disorder	(61)
SLED1	19p13.2		(58)
SLED2	18q21.1	Anti-dsDNA antibodies	(58)
SLED3	10q22.3		(58)
SLEH1	11q14	Hemolytic Anemia	(57)
SLEH1	11q14	Nucleolar antinuclear antibody	(64)
SLEN1	10q22.3	Renal disease	(62) and confirmed in (67)
SLEN2	2q34–35		(62) and confirmed in (67)
SLEN3	11p15.6		(62)
SLER1	5p15.3	Self-reported rheumatoid arthritis	(59) and confirmed in (65)
SLEV1	17p13	Vitiligo	(60) and confirmed in (402)
	1q22–23	Thrombocytopenia	(63)
	11p13		(63)
	11p13	Discoid lupus erythematosus	(66)

Genes within SLE Significantly Linked Regions (Table 6-4)

(1) SLE-Associated Genes at 6q21

The General Review of the MHC Region

The MHC (also referred as HLA) region contains more than 200 identified loci within about 4 × 106 base pairs (75). It is the most gene-dense region of the human genome that has been completely sequenced thus far (76). Historically, the MHC region is known to specify histocompatibility genes and can be subdivided into class II (centromeric), class III, and class I (telomeric) regions. The class I and class II molecules are the most polymorphic human proteins known to date. Since these molecules shape the immune repertoire of an individual, the extreme polymorphism is thought to evolve in response to infectious pathogens. Perhaps it is the reason why the MHC is associated with more diseases than any other region of the human genome, and is linked to most, if not all, autoimmune disorders (76 ,77).

Table 6-4: Genomic Segments That Have Reached the Threshold for Significant Linkage to SLE and Have Been Confirmed in an Independent Cohort

Cytogenetic Location	Locus Name	Candidate Genes
1q23		CRP, FCGR2A, FCGR3A, FCGR2B
1q31		
1q41–42	SLEB1	PARP, TLR5
2q35–37	SLEB2	PDCD1
4p16–15.2	SLEB3	
6p21–11		MHC haplotype
12q24	SLEB4	
16q12–13		NOD2/CARD15, OAZ

The MHC class I region contains the HLA-A, B, C, E, F, G, H, J, and X loci (75 ,76 ,78). Each individual expresses at least three different class I proteins encoded by HLA-A, B, and C in combination with a nonpolymorphic protein—ß2 microglobulin. The gene for ß2 microglobulin, despite it encodes a part of the MHC class I molecule, is located on chromosome 15. The MHC-class I A, B, and C molecules are extremely polymorphic, expressed on all nucleated cells, and most highly in hematopoietic cells. The additional genes in the class I region, E, F, G, H, J, and X loci, are class I-like genes encoding class IB molecules, which are important in innate immunity such as mediating recognition by natural killer (NK) cells.

The MHC class II region includes the genes for the α and ß chains of the antigen-presenting class II MHC-molecules HLA-DR, DP, and DQ, the genes encoding the DMα and DMß chains, and the genes encoding the α and ß chains of the DO molecule (encoded by DNα and DOß) (75 ,76 ,79). Additionally, the LMP 2 and 7 genes encoding proteosome subunits (necessary for generating peptide fragments form endogenous proteins to bind class I molecules), and the TAP-1 and 2 genes (jointly encoding a TAP heterodimer to transport peptide from the cytosol to the endoplasmic reticulum) are also in the class II region. The DM molecules act as a chaperone and catalyze peptide binding to class II- DR, DP, and DQ molecules, while DO molecules serve as a negative regulator of DM. The class II molecules are highly polymorphic, there are 239 DRß alleles, 20 DQα, 35 DQß, 12 DPα, and 80 DPß alleles found mainly in

Caucasian populations with the exception of a single DRα allele. The true polymorphic nature of these molecules awaits detailed studies worldwide. An individual can use DR, DP, or DQ molecules to present peptide antigens to T cells that are recognized by T cell receptors initiating immune responses. These MHC class II molecules are glycosylated heterodimer membrane proteins normally expressed in a subset of hematopietic cells and thymic stromal cells. Upon exposure to inflammatory cytokine interferon-γ, other cell types can be induced to express class II molecules.

It is worth noting that there is no wild type for class I and II molecules. Particular alleles of these antigen-presenting molecules that have been associated with autoimmune diseases are also commonly found in a normal, unaffected population (76). The presence of multiple class I and II loci allows a broad spectrum of peptides that can be presented to T cells of an individual. Allelic variation of these molecules appears to be restricted to the amino-terminal domains (α1 and α2 domains of class I molecules and ß1 domain of class II molecules) at positions that line the peptide-binding cleft or at exposed surfaces of the outer domain of the molecule. Additionally, most individuals are heterozygotes expressing different alleles inherited from both parents at class I and II loci. The large number of different alleles of these antigen- presenting molecules also allows a diverse immune response to a pathogen in any population. The distribution of allelic frequency of class I and II molecules varies among ethnic groups, and may show regional differences as well. This frequency variation and linkage disequilibrium of the MHC region give rise to various extended haplotypes frequently observed among separate populations.

The MHC class III region contain genes encoding the complement component C4 (C4A and C4B genes), C2 and Factor B (Bf gene), cytokine tumor necrosis factor alpha (TNF-α) (TNFA gene) and lymphotoxins (LTA and LTB genes), and heat-shock protein HSP 70. Physically near the two C4 genes is the gene encoding 21-hydoxylase, an enzyme involved in steroid synthesis. Within the class III region C4A and C4B are the most polymorphic genes including at least 14 C4A and 17 C4B alleles (80 ,81). Frequent deletions and gene duplication events of the C4 genes further contribute to their genetic polymorphism (82 ,83 ,84).

Genetic Association of MHC Alleles with SLE

MHC Class II Molecules and SLE

Approximately 30 years ago, positive association between HLA-B8 and SLE was reported (85 ,86 ,87). Later studies showed weak or no association between SLE and class I molecules, but very consistent association with HLA-DR2 and DR3 in many populations (88 ,89 ,90). The more recent DNA typing has subdivided the previous DR2 and DR3 specificity into continuously increasing numbers of DRB1 alleles. Case-control studies have demonstrated the association between SLE and HLA-DR3 (or HLA-DRB1*0301; one of the allele from the previous DR3 specificity) in many Caucasian populations including American Caucasians, Australians, British Caucasians, Spaniards, Italians, Germans, Greeks, Scandinavians and Koreans (91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113). The association of SLE susceptibility and DR2 (or HLA-DRB1*1501; one of the allele from the previous DR2 specificity) has also been shown in studies of Caucasians from the United States, Australia, United Kingdom, Germany, Dutch, as well as Asians from Japan, China, Korea, and Singapore (94 ,96 ,103 ,106 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123). There are reports showing no association between SLE susceptibility and DR3 and/or DR2, which include studies of Bulgarians, Greeks, Indians, and Chinese in Taiwan (124 ,125 ,126 ,127 ,128 ,129). Taken into account of the heterogeneous manifestations of SLE including various autoantibodies, the confirmed association of DR2 and DR3 with SLE is an important and remarkable finding. The estimated relative risk of either DR2 or DR3 for the development of SLE is approximately two- to threefold in Caucasians. A recent multicenter study of 534 SLE patients and 958 controls from seven European countries has shown association of DRB1*03, DRB1*15, and DRB1*16 with SLE with odds ratio of 2.2 (95% confidence interval [CI] 1.8 to 2.9), 1.6 (1.2 to 2), and 1.4 (1.1 to 2), respectively (130).

In Mexican Americans (who have ancestry of Mexicans, European Caucasians, and Native Americans), HLA-DRB1*0301(DR3) (131 ,132) and DRB1*08 (DR8) (100 ,132) have been associated with SLE. This result was not confirmed in a study of Mexican Mestizo population (133). African Americans are known to have varying degrees of African, European Caucasian, and Native American ancestry. Several studies of African Americans yielded weak and varying associations of DR alleles (none, DR2, DR3, or DR7) with SLE (100 ,105 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141). In genetic studies of Mexican Americans and African Americans, it is especially challenging to match ethnic ancestry of the cases and controls.

The lack of consistency of the observed association might be derived from the unmatched SLE cases and normal controls (21). The family-based association test, such as the TDT that uses intrafamily controls (28), can avoid this problem in assessing the association between MHC class II alleles and SLE susceptibility. Association between the DRB1*1501(DR2) and SLE has been observed in our 91 Caucasian families using the TDT (142).

MHC Class II Alleles and Autoantibodies

MHC class II alleles may be more strongly associated with autoantibody subsets than with the disease status of SLE because of their pivotal role in T cell dependent antibody responses. A few examples of genetic association between class II alleles and specific autoantibodies are described below.

Anti-Ro (SSA) and anti-La (SSB): These antibodies are frequently present in patients affected with either SLE or Sjogren syndrome. These antibodies were initially associated with HLA-DR3 (114 ,143 ,144 ,145) or DR2 in Caucasians. Hochberg et al. showed that the presence of both anti-Ro and anti-La together with DR3 occurred

frequently in SLE patients with older age of disease onset, while anti-Ro in the absence of anti-La and DR2 were present in patients with younger age of disease onset (136). Subsequently, antibodies to Ro and La seem to be most significantly associated with the HLA-DQ alleles in linkage disequilibrium with DR2, DR3, and other MHC haplotypes (146 ,147 ,148). The strong association between anti-La and DR3 (OR = 71) and DQB1*0301 (OR = 35) was recently observed in a UK case-control study (112). Molecularly, they are commonly the HLA-DQA alleles with glutamine at position 34 and DQB alleles with leucine at position 26 (149 ,150). The anti-Ro response has been resolved into antibodies to 52kD and 60kD Ro/SSA molecules. The response to the 52kD Ro protein has been associated with HLA-DR3 and DRB1*0301, DQA1*0501, and DQB1*0201 (151 ,152). The presence of anti-Ro/SSA alone is mainly associated with DR2, and DQw1 (153), while the presence of both anti-Ro/SSA and anti-La/SSB is associated with HLA- B8, DR3, DRw52, and DQw2 (153).

Antiphospholipid antibodies: These antibodies (anticardiolipin [aCL] antibodies and lupus anticoagulant [LAC]) are frequently found in patients affected with primary antiphospholipid syndrome (APS) and a subset of SLE patients. Clinically, they are associated with both venous and arterial thrombosis, and recurrent fetal loss. An increased frequency of class II alleles HLA-DR7, DR4, and DRw53 (DRB4*0101) were reported in studies of primary APS (154 ,155 ,156) and supported by studies of patients affected with SLE or with secondary APS (157 ,158 ,159 ,160 ,161). The DRB1*0402 allele (one of the alleles of DR4 specificity) was highly represented in both aCL and anti-ß2 glycoprotein I positive patients from a large cohort of European SLE patients (161). A sharing of HLA-DR4 haplotype in affected members was noted in two families multiplex for SLE and manifestations of APS (162 ,163). However, a study of seven families in which 30 of the 101 family members met criteria for APS failed to show linkage of HLA with APS (164). Segregation studies of these families suggested an autosomal dominant (or codominant) inheritance of the disease. Debastiani et al. (165) concluded that the genetic predisposition to APS is partially resulted from HLA alleles including DR4, DR7, and DRw53. DR4 and DR7 are independently associated with aCL while the association of DRw53 may be linked with either DR4 or DR7. A subset of aCLs bind to ß2 glycoprotein I (ß2GPI), a phospholipid-binding plasma protein. In a combined cohort of patients with primary APS, SLE, or another connective tissue disease, anti-ß2GPI autoantibodies were found to be associated with HLA-DQB1*0302 carried on DR4 haplotypes and DRB4*0101 in Mexican Americans, with HLA-DR6 (DR13) haplotype DRB1*1302-DQB1*0604/5 in blacks, but not with HLA-DR7 in any of the studied Caucasians, Mexican-Americans, and African Americans (166). When analyzed as a combined multiethnic cohort, HLA-DQB1*0302, and the combined DQB1*0301, *0302, and *0303 alleles showed the most powerful association with anti- ß2GPI (166). ß2GPI is a member of the complement control protein (CCP) superfamily. Genetic variations of the 5th CCP domain of ß2GPI (at amino acid positions 247, 306, and 316) that may affect its binding to phospholipids have been suggested to influence the generation of anti-ß2GPI antibodies in APS patients (166 ,167 ,168 ,169 ,170). A correlation between the valine/leucine polymorphism at position 247 and the production of anti-ß2GPI in primary APS patients was observed in British Caucasians (168), and was subsequently supported by studies of Asian patients with APS (especially as a major risk factor in those V/V homozygotes) but not of American Caucasians and African Americans (169). Kamboh et al. reported that polymorphisms at codons 306 and 316 had a gene-dosage effect on plasma concentrations of ß2GPI, and the Trp to Ser at codon 316 protected against the production of anti-ß2GPI in American Caucasian SLE patients (171). The protective association of this polymorphism was not supported by studies of SLE and APS patients from the Netherlands (172) and the United States (170). Instead, Gushiken et al. suggested that Ser316 polymorphism may be an independent risk factor for thrombosis in patients with SLE (170).

Anti-Sm and RNP antibodies: Anti-Sm antibodies are highly specific for SLE including the binding specificity to U1, U2, U4, U5, and U6 small nuclear ribonuclearproteins (snRNPs). These autoantibodies are present frequently in African-American SLE patients (173). Anti-RNPs often occur with anti-Sm in SLE patients, or in the absence of anti-Sm in patients with mixed connective-tissue diseases (MCTD). Many class II alleles have been implicated in association with anti-Sm or anti-RNP including HLA-DR4 in Caucasians (144 ,174) and Asians (175) and DR2 in Caucasians (174) and blacks (176), as well as HLA-DQB1*0302 in Caucasians (175) or in Mexican Americans (131).

Anti-dsDNA antibodies: These antibodies may contribute to the development of glomerulonephritis. This antibody response has been associated with HLA-DR3 (177), DR2 (114 ,178), and DR7 (179). Their respective linked HLA-DQ alleles may also responsible for the observed associations.

In summary, the MHC associations with many autoantibodies including anti-dsDNA, antiphospholipid, anti-RNP, and Sm are very complex and inconclusive at present. Other MHC class II genes including DP, DM, Tap1, and Tap2 alleles have either no or weak and inconsistent associations with SLE.

SLE-Associated Genes within MHC III Region

Complement Component C2 and C4

The complement component genes within the class III region include C2, C4A, C4B, and factor B. They are closely linked and usually inherited as a single group termed a

complotype. Hereditary C2 deficiency in Caucasians is located on an HLA-A25, B18, DR2 (DRB1*1501), DQB1*0602 haplotype (180 ,181). This deficiency is the result of a 28-bp deletion leading to a splicing defect of the C2 transcript (182). Heterozygous deficiency is common in Caucasian populations (approximately 1% to 2%), and is increased among SLE patients in several studies (181 ,183 ,184), but not in another report (185). Homozygous C2 deficiency is present in approximately 1 in 10,000 Caucasians, and about 30% of them develop SLE or lupus-like disease (186 ,187). These patients frequently have anti-Ro (but not anti-La) autoantibodies (188) (189). In summary, C2 deficiency may represent an infrequent risk factor for SLE in Caucasian patients (181), but not in Asians (124) or African Americans (184).

Plasma C4 is the protein product of two C4A genes and two C4B genes. Although double homozygous deficiency of C4 (producing no C4) is rare, approximately 70% of these individuals develop SLE or lupus-like disease (190 ,191 ,192 ,193 ,194 ,195 ,196 ,197). More commonly, individuals have homozygous null alleles at either C4A or C4B locus, often with reduced levels of serum C4. Functional differences between C4A and C4B have been demonstrated. Many Caucasian populations studied revealed an association of C4A deficiency with SLE in which C4A*QO (the null allele of C4) allele frequencies are 0.25 to 0.41 in patients versus 0.10 to 0.23 in controls (96 ,198 ,199 ,200 ,201 ,202 ,203). The C4A*QO is most often caused by the deletion of the C4A and 21-OHA genes on the HLA-B8, Cw7, DR3, C4A*QO, C4B1 haplotype, a common MHC haplotype in Caucasians (202 ,204 ,205 ,206). Because the frequency of DR3 is also increased in patients, and because the C4A null allele is not increased in DR3-negative patients (96 ,207), it is difficult to determine the relative contribution of separate loci within the extended MHC haplotype. Studies of Asians in whom C4A null alleles are within other MHC haplotypes have supported that the lack of C4A protein may predispose to SLE. A recent review of more than 35 publications has concluded that 40% to 60% of SLE patients from multiple ethnic/race groups (including northern and central Europeans, Anglo-Saxons, U.S. Caucasians, African Americans, Asian Chinese, Koreans, and Japanese) have heterozygous or homozygous deficiencies of C4A genes (208). Among different ethnic groups, various molecular mechanisms result in C4AQ0 (the null allele of C4) with the estimated relative risk in the range of 2.3 to 5.3 for SLE (208). The significant association between C4AQ0 and SLE in the context of multiple MHC haplotypes, ethnic/racial groups, and molecular mechanisms strongly support the notion that no or low expression level of C4A protein predisposes to SLE susceptibility. C4B deficiencies have been associated with SLE in Spanish, Mexican, and Australian Aborigine populations (208). Taken together, both C4A and C4B proteins are important regulators of autoimmunity, which is supported by the observation that C4 deficient mice spontaneously develop autoantibodies to dsDNA and have glomerular depositions of immune complexes (209).

Tumor Necrosis Factor

In addition to complement components C2 and C4 loci, within the MHC class III region the loci for TNF encoding TNF-α, TNF-ß, and lymphotoxin-ß have also been implicated in SLE susceptibility. Polymorphisms of the TNF-α gene, which is located within the MHC class III region, have been associated with SLE. Reduced levels of TNF-α production associated with genetic polymorphisms at or near the TNF-α, gene were observed in one lupus-prone mouse model and in DR2 positive SLE patients (210 ,211 ,212). Compared to the DR3 positive SLE patients, DR2 positive patients were also associated with increased incidence of lupus nephritis (210). In mice, recombinant TNF-α replacement could delay in the development of lupus nephritis (211 ,212). In humans, treatment with a monoclonal antibody to TNF-α (Remicade, Centocor, Malvern, PA) in rheumatoid arthritis patients occasionally induced anti-dsDNA antibody and a self-limiting clinical lupus syndrome (213). These findings suggest that (1) reduced levels of TNF-α may predispose to SLE, and (2) polymorphisms of TNF-α gene may influence its level of production.

In recent years, polymorphisms in the promoter region of the human TNF-α gene have been studied extensively in association with many autoimmune diseases (reviewed in (214)). Among them, -308A TNF-α has been a major focus of these studies for its association with SLE either independently or as a part of an extended MHC haplotype HLA-A1-B8-DRB1*0301-DQ2 (215) in Caucasians (109 ,216 ,217 ,218), Asians (124), African Americans (219) and Mexican Mestizos (133). The -308A TNF-α allele has been shown to confer higher transcriptional activity than the –308G TNF-α allele (220). However, neither the disease association nor the enhanced activity has been consistently demonstrated in other similar studies (214 ,220). Therefore, additional studies are needed to help clarify the role of genetic polymorphisms of the TNF-α in susceptibility to SLE.

MHC Haplotypes and SLE

The early finding of the association of B8 with SLE is likely attributable to B8-DR3 (DRB*0301 according to the new nomenclature) extended haplotype that contains a complement C4A null allele (C4A*QO), DQA*0501, and DQB1*0201. This haplotype is fairly common in Caucasian populations at a frequency of approximately 25%, and is associated with multiple autoimmune disorders. The other DR3 containing extended haplotype—HLA-B18-C4B*QO-DRB1*0301-DQA1*0501-DQB1*0201 frequently found in Spain and Mexico has also been associated with SLE. Null alleles of C4 (especially C4A*QO) have been implicated in the pathogenesis of SLE, while the rare deficiency of all four C4 genes clearly confers increased risk for lupus-like symptoms. The DR2-containing SLE-associated haplotype (HLA-B7-DRB1*1501-DQA1*0102-DQB1*0602) frequently found in Western Europeans and Asians is not linked to a C4 null allele. Various studies have used different ethnic populations to investigate the role of C4A null in SLE. Because of the

tight linkage disequilibrium of the MHC region, it has been difficult to determine whether a single locus or multiple independent loci mediate the observed association of extended haplotypes. In summary, the MHC region is likely to contain multiple genes influencing susceptibility to lupus via separate pathways. The impact of these genes appears to be quite variable among the studied groups using the current approaches. It is generally agreed that determination of relative contribution of each implicated locus within the MHC is a very difficult but important task for the next decade.

Finemapping of HLA Region

The genomic segment harboring the HLA is one of the eight major SLE susceptibility loci that have reached the threshold for significant linkage and have been confirmed in at least one independent cohort using guidelines proposed by Lander and Kruglyak (55). Fine mapping of the linked region have narrowed the search to a chromosomal segment containing the HLA class II gene of three risk haplotypes: DRB1*1501 (DR2)/DQB1*0602, DRB1*0301 (DR3)/DQB1*0201, and DRB1*0801 (DR8)/DQB1*0402 (221). This study used approximately 100 microsatellite markers and a collection of >300 families (mainly Caucasians) to narrow the SLE-associated haplotype blocks to approximately 500 kb except the DRB1*0301 containing block which is approximately 1 Mb because of the extremely strong linkage disequilibrium. Nearly two thirds of Caucasian SLE patients harbor at least one of the three risk haplotypes, in which the DRB1*0801 containing haplotype is relatively rare. The estimated haplotype relative risk is in the range of 1.5 to 2.3 for harboring one copy and 1.7 to 5.2 for any two copies of risk haplotypes. Of interest, both the class I and III regions, containing the genes encoding TNF-α, and complement components C2 and C4, have been excluded in the DRB1*1501 containing risk haplotype (221).

(2) SLE-Associated Genes at 1q23

CRP (1q23)

The genes for acute phase reactants CRP and serum amyloid P component (SAP) are positional candidates for a murine lupus susceptibility locus (222). Both of these proteins bind to major autoantigens in SLE such as membrane components and nuclear materials released during cell death and/or exposed on the cell surface during apoptosis (223). Abnormally low CRP levels in SLE was attributed to reduced synthesis (224). Low basal levels of serum CRP were associated with inheritance of minor alleles at two SNPs of the CRP gene (rs1800947 located within codon 188 and rs1205 in the 3′ UTR), and the minor allele of the latter SNP was also associated with SLE in 586 UK simplex families (225). Another study showed two CRP promoter SNP haplotypes (rs3093062 located at –409 and rs391244 at –390), which could influence binding affinity to transcription factors and the promoter activity, were associated with basal levels of serum CRP in 267 healthy controls independent of age, gender, or race (226). Of interest, CRP may prevent inflammation and tissue damage as shown that a single dose of CRP can reverse lupus nephritis and nephrotoxic nephritis in a murine model (227).

FCGR2A, FCGR3A, and FCGR2B (1q23–24)

The Fcγ receptors expressed on the cell membrane of different leukocytes are a family of molecules that bind to the Fc portion of IgG molecules that each recognizes one or a few closely related IgG isotypes (75 ,228). The low affinity FcγRII and FcγRIII bind to polymeric IgG present in immune complexes, which mediate internalization of immune complexes, antibody-mediated cytotoxicity, and the release of cytokines (229). These receptors have cytoplasmic tails containing either the immunoreceptor tyrosine-based activation motif (ITAM) or the immunoreceptor tyrosine-based inhibition motif (ITIM). Co-aggregation of ITAM-containing FcγR molecules triggered by binding to immune complexes results in cell activation, phagocytosis, and the release of proinflammatory cytokines. In contrast, co-aggregation of ITAM and ITIM-bearing FcγR molecules causes abrogation of Ca2+ influx, inhibition of phagocytosis, and release of pro-inflammatory cytokines. The important role of impaired clearance and tissue deposition of immune complexes in the pathogenesis of SLE has led to the identification of functional polymorphisms of FcγRII and FcγRIII genes and their association with SLE as described below.

FcγRIIa (CD32) molecules, expressed on cell membranes of monocytes, macrophages, neutrophils, and platelets, is an ITAM-containing stimulatory receptor (230). FcγRIIa is the major receptor for the IgG2 subclass, but it is capable of binding to all IgG isotypes (230 ,231). A functional SNP that results in either the histidine [H] or the arginine [R] residue at codon 131 are two codominantly expressed alleles in multiple studied ethnic groups, in which the R131 binds less efficiently to IgG2 resulting in delayed clearance of immune complexes (229). In lupus nephritis patients of Dutch Caucasians, the R131 allele and R131 homozygotes were significantly overrepresented (231). Simialrly, African-American lupus nephritis patients had significant under-representation of FcγRIIa-H131 homozygosity (232). This skewed distribution of FcγRIIa alleles was further confirmed in Korean lupus nephritis patients and SLE patients as a whole (233), supporting the conclusion that the FCγRIIa-R131 allele increases the risk for lupus nephritis. However, this notion was not supported in many similar studies including American, British, Italian, Dutch, or German Caucasians, Afro-Caribbeans, Chinese, or Koreans. Despite the lack of association in German Caucasians, SLE patients homozygous for the FcγRIIa-R131 allele had higher frequencies of proteinuria, hemolytic anemia, hypocomplementemia, and an earlier age of onset of SLE. There was a strong trend toward skewing of FcγRIIa in Dutch SLE patients, and in a subset of 13 SLE patients, homozygous R/R patients showed significantly slower clearance of in vivo clearance of IgG-coated erythrocytes than

H/R and H/H patients (238)). These data are consistent with the interpretation that either the FcγRIIa-R131 allele can be a weak risk factor or a disease modifier for certain clinical manifestations. FcγRIIa-R131 may not be a genetic risk for the development of SLE, but may be a risk factor for lupus nephritis, especially in SLE patients positive for IgG2 antibodies to C1q. The investigators postulated that IgG2 autoantibodies to C1q might be particularly nephritogenic in lupus patients with R131/R131 genotypes because of poor efficiency in clearing IgG2 containing immune complexes. A meta-analysis of 17 studies concluded that the low-binding R131 allele confers a 1.3-fold risk for developing SLE, but not for lupus nephritis (234). The modest relative risk for SLE (and no significant risk for lupus nephritis) might be an underestimate influenced by genetic heterogeneity, genotyping errors, and/or low-penetrance of genotypic effects. Genotyping errors are a serious concern because extensive sequence homology among FcγR genes makes it difficult to achieve highly specific PCR-based genotyping assays, especially in earlier studies when the human genome sequence database was not available.

FcγRIIIa (CD16) expressed on cell surfaces of NK cells, monocytes, and macrophages binds to both IgG1 and IgG3 subclasses (228 ,230). A common polymorphism of T to G substitution at position 559 results in an amino acid substitution from phenylalanine (F) to valine (V) (at amino acid 176 counting from the leader sequence or at amino acid 158 of the mature sequence) (235). Individuals homozygous for F/F bind IgG1- and IgG3-containing ICs less efficiently than those with V/V genotypes (reviewed in (229)). Wu et al. (1997) showed a strong association between the low affinity allele and SLE in an ethnically diverse population, and especially an under-representation of homozygous V/V in lupus nephritis patients (235). This skewed allele distribution of FcγRIIIa-158V/F in SLE patients was further supported by studies of Caucasians (236)and Koreans (237), but not by similar studies of Dutch Caucasian (238) and African Americans (239). Seligman et al. have recently demonstrated an association between the low affinity FcγRIIIa-158F allele and risk of lupus nephritis among Caucasians but not among non-Caucasians (240). In contrast, the low affinity FcγRIIa-131R allele was not associated with lupus nephritis in the examined Caucasians, Hispanics, African Americans, and Asian/Pacific Islanders (240). Taken together, association between the FcγRIIIa-V/F158 polymorphism and susceptibility to SLE and/or to lupus nephritis has been reported in some but not in other similar studies. A meta-analysis of more than one thousand subjects in each of the three categories (lupus nephritis, SLE without renal involvement, and non-SLE controls) has concluded that the F158 allele confers a 1.2-fold risk for developing lupus nephritis in patients of European, African, and Asian descent, but not for SLE susceptibility per se in the absence of nephritis (241).

FcγRIIa and FcγRIIIa are transmembrane proteins. In contrast, FcγRIIIb is attached to cell membrane by a phosphatidylinositol anchor. FcγRIIIa and FcγRIIIb both bind to IgG1 and IgG3 but are expressed in different cell types; the former mainly in NK cells and macrophages and the latter in neutrophils. Earlier reports of a few SLE patients with rare alleles deficient in surface expression of FcγRIIIb suggested its possible involvement in the development of SLE (242 ,243). Additionally, two common variants of FcγRIIIb gene, NA1 and NA2 alleles, were identified with differences in four amino acids (244). Functionally, individuals homozygous for NA2 have lower capacity to mediate phagocytosis than NA1 homozygotes. Hatta et al. have reported that individuals with FcγRIIIB-NA2/NA2 are at risk for lupus nephritis in a Japanese population (245). Of interest, in this study genetic association between alleles of FcγRIIa, FcγRIIIa, or FcγRIIIb and SLE were simultaneously compared. Only the FcγRIIIB-NA2 but not the FcγRIIA-R131or FcγRIIIA-158F was a risk factor for SLE. However, a similar study of Dutch Caucasians found NA1 allele was at a significantly higher frequency among lupus nephritis patients than non-nephritis patients (238). Another study conducted in a combined American and Dutch Caucasian cohort, however, found significant association of the FcγRIIIA-158F allele with SLE, but not the FcγRIIA-R131 or FcγRIIIB-NA2 allele (236).

Since IgG2 and IgG3 are major subclasses of ICs deposited in renal biopsies of lupus nephritis patients (246), the relative importance of FcγRIIa-H/R131 and FcγRIIIa-V/F158 polymorphisms might depend on the IgG subclass of pathogenic autoantibodies in each patient. FcγRIIa-R131 and FcγRIIIa-F158 may not be independent risk alleles, they often are inherited together on the same chromosome as a single risk haplotype for SLE (247), and the presence of multiple at-risk alleles of genes affecting immune complex clearance may interact to enhance risk for SLE (248). Haplotype studies suggest LD between FcγRIIa-H/R131 and FcγRIIIa-V/F158 in some but not in other studied populations (discussed in (241)). Although both of these gene variants have shown evidence for linkage to SLE (45 ,249), they may not be sufficient to account for the observed linkage at 1q23 to SLE, suggesting the possibility of additional SLE susceptibility genes in their flanking regions. Other neighboring genes have also been associated with SLE including Fc_RIIb and CRP.

FcγRIIb, expressed on B cells, monocytes/macrophages, dendritic cells, and mast cells, contains an immunoreceptor tyrosine-based inhibitory motif in its cytoplasmic tail to negatively regulate cell activation. The FcγRIIb-I/T 232 polymorphism that may alter the inhibitory function (250) has been associated with SLE susceptibility in Japanese (251), but not in African American, U.S. Caucasian, and Swedish populations (247 ,250). Recently, the association of FcγRIIb-T232 allele and SLE has been confirmed in Thais and Chinese (252 ,253), suggesting it may be a common susceptibility gene in Asian populations. Recently, 10 SNPs of the human FcγRIIb have been shown to define two haplotypes, in which the less frequent promoter haplotype exhibits increased expression of the reporter gene in both B cells and myeloid cell lines and has been associated with SLE in US Caucasians (OR = 1.65; p = 0.0054) (254 ,255). The FcγRIIb haplotype is not in linkage disequilibrium with the previously identified FcγRIIa and FcγRIIIa polymorphisms; hence the

association of the high-expression haplotype with SLE demonstrates a role for FcγRIIb in SLE susceptibility independent of the stimulatory FcγRIIa and FcγRIIIa.

Fc receptor-like genes (FCRLs, also known as FCRHs) clustered at 1q21–22, encode a set of proteins with high structural homology with classical FcγRs. Recently, a functional SNP (-169 C->T) in the promoter region of FCRL3 was associated with multiple autoimmune diseases including SLE in a Japanese case-control study (256). Whether this finding has implications in other ethnic groups awaits further studies.

TCRZ (1q23)

Decreased expression of TCR ζ chain in studies of the TCR/CD3-mediated signaling was observed in the majority of SLE patients, and this defect is independent of disease activity, medication, or clinical manifestations (257). Each of the TCR ζ chain or the CD3-γ, -δ, -ε chain contains one or more ITAM. They associate non-covalently with the antigen-binding TCR α/ß (or γ/δ) chains to mediate signal transduction in T lymphocytes. A critical early event in the signal transduction pathway of TCR activation is phosphorylation of ITAM and its subsequent dephosphorylation. Given the known T cell defects in SLE patients and the pivotal role of ζ chain in T cell activation, several studies focused on this gene as a candidate susceptibility gene for SLE. The absence of exon 7 (encoding the GTP/GDP binding site proximal to the third ITAM) of TCR ζ mRNA was observed in two Japanese SLE patients (258). Subsequently, frequent mutations in the cloned PCR products of TCR ζ mRNA were demonstrated in 6/8 Japanese SLE patients, but not in two systemic sclerosis patients and two normal controls (79). However, no unique mutations in TCR ζ mRNA were found in a study using direct sequencing of PCR products derived from Caucasian, African American, Hispanic, Chinese, or Japanese SLE patients living in North America (259). Instead, novel single-nucleotide polymorphisms of the ζ chain gene were shared similarly among normal and SLE patients (259). These investigators suggest that polymorphisms of TCR ζ chain gene are unlikely to play an important role in genetic susceptibility to SLE. It appears that the discrepancy in mutations of TCR ζ mRNA of the reported SLE patients may be attributed to how mutations are detected. Mutations in the cloned PCR products but not in PCR products from the total amplified ζ mRNA suggest that these mutations are present somatically in a small proportion of mRNA without corresponding alterations in genomic DNA. This interpretation is consistent with recent findings that peripheral B and T cells derived from SLE patients contain abnormal mRNA transcripts (260 ,261).

(3) SLE-Associated Genes at 1q41–42

PARP (1q41-42)

Poly (ADP-ribose) polymerase (PARP) is a zinc-finger DNA-binding protein that is involved in DNA repair and apoptosis (262). The potential involvement of PARP in SLE was previously suggested by its subnormal levels of activity and of mRNA in SLE patients, and by its intermediate levels in unaffected relatives of SLE patients (263 ,264 ,265). Autoantibodies that bind to the two zinc-finger motifs of PARP are frequently found in patients with autoimmune rheumatic and bowel diseases (266). These autoantibodies do not significantly affect PARP enzyme activity, but efficiently prevent caspase-3-mediated PARP cleavage during apoptosis and prolong cell survival, which can cause failure to eliminate autoimmune cells and sustain autoimmune stimulation (267). Recently, genetic polymorphisms of the PARP gene were tested for association with SLE because the gene is located within the genomic interval linked to human SLE in several independent studies (38 ,39 ,45 ,47 ,48).

Tsao et al. originally linked a 15-cM region on chromosome 1q41-q42 with susceptibility to SLE, and subsequently narrowed this region approximately 5-cM (47). Three positional candidate genes within the narrowed region were tested for an association with SLE using the transmission-disequilibrium test (TDT) (28). The TDT tests whether alleles of the test candidate gene are preferentially transmitted from heterozygous parents to the oldest affected offspring in each nuclear family, thus can avoid the potential spurious association caused by the population admixture in the case-control study. Of the three tested candidate genes, only PARP alleles showed an overall skewed transmission of to affected offspring using a multiethnic cohort containing124 families (47). A particular PARP allele (85 bp allele) appeared to be preferentially transmitted to affected offspring but not to unaffected offspring. Tsao et al. concluded that PARP might be the susceptibility gene within the chromosome 1q41-q42 region, or might be close to it (47).

Subsequently, case-control studies in French and German Caucasians found no significant association of the 85 bp PARP allele with SLE (268 ,269). Additionally, no association of the 85 bp PARP allele with SLE susceptibility was observed using the TDT analysis of three multi-ethnic cohorts comprised of 187 sibpair families, 126 multiplex families, and 119 simplex families (270). A case-control study of PARP alleles in African Americans found a significant difference between the PARP allele frequencies in SLE patients and controls, and a significant deviation from Hardy-Weinberg equilibrium (HWE) in SLE genotypes, but not in controls (271). Departure from HWE at a marker locus can provide evidence for linkage disequilibrium between the marker and susceptibility locus, and for heterogeneity of disease susceptibility (272 ,273). Considering this growing body of studies, it appears that the studied PARP polymorphism is not a risk factor for SLE (274), but may be in linkage disequilibrium with the SLE susceptibility locus within the 1q41-q42 region (271). An additional positional candidate gene, HRES-1, has been recently suggested (275). HRES-1 is an endogenous retrovirus related to human T lymphotropic virus. Many SLE patients have autoantibodies to the HRES-1 encoded nuclear protein (276). Further studies will help identify the gene variant that increases risk for SLE within the 1q41-q42 region linked to lupus susceptibility.

TLR5 (1q41–42)

The critical role of toll-like receptors (TLRs) in activating innate and adaptive immune response by differentially recognizing microbial or nonmicrobial components raises the possibility of their potential role in the development of autoimmune disease (277 ,278). One member of TLRs, TLR5, maps to chromosome 1q41, a chromosomal region linked to SLE susceptibility (36 ,45 ,49) and syntenic to murine lupus susceptibility region Sle1d (71), encodes a protein acts as an innate immune receptor for bacterial flagellin (279). A C to T alteration of the C1174T SNP changes the encoded position 392 amino acid from arginine to a stop codon (R392X), which results in truncating the extracellular domain and eliminating the transmembrane domain and cytoplasmic tail (280). The ligand-binding domain of TLR5 bearing the minor T allele of this SNP (which encodes the stop codon) is unable to mediate flagellin signalling (280). Using the family-based transmission disequilibrium test, the allele 1174C (but not the stop codon encoding 1174T allele and the other 3 TLR5 SNP alleles) was preferentially transmitted to 199 Caucasian SLE patients from their 326 parents (281). In contrast, the allele 1174C was not preferentially transmitted to their 75 unaffected siblings. The decreased frequency of the stop codon allele in SLE patients (3%) in comparison to unaffected individuals (6%) is consistent with a protective association. Subjects with the stop codon produced less pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) compared to those with the wild-type genotype, suggesting the stop codon SNP allele may contribute to protection from the development of SLE (281). These observations support a novel role for flagellated bacteria and the innate immune response in the pathogenesis of SLE.

(4) SLE-Associated Genes at 2q37

PDCD1

PDCD1 has recently been associated with SLE (14). PDCD1 is a member of the CD28/CTLA4/ICOS costimulatory receptor family, which delivers inhibitory signals that have profound effects on T- and B cell immunity (reviewed in (282)). Mice deficient in PDCD1 develop glomerulonephritis and arthritis in the C57BL/6 background (283), and autoimmune dilated cardiomyopathy in the BALB/c background (284). A large study of approximately 2,500 individuals has shown association between an intronic SNP (PD1.3A; i.e., the minor A allele of 7146 G/A) in PDCD1 and SLE susceptibility in Europeans and Mexicans (14). This association between the PD-1.3A allele with susceptibility to SLE has been confirmed in another study comparing European controls to 224 SLE patients recruited in California of European American descent (285), but not in many subsequent case-control, population-based studies of Northern Sweden (286), Denmark (287), Spain (288), Eastern United States (289), and China (12). The observed contradictive results may be attributed to, at least in part, variations of the allele frequency and the haplotype structure of PDCD1 among populations. This SLE-associated SNP affects a binding site of a transcription factor RUNX1 in an intronic enhancer, suggesting a potential functional mechanism predisposing to SLE (14). Considering currently available data, the minor A allele of 7146 G/A of PDCD1 may contribute to susceptibility to SLE and/or lupus nephritis in a few specific populations, but appears not to be a strong predisposing polymorphism for SLE (and/or lupus nephritis) in most studied populations.

(5) SLE-Associated Genes at 16q12

The 16q12 region is linked to SLE, but also to other autoimmune diseases (290 ,291 ,292 ,293), suggesting the possibility of a shared susceptibility gene interacting with other susceptibility loci to manifest multiple autoimmune disorders. In SLE, evidence for genetic interaction of the 16q12 locus and other susceptibility loci has been reported by two groups (52 ,294). The identification of three SNPs of NOD2/CARD15 as independent risk factor for Crohn disease has made this gene an excellent positional candidate gene for SLE. However, case-control studies in Spanish and Chinese populations have shown no association between NOD2/CARD15 and SLE (295 ,296). Fine mapping of 16q12 has supported the presence of SLE susceptibility gene(s) within this genomic interval (54), and led to the identification of a novel gene OAZ (OLF1/EBF-associated zinc finger protein) associated with SLE and/or lupus nephritis in Chinese (297 ,298). Whether OAZ plays a role in other ethnic groups is not known at present. Further studies are in progress to identify the causal gene variants and to delineate their roles in developing SLE.

(6) Other Linked Regions

These regions include 1q31, 4p16, and 12q24, and the genomic intervals identified by disease stratification listed. Thus far, no candidate susceptibility genes within these genomic intervals have been associated with SLE.

(7) Other Candidate Susceptibility Genes Associated with SLE (Table 6-5)

These genes may not be located within genomic segments exhibiting strong linkage to SLE, but their gene polymorphisms have been associated with SLE in multiple cohorts.

C1q (Mapped at 1p36)

Three genes (a, b, and c genes) encode the first component of the complement system. Homozygous deficiency in any of the three C1q genes is almost invariably associated with SLE (Morgan & Walport, 1991). This is a powerful disease susceptibility gene since >90% identified individuals with this deficiency developed SLE. However, homologous C1q deficiency is extremely rare in the population (approximately 40 cases in the world reported thus far). Despite various ethnic

and genetic backgrounds, these patients develop disease at a young age without a female predominance. They share a nearly invariant feature of severe photosensitive skin rash, often with high-titers of anti-Ro autoantibodies (188).

Table 6-5: Genes within the Associated with SLE

Names	Gene Symbols	Chromosomal Locations	Locus Name
Genes within SLE linked regions			
C-reactive protein	CRP	1q23	
IgG Fc receptor IIa (CD32)	FCGR2A	1q23	
IgG Fc receptor IIb	FCGR2B	1q23	
IgG Fc receptor IIIa	FCGR3A	1q23	
Fc receptor-like 3	FCRL3	1q21.2–22	
Poly(ADP-ribose) polymerase	PARP	1q42	SLEB1
Toll-like receptor 5	TLR5	1q41–42	SLEB1
Program cell death 1	PDCD1	2q37	SLEB2
MHC class II genes	DRB1	6p21	
MHC class III genes	C2, C4, TNF	6p21	
Caspase recruitment domain-containing protein 15	NOD2/CARD15	16q12	
OLF1/EBF-associated zinc finger protein	OAZ	16q12	
Genes not within SLE linked regions			
Complement component C1q	C1q	1p36	
Tumor necrosis receptor 2	TNFR2	1p36	
Interleukin –10	IL10	1q32	
Complement receptor 1	CR1	1q32	
Interleukin –6	IL6	7p21-p15	
Mannose binding lectin	MBL	10q11	
Monocyte chemoattractant protein 1	MCP1	17q11.2–12	
Interleukin-4 receptor	IL4R	16p11–12	
The MHC class II transactivator	MHCIITA	16p13	
Interferon α receptor 1	IFNAR1	21q22.11	
Interferon α receptor 2	IFNAR2	21q22.11	
T cell receptor γ locus	TCRG	7p15–14	
T cell receptor ß locus	TCRB	7q35	
T cell receptor α locus	TCRA	14q11.2	
T cell receptor δ locus	TCRD	14q11.2	
Ig heavy chain locus	IGH	14q32–33	
Autoimmunity genes			
Protein tyrosine phosphatase 22	PTPN22	1p13	
Program cell death 1	PDCD1	2q37	
Cytotoxic T lymphocyte antigen 4	CTLA4	2q33	
Type 1 IFN pathway genes			
Tyrosine kinase 2	TYK2	19p13.2	
IFN regulatory factor 5	IRF5	7q32	

To understand how the absence of C1q can predispose to SLE, C1q-deficient mice were established. A significant portion developed glomerulonephritis with immune deposits in certain genetic backgrounds but not in others. These data are consistent with genetic interactions, suggesting that either a single susceptibility gene (C1q) is necessary but not sufficient for expression of murine SLE, or genetic modifiers are present in resistant strains to suppress phenotypes mediated by C1q. Interestingly, significantly higher numbers of glomerular apoptotic bodies were observed in C1q deficient mice independent of their disease status, but not in wild type mice. These in vivo findings are consistent with the previous in vitro data showing direct binding of C1q to apoptotic blebs on UV-irradiated keratinocytes in the absence of antibody. The investigators postulate that C1q plays a role in the maintenance of immunological tolerance by clearing apoptotic cells, thus preventing the autoimmune response to autoantigen–containing apoptotic blebs.

TNFR2 (1p36)

The p75 tumor necrosis factor receptor (TNFR2), the larger of the two membrane receptors that bind to either TNF-α or TNF-ß, mediates effector functions in different cell types. TNFR2 may be particularly important in T cells for being the major TNF receptor on circulating T cells and the

major known mediator for autoregulatory apoptosis of CD8+ T cells (299). Since TNF-α is a strong candidate gene for SLE susceptibility, molecules participating in the TNF-mediated pathway are also likely to influence the pathogenesis of SLE.

TNFR2 has been considered as a candidate gene within the murine susceptibility loci (the NZB-derived Nba-1, Imh-1, and Mott-1 loci) for SLE (300 ,301 ,302). The autoimmune phenotypes linked to these loci include fatal glomerulonephrits, IgM hypergammaglobulinemia, and formation of aberrant plasma cells (Mott cells). TNFR2 is located on the distal end of mouse chromosome 4, a region homologous to the short arm of human chromosome 1 (1p36) where tentative linkage to human SLE has been reported in two independent genome scans (38 ,44). A SNP of TNFR2 results in arginine instead of methionine at position 196 of exon 6, which was associated with SLE in Japanese (303) but not in a Spanish or UK population (304). Whether this amino acid difference has functional significance is yet to be determined. No association between SLE and a TNFR2 intronic microsatellite was found in a recent Italian case-control study (305). Thus, current evidence for association between SLE and polymorphisms of TNFR2 is weak and inconsistent. Further studies will help clarify whether genetic variants of TNFR2 are risk factor for lupus.

Interleukin-10 (1q32)

IL-10 is an excellent candidate gene for SLE susceptibility because (1) increased IL-10 production promotes B cell hyperactivity and autoantibody production (306), (2) the frequency of IL-10 secreting cells is elevated in peripheral blood samples of sporadic cases of SLE (307), and (3) studies of SLE patients and their relatives suggest genetic influences on IL-10 production (308 ,309). Llorente et al. showed that both SLE patients and their relatives from 13 Mexican extended pedigrees had higher levels of spontaneous release of IL-10 and mRNA than normal controls (308). Grondal et al. subsequently confirmed this finding in Caucasians; both SLE patients and their relatives from an extended Icelandic pedigree had higher numbers of IL-10 secreting cells than healthy controls (309). The increased production of IL-10 in SLE patients appears to be constitutive and not affected by disease activity or treatment. Additionally, environmental factors may impact on IL-10 production; spouses of SLE patients had significantly higher number of IL-10 producing cells compared to controls.

The molecular mechanisms that cause increased IL-10 production among SLE patients have been actively explored. Levels of IL-10 secretion have been correlated with specific haplotypes containing either two microsatellite markers or three single nucleotide polymorphisms within the IL-10 promoter (310 ,311 ,312). Association between these IL10 promoter polymorphisms and SLE has been reported in case-control studies of two Caucasian (305 ,310) and one Mexican-American (313) but not in similar studies of another Caucasian (314) or one Asian (315) population(s). Association between these IL10 promoter polymorphisms and SLE has been reported in case-control studies of Caucasians (310 ,316 ,317 ,318), Mexican-American (313) and Asians (319 ,320). However, this association was not observed in other groups (314 ,315 ,321 ,322 ,323 ,324). Mehrian et al. also reported synergistic effect between IL-10 and bcl-2 genotypes in susceptibility to SLE (313). To our knowledge, this finding has not been replicated in another cohort. In the two negative association studies with SLE, there was significant association with renal disease in lupus patients (314 ,315). Subsequent identification of new SNPs and SNP haplotypes within the IL10 promoter region have led to their correlation with a high or low IL-10 production (305 ,325), in which the high IL-10 haplotypes is associated with SLE in African Americans. Recently, a meta-analysis combining 15 relevant IL-10 studies showed a significant association between the G11 allele (23 CA repeat at –1.1 kb) and SLE (OR = 1.28, 95% CI = 1.03–1.59, p = 0.028), but no association of any of the IL-10 promoter SNPs (326). However, when stratified by ethnicity, IL-10 promoter –1082 G allele was associated with SLE in Asians (OR = 1.358, 95% CI = 1.015–1.816, p = 0.039). Taken together, IL-10 polymorphisms confer modest risk for the development of SLE in multiple ethnic groups.

CR1 (1q32)

Complement receptor 1 (CR1) is the binding receptor for complement component C3b and C4b expressed on the cell membrane of erythrocytes, granulocytes, all B and some T lymphocytes, monocytes, glomerular podocytes, and follicular dendritic cells (327). Functionally, it is important in effective removal of circulating immune complexes that have fixed complements. Excessive amounts of immune complexes in SLE may be caused by dysregulated overproduction of autoantibodies or impaired clearance of immune complexes. Wilson et al. (328) quantified the C3b receptors on erythrocytes from 38 SLE patients, 14 of their spouses, and 47 relatives, as well as from 113 normal subjects and concluded that the decreased number of C3b receptors frequently observed in lupus patients was inherited in an autosomal codominant manner. Subsequently, a HindIII restriction enzyme RFLP (7.4 or 6.9 kb fragment) that correlated with the number of CR1 on erythrocytes further supported the genetic basis of decreased CR1 receptors observed in SLE patients (329) (330 ,331). However, the correlation between HindIII RFLP and SLE susceptibility was not found in 9 SLE multicase Mexican families (332) or in Indian case-control study (333). Kumar et al. observed while the numbers of CR1 remained stable in consecutive patients among controls, they varied significantly in lupus patients correlating with serum levels of C3d and circulating immune complexes, and the severity of the disease (333). Thus, low levels of CR1 expression in erythrocytes among lupus patients may be an acquired defect.

CR1 exhibits allelic size variants that has different numbers of C3b-binding sites (327 ,330 ,334 ,335 ,336). Because this

molecular weight polymorphism may affect clearance of immune complexes, several studies investigated whether the smallest allele (the CR1-C allele with the lowest C3b binding site) was associated with SLE susceptibility. An association between SLE and the C allele was suggested in one large SLE multiplex pedigree (334), but not in case-control studies of a French (337), Hispanic, African-American, or American Caucasian populations (100 ,338). Recently, a meta-analysis, combined with 13 studies, showed no significant association of CR1 functional polymorphisms (329), which determine the quantitative expression of CR1 on erythrocytes with SLE (326). In contrast, the S structural variant, which is characterized by an additional C3b binding site on a fifth long homologous repeats of CR1 (335), was associated with SLE (OR = 1.54, 95% CI = 1.22–1.96, $p < 0.001$) (326).

IL-6 (7p21-p15)

Interleukin-6 is an important B cell growth and differentiation factor. Polymorphisms of the 3′ flanking region of the IL6 have been shown to be associated with SLE in Caucasians and African-Americans, which may enhance the stability of IL-6 mRNA thus contributing to elevated levels of IL-6 in SLE patients (339 ,340 ,341). However, this polymorphism appears only weakly associated with disease, as it was only present in the minority patients (342). Another SLE-associated polymorphisms are short AT-rich minisatellite alleles that were found exclusively in SLE patients (13% among the studied Caucasian and 9% among African Americans). To our knowledge, this finding has not been replicated in independent studies.

Mannose-Binding Lectin (10q11)

Mannose-binding lectin (MBL) binds mannose on surfaces of bacteria or viruses, initiates a pathway for opsonization of pathogens, and activates complement components by MBL-associated proteases. MBL deficiency results in low levels of circulating MBL, impaired opsonization of microorganisms, and frequent infections, which occurs in 5% to 10% of the general population (343). MBL is very similar to C1q in three-dimensional structure. The well-known association of complement deficiencies and SLE has prompted genetic studies of MBL in SLE patients. Case-control studies in Caucasians, African Americans, and Southern Chinese have found polymorphisms in the promoter region and exon 1, which correlate with lower levels of MBL, are significantly more frequent in SLE patients than in controls (344 ,345 ,346). Subsequently, Garred et al. (347) reported that homozygosity of MBL variant alleles could explain the increased risk for infections in a small subset of Danish SLE patients, in addition, it could represent a minor risk factor for developing SLE. Cardiovascular disease is an important complication in SLE patients. Homozygosity for MBL exon 1 variants has been associated with an increased risk (relative risk of 5.8 to 7.0 without and with correction for other known risk factors) of arterial thrombosis, but not of venous thrombosis in 91 Danish SLE patients (348).

Monocyte Chemoattractant Protein 1 (17q11.2–12)

Monocyte chemoattractant protein-1 (MCP1), a ß-chemokine, plays role in monocyte and lymphocytes recruitment and is considered as a main chemokine responsible for initiating the glomerulonephritis in animal models (349 ,350). The functional SNP (–2518G/A) was first reported o affect the expression level of MCP-1 in response to IL-1ß stimulation in vitro (351). Subsequent studies found the G allele of this SNP was related to the higher MCP-1 protein level in PBMC from SLE patients and associated with SLE and lupus nephritis in a case-control study (352). The association of –2518G was also observed for the development of cutaneous vasculitis, but not SLE or lupus nephritis in a Spanish population (353).

MHCIITA (16p13)

The MHC class II transactivator (CIITA) plays a pivotal role in the regulation of MHCII gene expression. An absence of CIITA was associated with severe immunodeficiency resulted from the defect production of MHC class II molecules (75). The transcription of human CIITA gene is controlled by four alternative promoters, which exhibits cell-type specific activity. The promoter I (pI) and pIII are responsible for the CIITA expression in dendritic cells and B cells respectively, while pIV mediates the interferon γ induced CIITA expression (354). The analysis of four promoters showed no sequence difference between rheumatoid arthritis, type I diabetes, and normal controls (355), but the sample size of this study is rather small. Subsequently, one Caucasian (356) and one Japanese study (357) revealed only pIII of four promoters is polymorphic. The pIII SNP and four exonic SNPs were not associated with SLE in the latter Japanese cohort (357). In contrast, an intronic SNP (485 A->A/G), not in LD with previous 5 SNPs (r2 = 0.0074–0.071), was associated with SLE in this group (357). Further studies are needed to test the CIITA polymorphisms in multi-ethnic groups with larger sample size for evidence of association with SLE.

Interleukin-4 Receptor (IL4R, 16p11–12) and Interferon-α Receptors (IFNAR, 21q22)

Cytokine profiles of SLE patients have been a subject of great interest. Because IL-4 and IL-4 receptor (IL-4R)-mediated signaling pathway is important for the commitment of the Th2 phenotype, functional polymorphisms of these two genes may predispose susceptibility to autoimmune diseases. A recent study of 50 Japanese SLE patients and 100 Japanese controls showed significant association of SLE with the IL-4R α chain gene polymorphisms (codons 50 and 551) but not with the IL-4 gene promoter polymorphism (358). One of an important cytokine that Th1 cell type

produces is IFN-α. Genetic association between SLE susceptibility and variants of IFN-α receptor 1 and 2 genes were recently reported in Japanese population (359 ,360). These findings await confirmation in other populations.

T Cell Receptor Genes

The most common T cell receptor (TCR) is the αß TCR present on the majority of T cells. Other T cells bear the γδ TCRs. The TCR α and δ chain genes reside on human chromosome 14, while the TCR ß and γ chain genes map to human chromosome 7. Within a TCR gene locus, the DNA rearrangement process brings together one variable gene segment, (one diversity gene segment), one joining gene segment, and one constant gene segment to encode gene products. A diverse TCR repertoire can present various processed peptides to MHC class I/II molecules to mount immune responses. A number of studies examining TCR RFLP in SLE patients have yielded weak and inconsistent results. Tebib et al. reported an association between the constant region of the TCR α chain and SLE in American Caucasians but not in Mexicans (361). Huang et al. did not support this observation in a study of North American Caucasians (14 SLE multiplex families as well as 41 cases and 88 controls) (362). Dunckley et al. found no association between TCR (α,ß, and γ) RFLP and SLE or MHC class II molecules (363). Frank et al. reported an association of TCR ß RFLP with anti-Ro(SSA) antibodies but not with SLE (364). No linkage of TCR α, ß, and γ chain genes to SLE was observed in 5 multiplex Caucasian families (365). The family studies had fairly small sample sizes and might lack power to detect linkage. Data available thus far fail to support the contribution of TCR gene variants to risk for SLE.

Immunoglobulin Heavy and Light Chain Genes

Earlier association studies of the immunoglobulin heavy chain locus (map to chromosome 14) and gene products (Gm allotypes) as well as the κ light chain gene (chromosome 2) and gene products (Km allotypes) yielded conflicting results. An association of Gm phenotype to SLE was observed in American and Australian Caucasians (366 ,367), Japanese (368), African Americans (369), but not in Hungarians or central Europeans (370 ,371). An association between Km phenotype and SLE was reported in North American Caucasians (372) but not in central Europeans (371). Kumar et al. found a significant association of a Ig heavy chain constant region polymorphism with SLE in unrelated Mexicans and Mexican SLE multiplex families but not in SLE patients from the United States (373). No association between the λ light chain polymorphism and SLE was observed by Blasini et al. (374). Since the Gm/Km allotypes or Ig RFLP can only detect limited polymorphism present within the vast variation of immunoglobulin gene loci, these studies do not rule out the possibility that immunoglobulin gene variants can confer genetic risk for SLE.

The expression of a particular V gene and receptor editing may regulate the production of autoantibodies (375). Olee et al. reported the deletion of the Humhv3005 gene (likely to encode heavy chains of rheumatoid factors) in 25% of the studied rheumatoid arthritis and SLE patients and 2% of the normal controls (376). A subsequent study from the same group concluded the deletion of this V gene contributed weak genetic risk for SLE (362).

Autoimmunity Genes (Table 6-5)

PDCD1: The minor allele of the intronic 7146G/A SNP of the PDCD1 gene has recently been associated with type 1 diabetes (13). Other SNPs and or SNP haplotypes of the PDCD1 gene have been implicated in nephropathy among SLE patients (287) and in susceptibility to rheumatoid arthritis (12 ,377). It appears that PDCD-1 polymorphisms may be a shared genetic factor for multiple autoimmune diseases in humans, similarly to observations in knockout mice. However, the specific disease-associated genetic polymorphism, and the resulting functional consequences leading to the occurrence of the disease have not been well characterized as yet. Of interest, two recent publications describe that individual SNPs affecting a transcription factor binding site (RUNX1) are associated with RA (378) and psoriasis (379), highlighting the potential importance of polymorphisms involved in regulation of gene expression in susceptibility to autoimmune diseases (380). These novel findings await validation by independent confirmation and further functional studies.

CTLA-4 (mapped at 2q33): The cytotoxic T lymphocyte antigen 4 gene (CTLA4), which encodes a vital negative regulatory molecule of the immune system, is a logical candidate gene for multiple autoimmune disorders. A systematic survey of 109 polymorphisms of a 2q33 interval containing the T-lymphocyte regulatory genes CD28, CTLA4, and inducible costimulator (ICOS) revealed polymorphisms within a noncoding 6.1kb 3′ region of CTLA4, which correlated with lower messenger RNA levels of the soluble alternative spliced CTLA4. These polymorphisms are associated with Graves disease, autoimmune hypothyroidism and type 1 diabetes (8). Genetic studies investigating polymorphisms within the CTLA4 promoter (-1722T/C, -1661A/G, -319C/T) and exon1 (+49G/A) in Korean, Spanish, European American, and African-American population studies yielded negative or inconsistent evidence for association with SLE (381 ,382 ,383 ,384). A SNP (CT60A/G) within the 3′UTR of CTLA4, one of the polymorphisms within the 6.1 kb interval associated with multiple autoimmune diseases (8), has recently been associated with SLE in a study of 396 Spanish SLE patients and 293 controls (384).

PTPN22 (1p13): Protein tyrosine phosphatases (PTPs) have been recognized as negative regulators for stimulatory signaling pathways, which are critical in maintaining homeostasis of cellular immunity. The PTPN22 gene is expressed primarily in cells of hematopoietic lineage. The minor T allele of the C1858T polymorphism, which

causes the R620W amino acid substitution and disrupts an important motif of PTPN22 for interaction with a negative regulatory kinase Csk (385), has recently been associated with multiple autoimmune diseases in Caucasians (15), including type 1 diabetes (T1D) (385 ,386 ,387 ,388), autoimmune thyroid disease (388 ,389), rheumatoid arthritis (RA) (390 ,391), and SLE (391 ,392), but not with multiple sclerosis (MS) (393). Supporting these findings, the PTPN22 missense SNP has been associated with four autoimmune diseases (T1D, autoimmune thyroid disease, RA, and SLE) in 746 affected individuals from a collection of 265 Caucasian families multiplex for at least 2 of the 9 autoimmune diseases (T1D, RA, SLE, MS, autoimmune thyroid disease, juvenile RA, inflammatory bowel disease, psoriasis, and primary Sjögren syndrome) (394). In this study, the absence of any evidence for association of this SNP with MS is strong, but the other four autoimmune diseases have very small sample sizes to draw definitive conclusions (394). Taken together, these recent findings support the hypothesis that genes shared in non-MHC regions contributes to multiple autoimmune diseases despite their diverse, and unrelated phenotypes (7). A note of caution is that all these studies use the population-based case-control design in which several studies have used the same control samples. A family-based TDT design containing >600 Caucasian trios failed to confirm the association between the PTPN22 missense SNP and SLE, and the 1858T allele frequency in cases from these trios was not different from the reported allele frequency of controls, but was increased in SLE patients with autoimmune thyroid disease (395). Most recently, a RA study (396) has reported other PTPN22 SNPs, independent of C1858T, may contribute to the development of RA. Considering current available data, genetic polymorphisms within the PTPN22 gene clearly confer risks for several autoimmune diseases, and the genetic contribution is more evident in individuals with multiple autoimmune diseases.

Type 1 IFN pathway genes associated with SLE (Table 6-5): Several groups have independently shown IFN-inducible gene expression patterns using peripheral blood samples from SLE patients (397 ,398 ,399). The type I IFN system has been proposed as having a pivotal role in the development and maintenance of SLE clinical symptoms (400). A recent study has shown the polymorphisms in the genes participating in type 1 IFN pathway (tyrosine kinase 2 and interferon regulatory factor 5 genes) are associated with SLE (401). Further studies are needed to clarify the importance of type 1 IFN in SLE.

Summary

There has been tremendous progress in genetic studies of SLE in recent years. Hundreds of families containing multiple members affected with SLE have participated in genetic studies. Linkage analyses of these families have suggested several chromosomal regions that are likely to contain SLE susceptibility genes. Within the linked regions, promising candidate genes have also been associated with SLE. The continued advances in the identification of haplotype-tagged SNPs and block boundaries through out the human genome and the high throughput SNP genotyping platform will greatly facilitate the identification of disease-associated polymorphisms. We anticipate that the identification of specific SLE susceptibility genes will further our understanding of the pathogenesis of this disease, and will facilitate the development of more targeted therapies in the near future.

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Chapter 7

The Genetics of Murine Systemic Lupus Erythematosus

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Although the etiology of systemic lupus erythematosus (SLE) is multifactorial, genetic susceptibility appears to be an important and possibly essential component. Elucidation of the processes leading to disease induction and maintenance will likely require knowledge of the key genetic alterations and their contributions. Deciphering the genetics of lupus, however, has proven to be formidable, largely because of the size of the human genome and the complexity of polygenic inheritance in heterogeneous human populations. Studies of inbred strains of mice that, spontaneously or by induction, develop SLE-like systemic autoimmunity have provided an excellent opportunity to examine genetic susceptibility and genes vital to the development of lupus in well-defined model systems. Mouse models are particularly useful in dissecting polygenic traits because of homogeneous genetic backgrounds, standardized environmental conditions, and the ability to obtain sufficient numbers for genetic mapping and for accurate phenotype determination. Furthermore, the mouse genome is well characterized and its gene composition is sufficiently similar to the human genome to allow comparative analysis. Mice are also the premier mammalian species for genetic manipulation and, importantly, their immune system is nearly identical to humans, making it possible to study in considerable detail the immunologic alterations associated with different genetic backgrounds. This chapter will cover the mouse lupus susceptibility loci, the candidate and known susceptibility genes for spontaneous or induced models of SLE, and other potential lupus-predisposing genes identified by genetic manipulation.

Spontaneous and Induced Mouse Models of SLE

Mouse models of systemic autoimmunity used in genetic studies include monogenic, polygenic, spontaneous, and induced diseases (Table 7-1). Descriptions of the major strains can be found in Chapter 18 . Most genetic studies have focused on the spontaneous models, particularly the NZB, NZW, MRL-*Fas*^{lpr}, and BXSB strains. autoimmunity in these mice is polygenically inherited, although both the MRL-*Fas*^{lpr} and BXSB strains have single genetic alterations (*Fas*^{lpr} and *Yaa*, respectively) that exhibit Mendelian transmission of lupus traits. A number of recombinant inbred lines, derived from crosses of lupus-prone and nonautoimmune strains (RI lines in Table 7-1), have also been generated. These lines develop a spectrum of phenotypes indicating polygenic inheritance of traits, however, the relatively small number of substrains have precluded more detailed analysis. RI lines, derived from the NZB and NZW strains (NZM/Aeg2410 and NZM/Aeg2328), have been useful for studying recessive susceptibility genes (see below). Two long-lived (-*II*) sublines derived from the BXSB and MRL-*Fas*^{lpr} strains have also been described (1 ,2). Both have less severe disease than the original parental strain from which they spontaneously arose, and may be caused by single mutations. Studies of the MRL-*Fas*^{lpr}-*II* mice revealed decreased interferon-γ (IFN-γ), as well as IgG2a and IgG3 subclasses, suggesting that a reduction in pathogenic Th1-type responses may play a role in disease resistance (3). Recently, the BXD2 RI line derived from the B6 and DBA/2 strains was discovered to develop lupus-like manifestations as well as erosive inflammatory arthritis (4). This suggests that significant autoimmune predisposing alleles are present in the two parental nonautoimmune strains, and perhaps other nonautoimmune strains as well, that can predispose to autoimmunity when the optimal combinations occur, although spontaneous mutation(s) cannot be excluded.

Genetic predisposition is also important for the induced mouse models, particularly mercury-induced autoimmunity (HgIA). Exposure of susceptible strains to mercury results in a constellation of immunopathologic manifestations including lymphoproliferation, hypergammaglobulinemia, autoantibodies, and immune complexes (5 ,6). Although most strains develop lymphoid hyperplasia and elevated immunoglobulin G (IgG) levels, autoantibody and immune complex deposits are dependent on H-2 haplotype and other background genes (7 ,8). Notably, the antinucleolar antibody response, which is mainly directed against fibrillarin (9) (a specificity also observed in scleroderma (10)), requires the I-A^{s} haplotype (8). Among the various backgrounds examined, the DBA/2 strain is the most resistant (7), and strains susceptible to

spontaneous lupus appear to be more sensitive to mercury exposure (11).

Table 7-1: Spontaneous and Induced Mouse Models of Lupus

- Spontaneous disease models
  - NZ and related strains
    - NZB
    - NZW
    - (NZB × NZW)F1
    - (NZB × SWR)F1
    - (NZB × NZW) recombinant inbred (RI) lines
    - "NZM/Aeg" lines (407)
    - (NZB × SM)RI lines "(NXSM)RI"
    - (NZB × C58)RI lines "(NX8)RI"
  - MRL (*Fas*^{lpr} and wild-type) and related strains
    - MRL-*Fas*^{lpr}*.II* (long-lived substrain) (2)
    - MRL-*Fas*^{lpr}, Yaa (209)
    - SCG/Kj-*Fas*^{lpr} (BXSB × MRL-*lpr*)RI (408)
  - BXSB and related strains
    - BXSB-*II* (long-lived substrain) (1)
    - (NZW × BXSB)F1
    - (NZB × BXSB)F1
  - (SJL × SWR)F1 (409)
  - (DBA/2 × C57BL/6) RI line 2 "BXD2" (4)
  - Palmerston North (410)
  - Motheaten strains (195,196,197)
- Induced disease models
  - Heavy metal–Induced autoimmunity (5)
  - Drug-induced lupus (411)
  - Pristane-Induced (412)
  - Anti-idiotypic (413)
- Graft-versus-host disease
- BCG-injected NOD (13,14)
  - 1–3-galactose-deficient mice (12)

Two induced models of lupus-like disease have been added since the previous edition (Table 7-1). Nonautoimmune prone galactose-α1–3-galactose-deficient mice exposed topically to commercial bovine thrombin preparations commonly used in human surgical procedures developed antibodies to the xenogeneic galactose-α1–3-galactose and, in a few cases, autoantibodies to clotting factors. Unexpectedly, they also produced anti-double-stranded DNA (dsDNA) and anticardiolipin autoantibodies, and developed immune complex-mediated GN with a higher frequency of disease in females (12). The other model, type I diabetes mellitus-susceptible NOD mice, were given intravenous *Mycobacterium bovis* (bacillus Calmette-Guérin), which prevented diabetes but, surprisingly, induced systemic autoimmunity manifested by accelerated hemolytic anemia, antinuclear antibodies, exacerbation of sialadenitis, and immune complex-mediated glomerulonephritis (GN) (13). Although it was initially hypothesized that susceptibility to two different types of autoimmune diseases might be because of common autoimmune–predisposing genes, later mapping studies revealed no colocalization of loci predisposing to lupus or diabetes other than the MHC region (14). These models provide striking examples of the complex interactions between genetic susceptibility and environmental factors that influence predisposition to SLE and other autoimmune diseases, and demonstrate the utility of mouse models to help address this issue.

In addition to the above models, gene knockout/knockin, transgenic, or mutagenic manipulation of non-autoimmune background strains has generated novel autoimmune mouse models with manifestations similar to spontaneous SLE. Finally, interval-specific congenic strains with introgressed genomic regions encompassing susceptibility loci have also provided another important resource for genetic studies.

Lupus Susceptibility Loci and Genes

The forward genetics approach to identify genes predisposing to quantitative traits generally entails four main steps. First, mapping of traits is performed by genome-wide scans using evenly distributed markers spanning the chromosomes. Next, interval-specific congenic strains, each containing an introgressed genomic fragment encompassing a specific locus, are generated to confirm the mapping studies and more clearly define the effects of individual loci on normal immune responses and autoimmunity. Third, more precise mapping is performed by generating and screening panels of congenics with crossovers or smaller intervals, which may be accomplished in one or two stages, i.e., localization first to approximately 5 cM sized fragments and then to <1 cM sized fragments (15). The final step entails screening candidate genes within the narrowed intervals (typically 0.5 to 1.0 cM). Selection of candidates is based on function, expression profile, or other characteristics suggested by the phenotype of the interval congenics. The recently assembled annotated mouse genome sequence has greatly facilitated this process.

Genome-wide scans involving a variety of crosses have revealed multiple quantitative and binary trait loci. At least 75 named loci, distributed over all 19 autosomal chromosomes and linked to one or more lupus traits, have been reported and are listed by chromosome and chromosomal location (distance in cM and Mb from the centromere) in Table 7-2 . Lupus susceptibility loci were not only identified in lupus-prone strains, but in many nonautoimmune backgrounds as well, including the 129, B6, BALB/c, C57L, DBA/2, NOD, and SWR strains. The large number of loci may be somewhat overestimated since suggestive linkages are included and since some loci, identified by different groups, overlap and may be identical. Conversely, several loci (*Sle1*, *Nba2*, *Sle2*), upon more precise mapping with subinterval congenic lines, were found to be composed of multiple subloci (16 ,17 ,18), suggesting underestimation of the number of loci. Based on the mapping studies, there is considerable

genetic heterogeneity even among inbred strains with susceptibility to spontaneous lupus not because of a few common predisposing loci, but rather to a large pool of loci, with individual strains containing different sets of common and unique predisposing variants.

Table 7-2: Loci Predisposing to Lupus-Related Traits in Spontaneous Disease Models

Name	Chr	cM/Mb	Marker	Cross	Major Associations	Parental Allele	Ref.
Bxs4	1	7.7/20.0	*D1Mit3*	B10 × (B10 × BXSB)F1	LN	BXSB	(67)
Bxs1	1	32.8/64.1	*D1Mit5*	BXSB × (B10 × BXSB)F1	GN/ANA/spleen	BXSB	(66)
Bxs2	1	63.1/120.2	*D1Mit12*	BXSB × (B10 × BXSB)F1	GN/ANA/spleen	BXSB	(66)
Bana3	1	60.9/153.0	*D1Mit396*	(NOD × Ba) × NOD BC	ANA (*M. bovis*)	NOD	(14)
Swrl1	1	87.9/168.3	*D1Mit15*	B × (SWR×B)F1	dsDNA/histone	SWR	(414)
Sle1	1	87.9/168.3	*D1Mit15*	(NZM × B6) × NZM (NZM × B6)F2	GN dsDNA/GN/spleen	NZM (NZW)	(101) (415)
Hmr1	1	87.9/168.3	*D1Mit15*	(SJL × DBA/2)F2	glom. dep. (HgIA resistance)	DBA/2	(73)
		92.3/169.1	*D1Mit36*	(B × DBA/2)F2	glom. dep. (HgIA resistance)	DBA/2	(73)
Cgnz1	1	92.3/169.1	*D1Mit36*	(NZM2328 × C57L)F1×NZM2328	chronic GN	NZM2328 (NZW)	(416)
Lbw7	1	92.3/169.1	*D1Mit36*	BWF2	chr/spleen	NZB	(20)
Nba2	1	92.3/168.9	*D1Mit111*	(B × SM) × W	GN	NZB	(102)
		92.3/ND	*D1Mit148*	(B × SM) × W/(B6.H2^{z} × B) × B	ANA/gp70/GN		(417)
		94.2/172.6	*Crp/Sap*	((B6.H2^{z} & Ba.H2^{z}) × B)F1 × B	GN		(418)
Bxs3	1	100.0/175.6	*D1Mit403*	BXSB×(B10×BXSB)F1	dsDNA	BXSB	(66)
Agnz1	1	101.0/181.8	*D1Mit37*	(NZM2328×C57L)F1×NZM2328	acute GN	NZM2328 (NZW)	(416)
Wbw1	2	86.0/152.3	*D2Mit285*	(W×PL)F1 × B	mortality/GN	NZW	(419)
Sles2	3	35.2/78.6	*D3Mit137*	(B6.NZMc1 × NZW)F1×NZW	dsDNA/GN (resistance)	NZW	(19)
Bxs5	3	39.7/87.0	*D3Mit40*	B10 × (B10 × BXSB)F1	ANA/IgG3	BXSB	(67)
Lprm2	3	64.1/130.9	*D3Mit14*	(MRL-*lpr* × C3H-*lpr*)BC & F2	vasculitis (resistance)	MRL	(58, 61)
Arvm1	4	19.8/46.2	*D4Mit89*	(MRL-*lpr* × C3H-*lpr*)BC & F2	vasculitis	MRL	(61)

Lprm1	4	32.5/63.6	*D4Mit82*	MRL-*lpr* × (MRL-*lpr* × C3H-*lpr*)F1	vasculitis	MRL	(58)
Acla2	4	40.0/82.0	*D4Mit79*	NZW × (NZW × BXSB)F1	CL	BXSB	(68)
Sle2	4	44.5/94.0	*D4Mit9*	(NZM × B6) × NZM	GN	NZM	(101)
Spm1	4	45.9/98.3	*D4Mit58*	(B6 × NZB)F1 × NZB	spleen	NZB	(420)
Adaz1	4	49.6/ND	*D4Mit36*	(NZM2328 × C57L)F1 × NZM2328	dsDNA	NZM2328	(416)
Lbw2	4	55.6/123.8	*D4Nds2*	BWF2	mortality/GN/spleen	NZB	(20)
Sles2	4	57.6/123.4	*D4Mit12*	(B6.NZMc1 × NZW)F1 × NZW	dsDNA/GN (resistance)	NZW	(19)
Arvm2	4	57.6/123.9	*D4Mit147*	(MRL-*lpr*×C3H-*lpr*)BC & F2	vasculitis	MRL	(61)
Asm2	4	65.0/110.8	*D4Mit199*	MRL-*lpr*×(MRL-*lpr*×C3H-lpr)F1	sialadenitis	MRL female	(60)
nba1	4	65.7/130.7	*Epb4.1*	BWF1 × W	GN	NZB	(421)
Lmb1	4	69.8/123.4	*D4Mit12*	(B6-lp-r×MRL-lpr)F2	Lprn/dsDNA	B6	(57)
Imh1/Mott	4	69/ND 69.8/140.0	*D4Mit66* D4Mit48	BWF1 × W	hyper IgM/GN/dsDNA	NZB	(422, 423)
Aia1	4	75/ND	—	NZB × NZC	RBC	NZB	(424)
Sle6	5	20.0/ND	*D5Mit4*	(B6.NZMc1 × NZW)F1 × NZW	GN	NZW	(19)
Lmb2	5	41.0/72.0	*D5Mit356*	(B6-*lpr* × MRL-*lpr*)F2	Lprn/dsDNA	MRL	(57)
Lprm4	5	54.0/ND	*D5Mit23*	MRL-lpr × (MRL-*lprx*C3H-lpr)F1	spleen	MRL	(58)
Lbw3	5	84.0/140.7	*D5Mit101*	BWF2	mortality	NZW	(20)
Lbw4	6	64.0/ND	*D6Mit25*	BWF2	mortality	NZB	(20)
Sle5	7	0.5/ND	*D7Mit178*	(NZM × B6)F2	dsDNA	NZM(NZW)	(415)
Lrdm1	7	6.0/20.3	*Pou2f2*	(MRL-*lpr* × CAST)F1 × MRL-*lpr*	GN	MRL	(59)
Sle3	7	16.0/32.0	*D7Mit25*	(NZM × B6)F2	GN	NZM(NZW)	(415)
Lbw5	7	23.0/38.3	*D7Nds5*	BWF2	mortality	NZW	(20)
Nba5	7	23.0/38.3	*7Nds5*	B6 × (B × B6.*Yaa*)F1	gp70IC	NZB	(54)
Lmb3	7	27.0/51.7	*D7Mit211*	(B6-*lpr* × MRL-*lpr*)F2	Lprn/dsDNA	MRL	(57)
Sle3	7	28.0/50.5	*p*	(NZM × B6)×NZM	GN	NZM (NZW)	(101)
Aem2	7	28.4/75.3	*D7Mit30*	(B6 × NZB)F1×NZB	RBC	NZB	(420)
Myo1	7	69.0/136.8	*D7Mit14*	NZW×(NZW×BXSB)F1	MI	BXSB	(68)
Pbat2	8	11/28.4	*D8Mit96*	NZW×(NZW×BXSB)F1	platelet	BXSB	(68)
sbb1	9	17.0/37.0	*D9Mit67*	(B6 × Ba)F2-Fcγ RIIb-/-	spleen (Fc γg RIIb ko)	BALB/c	(75)
baa1	9	28.0/49.5	*D9Mit22*	(W×Ba)F1×W	IgM ssDNA/IgM histone	Balb/c	(425)
Gp1	9	57.9104.8	*D9Mit53*	BXSB×(B10 × BXSB)F1	gp70IC	BXSB	(426)
Bana2	10	0.0/20.3	*D10Mit213*	(NOD × Ba) × NODBC	ANA (M. bovis)	BALB/c	(14)
Asm1 *40/ND*	10	38/70.3	*D10Mit115* *D10Mit259*	MRL-lpr ×(MRL-lprxC3H-lpr)F1	sialadenitis	MRL	(60)

Aem3	10	41.5/82.5	*D10Mit42*	(B6 × NZB)F1 × NZB	RBC	NZB	(420)
Lmb4	10	51.0/92.4	*D10Mit11*	(B6-*lpr* × MRL-*lpr*)F2	Lprn/GN	MRL	(57)
Lbw8	11	28.0/53.4	*IL4*	BWF2	chr	NZB	(20)
sbb2	12	6.0/ND	*D12Mit12*	(B6 × Ba)F2-Fc*g* RIIb-/-	ANA (Fc*g* RIIb ko)	B6	(75)
Lrdm2	12	27.0/ND	*D12Nyu3*	(MRL-*lpr* × CAST)F1 × MRL-*lpr*	GN	MRL	(59)
Spg3	13	35.0/54.9	*D13Mit250* B6x(BxB6.*Yaa*)F1	B6 × (W × B6-*Yaa*)F1	gp70, gp70IC	NZW, NZB	(54,56,427)
Swrl2	14	27.5/56.9	*D14Mit37*	B × (SWR × B)F1	GN/dsDNA	SWR	(414)
Myo2	14	39.0/66.9	*D14Mit68*	NZW × (NZW × BXSB)F1	MI	BXSB	(68)
Lprm3	14	44.0/82.4	*D14Mit195*	MRL-*lpr* × (MRL-*lprx*C3H-*lpr*)F1	GN (resistance)	MRL	(58)
Paam1	15	17.8/31.9	*D15Mit111*	MRL-*lpr* × (MRL-*lprx*C3H-*lpr*)F1	arthritis in males	MRL	(428)
Lprm5	16	21.0/27.8	*D16Mit3*	MRL-*lpr* × (MRL-*lprx*C3H-*lpr*)F1	dsDNA	MRL	(58)
Bah2	16	34.6/31.1	*D16Mit58*	(NOD × Ba) × NODBC	RBC (M.bovis)	BALB/c	(14)
nwa1	16	38.0/ND	*D16Mit5*	(W × Ba)F1 × W	histone	NZW	(425)
nwa1	16	38.0/ND	*D16Mit5*	(B × W)F1 × W	GN/dsDNA	NZW	(429)
sbb3	17	16.0/25.5	*D17Mit198*	(B6 × Ba)F2-Fcγ RIIb-/-	ANA/spleen (Fcγ RIIb ko)	BALB/c	(75)
Acla1	17	18.2/31.3	*D17Mit16*	NZW × (NZW × BXSB)F1	CL	NZW/BXSB	(68)
Sles1	17	18.8/32.4	*D17Mit34*	(B6.NZM*c1* × NZW)F1 × NZW	GN/dsDNA (resistance)	NZW	(19)
Pbat1	17	18.9/32.7	*D17Nds2*	NZW × (NZW × BXSB)F1	platelet	NZW/BXSB	(68)
Bana1/ Bah1	17	20.4/35.2	*D17Mit24*	(NOD × Ba) × NODBC	ANA/RBC (M.bovis)	NOD	(14)
Wbw2	17	24.0/46.1	*D17Mit177*	(W × PL)F1 × B	mortality/GN	NZW	(419)
Agnz2	17	55.7/85.3	*D17Mit130*	(NZM2328 × C57L)F1 × NZM2328	acute GN	C57L	(416)
Swrl3	18	20/39.7	*D18Mit17*	B × (SWR × B)F1	dsDNA/histone	SWR	(414)
Lbw6	18	47.0/63.8	*D18Mit8*	BWF2	mortality/GN	NZW	(20)
nwa2	19	41.0/41.9	*D19Mit11*	(W × Ba)F1 × W	ssDNA	NZW	(425)

Table includes only named loci with linkages p <0.01 or *lod*>1.9. Loci are listed by their approximate chromosomal locations based on the marker with the highest association. Chr, chromosome. cM distances are from the Mouse Genome Informatics (http://www.informatics.jax.org/) and Mb distances from the Ensembl Mouse Genome Database (http://www.ensembl.org/Mus_musculus). NM, not mapped to mouse genome. Abbreviations for mouse strains (Cross column): B, NZB, B6, C57BL/6, B10, C57BL/10, Ba, Balb/c, *CAST, CAST/Ei, Ipr, Fas*^{lpr}, NZM, NZM/Aeg2410, W, NZW, (MRL-*lprx*C3H-*lpr*)BC & F2, both MRL-*lprx*(MRL-*lprx*c3h-*lpr*)F1 & (MRL-*lprx*C3H-*lpr*)F2 crosses. Original phenotypes that mapped to loci are shown: chr, antichromatin autoantibody, CL, anticardiolipin autoantibody, dsDNA, anti-dsDNA autoantibody, GN, glomerulonephritis, gp70, gp70 immune complexes, histone, antihistone autoantibody, LN, lymphadenopathy, Lprn, lymphoproliferation, MI, myocardial infarct, platelet, antiplatelet autoAb and thrombocytopenia, RBC, antiRBC autoAb, spleen, splenomegaly. Autoantibodies are IgG unless otherwise specified.

Loci Identified in Crosses of NZB and NZW Mice

The NZB and NZW strains are the most extensively studied of the lupus-prone mice. Genome-wide scans of intercrosses, backcrosses, and crosses to normal background strains resulted in the identification of 13 named NZB and 20 named NZW loci, covering 7 and 10 autosomal chromosomes, respectively, that contribute to one or more lupus-related traits (Table 7.2). Four NZW chromosomal regions associated with suppression of lupus (designated *Sles1–4*) were also identified (19). Loci were also mapped in these crosses to chromosomes from several nonautoimmune strains, including B6, BALB/c, C57L, and SWR.

Several loci, in addition to the MHC region, appear be confirmed in more than one cross. These include *Sle1/Cgnz1/Agnz1* (NZW-derived) and the overlapping *Lbw7/Nba2* (NZB) on chromosome 1, *Lbw2/Sle2/nba1/Imh1/Mott/Spm1* (NZB) on chromosome 4, *Sle3/Sle5/Lbw5* (NZW) and *Nba5/Aem2* (NZB) on chromosome 7, *Lbw8* (NZB) on proximal chromosome 8, *Sgp3* (NZB and NZW) on chromosome 13, and *nwa1* (NZW) on chromosome 16 (Table 7.2). Many of these loci have also been verified by interval congenic lines. In addition to identification of individual loci, mapping studies indicate that inheritance of lupus traits is multiplicative, dependent on the number and specific combination of susceptibility loci (epistasis) and that different sets of loci contribute to different traits, i.e., lymphoid hyperplasia, autoantibody production, GN, and mortality (19 ,20 ,21 ,22).

The NZW *Sle 1* locus on chromosome 1 is the best characterized. This interval overlaps with a region on human chromosome 1 linked to SLE (23 ,24 ,25) and therefore, may have relevance to human disease. *Sle1* congenic mice (B6.NZM*c1*) develop elevated IgG antinuclear antibodies (particularly targeting H2A/H2B/DNA subnucleosomes), but no GN (26). Bi- or tri-congenics of *Sle1* with *Sle2* (chromosome 4) and/or *Sle3/Sle5* (chromosome 7) intervals, however, result in GN and early mortality with severity dependent on the specific combination of loci (26 ,27). Bone marrow transfer studies have shown that the *Sle1* interval is functionally expressed in both B cells and T cells (28). More recently, others have generated interval-specific NZM2328 congenic mice (NZMC57L*c1*) in which the susceptible NZW *Cgnz1* interval on chromosome 1 (essentially *Sle1*) has been replaced with the nonautoimmune *Cgnz1* interval from C57L. Such congenics have significant reductions in anti-dsDNA and GN (29) and clearly demonstrate the significance of this single interval in disease susceptibility.

The *Sle1* locus has subsequently been shown using subinterval congenic mice to consist of a cluster of at least three loci (*Sle1a-c*), of which *Sle1b* appears to be the most potent (16 ,30). *Sle1a-c*, however, do not induce severe GN when combined individually with other SLE loci, indicating the importance of the entire interval. Physical mapping and cloning of the 900kb genomic segment of *Sle1b* identified 24 expressed genes and 2 pseudogenes (31). Of significant interest was the fact that this interval contained the SLAM/CD2 family genes, which encode surface molecules on hematopoietic lineage cells that mediate stimulatory or inhibitory signals. Furthermore, extensive polymorphism was discovered between the B6.*Sle1b* (*Sle1b* haplotype) and B6 genomes involving 10 genes, *Usp23, Nit1, Refbp2, Cd229, Cs1, CD48, CD84, Ncstn, Copa*, and *Pxf*. These differences included the expansion of *Cd224* in B6 mice to a 4-locus cluster, from which transcripts from 3 of the genes could be detected. Among the SLAM/CD2 family genes, however, the most likely lupus-promoting candidates appear to be CD48, CD150, CD84, and Ly108, although the entire haplotype may play a role. The NZW *Sle1b* haplotype is present in most inbred strains, including 129/SvJ, A/J, AKR/J, BALB/cJ, C3H/HeJ, CBA/J, CE/J, DBA/2J, DDY, Jc1, LP/J, MRL/MpJ, NOD/Lt, NZB/B1WJ, P/J, PL/J, SB/Le, SEA/GnJ, SJL/J, SM/J, WB/Re, PERA/EiJ, PERA/RkJ, PERC/EiJ, SK/CamEiJ, and SF/CamEiJ. Contrastingly, the B6 haplotype is limited to B6, C57BR/cdJ, C57L/M, RF/J, MOLF/EiJ and MOLE/EiJ. Thus, the *Sle1b* and B6 haplotypes are almost certainly of ancestral origin. Interestingly, a 129 locus on chromosome 1 that overlaps with *Sle1b* was shown to promote lupus when backcrossed onto the B6 background (32). It is possible that this might be due to the *Sle1b* haplotype, which suggests that chromosome 1 intervals from most inbred strains would have a similar effect.

The complement receptor 2 (*Cr2*) gene has been identified as a candidate gene for *Sle1c* (33). Comparison of the *Cr2* alleles of NZM2410/NZW and B6 strains revealed considerable polymorphism, with differences in 16 nucleotide residues (11 resulting in amino acid changes) and a 3 nucleotide insertion/deletion (33). Most significant, was a C→A (His→Asn) mutation at residue 1342 located in either the external domain short consensus repeat 7 (SCR7) of CR1 or the SCR1 of CR2, which introduced a new N-linked glycosylation site within the ligand-binding domain. This mutation reduced C3dg binding, CR1/CR2-mediated signaling, and IgG response to T-dependent antigens. Based on the known structure of CR1/CR2, glycosylation of the Asn residue apparently altered the function of CR2 by inhibiting its dimerization (33). The *Cr2* gene in mice encodes by alternative splicing both CR1 and CR2 glycoproteins (34 ,35). CR1/CR2 is expressed primarily on the surfaces of mature B cells and follicular dendritic cells, and binds C3 and C4 split products on antigens or immune complexes. CR2 reduces the B cell activation threshold and plays a role in both B cell apoptosis and antigen processing/presentation, particularly within the germinal center. Mice lacking *Cr2* have reduced T-dependent responses, generation of memory B cells, and germinal center formation. CR2 appears to help establish tolerance by enhancing the presentation of self-antigens, as suggested by the accelerated disease

observed in *Fas*^{lpr} mice when combined with *Cr2*-deficiency (36 ,37). However, to what extent transfer of the *Sle1b* SLAM/CD2 haplotype (present in the 129 genome where the knockout of *Cr2* was generated) contributes to lupus-like disease in *Cr2*-deficient mice remains to be determined.

B6 congenic mice containing the NZB chromosome 1 interval (B6.*Nba2*) have also been generated and characterized (38). These mice, similar to the *Sle1* congenics, spontaneously produce IgG anti-DNA and antichromatin autoantibodies, but do not develop GN. Furthermore, combining this interval with the *Yaa* also leads to higher levels of autoantibodies, including those to DNA, chromatin and gp70, as well as the development of severe lethal GN. At least part of this effect must be because of the *Sle1b* SLAM/CD2 haplotype present in NZB mice. In this regard, other studies attempting to more precisely map alterations in B cell activation induced by the NZB chromosome 1 interval, suggest that the *Nba2* interval is also composed of more than one susceptibility gene (17).

Analysis of microarray expression profiles of spleen cells from B6 and B6.Nba2 congenic identified *Ifi202* as a potential candidate gene for the *Nba2* (38). In mice containing the NZB *Nba2* interval, there was more than 10-fold increase in *Ifi202* and decrease in *Ifi203* (both within the *Ifi200* cluster), and impressively, of 11,000 genes these were the only differences detected. The specific polymorphism responsible for the increased expression is not known although several differences identified in the promoter region of the *Ifi202* gene are suspected. *Ifi202* is an interferon-inducible family of two genes (*Ifi202a* and *Ifi202b*) that has been suggested to play a role in cell survival, proliferation, and differentiation (39). *Ifi202* is also upregulated by IL-6 through STAT3 activation (40).

Another candidate for *Nba2* is the inhibitory Fc receptor, Fcγ RIIb, which on B cells inhibits B cell antigen receptor signaling following the engagment of immune complexes and colocalization of Fcγ RIIb and the antigen receptor (41). Inhibition is mediated by dephosphorylation of the antigen receptor by SHIP and possibly to a lesser extent SHP-1, which are bound to the immunoreceptor tyrosine-based inhibition motif on Fcγ RIIb (42 ,43). In NZB mice, there are two deletions in the promoter region associated with lower expression of Fcγ RIIb in germinal centers and with hypergammaglobulinemia (44). A more recent study, however, found that lower levels of Fcγ RIIb on germinal center B cells in NZB mice was a result of impaired upregulation, and that similar reduced levels were also present in several other lupus-prone strains indicating a lack of correlation with genetic background (45).

Congenic mice for the chromosome 4 loci have also been studied. B6.NZM*c4* congenic mice, which contain the *Sle2* locus (a mixture of NZW genome on the acromeric portion and NZB genome on the telomeric part of the interval), were found to develop generalized B cell hyperactivity, expansion of B1 cells and increased polyclonal IgM levels, but no increase in IgG antinuclear antibodies or GN (46). It was suggested that the expanded B1 cell population, which expresses higher levels of costimulatory molecules such as B7, might promote autoimmunity by enhancing self-antigen presentation T cells (47). More recently, BWF1 congenics that contain one or no copies of the NZW chromosome 4 interval (*Lbw2*) showed reduced B cell activation to LPS, decreased IgM levels and autoantibodies, less glomerular immune complex deposits and GN, and reduced mortality, but unexpectedly no difference in levels of IgG autoantibodies (48). Nevertheless, spontaneous IgG autoantibody–secreting cells were significantly reduced and the number of these cells correlated with amount of kidney deposits, but not serum levels.

Furthermore, kidney eluates did not demonstrate significant difference in the autoantibody repertoire in deposits from BWF1 mice with one or no copy of the NZB *Lbw2* locus. Thus, it was concluded that serum levels of IgG autoantibodies did not reflect the actual differences in production of autoantibodies and that the primary defect of *Lbw2* is B cell hyperactivity. Interestingly, congenic NZM2328 mice with replacement of the *Adnz1* chromosome 4 interval that overlaps *Sle2* and *Lbw2* (NZM.C57L*c4*) developed severe GN similar to wild-type NZM2328, but had markedly reduced to normal levels of anti-dsDNA antibodies (29). This model is particularly interesting since elucidation of the responsible genetic alteration should yield significant insights about the etiopathogenesis of antinuclear antibodies. Overall, findings in these various interval congenic mice suggest the presence of multiple subloci within the NZ chromosome 4 interval and/or considerable influence of background genes.

A NZB C1q polymorphism located within the *Nba1/Lbw2/Imh1/Mott* interval on chromosome 4 that down-regulates C1q levels is an attractive candidate since deficiencies of the early complement components (C1q-s, C2, or C4) predispose to SLE in humans (49). Moreover, homozygous C1q knockout mice develop a strain background-dependent loss of tolerance to nuclear antigens and abnormal accumulation of apoptotic bodies in the kidney glomeruli, suggesting that C1q may prevent systemic autoimmunity by playing a nonredundant role in the clearance of apoptosis byproducts (50 ,51). More recent fine mapping, however, has indicated that C1q is not within the *Lbw2* interval (48).

The chromosome 7 interval is similar to chromosome 1 in that both NZW and NZB loci have been mapped to this region. In terms of the NZW locus, the B6 congenic for *Sle3/Sle5* (B6.NZM*c7*) was found to develop elevated, but low, levels of antinuclear antibodies and a low incidence of GN, but when combined with other SLE loci promoted more severe lupus manifestations depending on the combinations (27 ,52). *Sle3* was initially thought to promote generalized T cell activation because of a marked increase in activated T cells, elevated CD4:CD8 ratios and resistance to activation-induced cell death in congenic mice (52). More recently, however, using bone marrow chimera experiments with allotype labeled B and T cells from B6 and B6.*Sle*3/5 mice, it was shown that the susceptibility genes was expressed in a nonlymphocyte bone marrow-derived population that affected T cell selection, survival, or both (53).

B6.*Yaa* mice congenic for the NZB chromosome 7 locus (B6.*Nba5*) have also been generated and characterized (54). Compared with B6.*Yaa* mice, congenics develop increased gp70 immune complexes and more severe GN, although the incidence was low and onset delayed compared with B6.*Yaa* congenic for the *Nba2* (NZB chromosome 1) locus. Remarkably, *Nba5* had no effect on either anti-DNA or antichromatin IgG autoantibodies. Thus, *Nba5* represents another locus that affects autoantibody specificity.

A CD22a variant in NZW and NZB mice located within the *Sle5* and *Lbw5* intervals on chromosome 7 has been suggested as a possible candidate (55). CD22a has a 794 base pair insertion within the second intron of a cluster of short interspersed nucleotide elements, which leads to aberrant alternative splicing. This is associated with reduced LPS-stimulated expression of CD22 in B cells to about half the level observed with the CD22b (B6 mice) allele. The CD22 is a candidate gene for *Sle5*, but it is not a candidate for *Lbw5*, since congenic NZB mice containing the NZW *Lbw5* interval have enhanced disease (unpublished observations).

The *Sgp3* locus on chromosome 13 has also been confirmed in B6 mice containing either the relevant NZW or NZB intervals (54 ,56). *Sgp3* was primarily associated with increased production of gp70, however, in autoimmune–prone *Yaa* mice *Sgp3* also enhanced GN and in some cases autoantibodies to DNA and chromatin.

Loci Identified in MRL-Fas^{lpr} Crosses

Although the *Fas^{lpr}* mutation promotes loss of tolerance and autoimmunity, the type of manifestations and severity of lupus-like disease are highly dependent on background susceptibility genes. This has led several groups to define lupus-related QTL in crosses of MRL-*Fas^{lpr}* mice, a strain that develops particularly severe spontaneous accelerated systemic autoimmunity (Table 7.2). Fifteen named QTL for one or more lupus traits have been identified on 8 of the 19 autosomal chromosomes. Many of the loci are linked to the different traits, such as sialadenitis, GN and vasculitis, which are likely caused by overlapping, but distinct, sets of susceptibility genes. Several loci on chromosomes 5 (*Lmb2, Lprm4*), 7 (*Lmb3, Ldrm1*), and 10 (*Lmb4, Asm1*) have overlapping intervals and may be identical (57 ,58 ,59 ,60). Interestingly, the Lmb1 locus (chromosome 4), which mapped to the nonautoimmune B6 background, had an additive effect on lymphoproliferation equal to the other *Lmb* QTL (57). This further demonstrates that so-called nonautoimmune mice can harbor bonafide susceptibility genes, but apparently the number and combination of such genes are insufficient for disease induction. Such genes undoubtedly account for the background effects observed when using different strain combinations to map QTLs.

The CD72c variant in MRL mice, which originated from the LG/J strain, has 13 amino acid substitutions compared with the CD72b allele (C3H strain), including acidic, basic, and neutral changes, and is a candidate for the *Arvm1* locus on chromosome 4 (61). CD72 is a member of the C-type lectin superfamily expressed on the surface of B cells (62). It is a negative regulator of B cell activation and also plays a role in B cell development.

Loci Identified in BXSB Crosses

The *Yaa* gene is the major predisposing gene in BXSB males, however, susceptibility is also highly dependent on other BXSB genes (63 ,64 ,65). Genome-wide searches to define those genes have identified 12 named BXSB-derived loci encompasssing 8 chromosomes in backcrosses of BXSB to C57BL/10 (B10) or to NZW strains (Table 7.2). In reciprocal male BXSB × B10 backcrosses, five QTL were found to be linked to one or more traits, including antinuclear antibodies, lymphoproliferation, and GN (66 ,67). Four were located in different regions on chromosome 1 and one was mapped to chromosome 3. Other loci with suggestive linkages were also identified on chromosomes 4, 10, and 13. Contrastingly, in a male BXSB × NZW backcross study, a completely different set of BXSB loci for other traits that included anticardioliolipin antibodies, antiplatelet antibodies, thrombocytopenia, and myocardial infarction, were found to map to chromosomes 4, 7, 8, 14, and 17 (68). Finally, in another study of female (BXSB × NZW)F2 mice, two BXSB loci were identified, one on chromosome 1 to splenomegaly and the other on chromosome 4 (*Lxw1*) to antichromatin autoantibodies (69). Interestingly, although female (BXSB × NZW)F1 and female (NZB × NZW)F1 mice both develop accelerated disease compared with parental strains, the genetic contributions of BXSB and NZB loci to this additive effect were completely different. This suggests that at least some of the susceptibility gene variants in these strains may not be derived from the common ancestral Asian and European mouse strains.

BXSB loci (*Bxs1–4*) on chromosome 1 have also been largely confirmed with 4 congenic B10.*Yaa* mice containing sizeable and overlapping chromosome 1 fragments of the BXSB genome (70). Three of these intervals, however, contain more than one *Bxs* locus, and therefore more precise mapping using congenic mice with smaller introgressed chromosomal regions will be required to verify the presence of these loci and to delineate their individual characteristics.

Loci in Other Spontaneous Lupus Crosses

A recent study documented significant lupus-like disease with immune complex GN in (129xB6) hybrids and identified loci on chromosomes 1 (129-derived), 3 (B6-derived), and 4 (B6-derived) that promote autoantibodies to nuclear antigens, including DNA and chromatin (32). Furthermore, B6 congenic mice containing a 129 chromosome 1 interval was sufficient to cause loss of tolerance to nuclear antigens and the production of autoantibodies. The 129 chromosome 1 region overlaps with *Sle1b* and the findings may be related to the previously described polymorphisms of the SLAM/CD2

locus (31). Since 129 × B6 mixed backgrounds are often used to define characteristics of mice with gene knockouts, these results suggest caution in ascribing lupus manifestations solely to the deficient gene, particularly if the gene is located on chromosome 1 (71).

Another recently identified model of lupus and rheumatoid arthritis is the BXD2 recombinant inbred strain (4). This line is one of approximately 80 B6xDBA/2 recombinant inbred strains originally developed at the Jackson Laboratory (72). BXD2 mice spontaneously develop a systemic autoimmune disease, characterized by anti-DNA autoantibodies, rheumatoid factor (RF), immune complex GN, severe erosive arthritis, and a reduced lifespan of 14 months (4). Mapping susceptibility loci using 20 recombinant BXD strains identified two loci, a DBA/2 locus on chromosome 2 that was linked to anti-DNA autoantibodies and a B6 locus on chromosome 4 linked to RF levels.

Loci Identified in Induced or Mutant Models of Lupus

Loci contributing to systemic autoimmunity have also been identified in several induced or mutant models of SLE. In mercury-induce autoimmunity, most strains are susceptible with the notable exception of the DBA/2. Mapping of F2 crosses between the DBA/2 and susceptible SJL or NZB, identified a single DBA/2 locus on chromosome 1 (*Hmr1*) that was linked to reduced glomerular immune complex deposition (73). Interestingly, antibodies to nucleoli, a characteristic specificity observed in this disease, was only affected by the H-2^s haplotype and no other background genes.

Intravenous *M. bovis* (bacillus Calmette-Guérin) given to type I diabetes mellitus-susceptible NOD mice prevents diabetes but, remarkably, induces systemic autoimmunity manifested by hemolytic anemia, antinuclear antibodies, immune complex-mediated GN, and exacerbation of sialadenitis (13). When backcrosses to BALB/c mice were analyzed for prediposing loci, hemolytic anemia mapped to two loci on chromosomes 17 (*Bah1*) and 16 (*Bah2*) and antinuclear antibody (ANA) to 17 (*Bana1*) 10 (*Bana2*), and 1 (*Bana3*). No locus was identified for GN. Interestingly, two of the four regions (*Bana3, Bah1/Bana1*) overlap with previously identified lupus-predisposing loci. Another interesting observation is that, other than the MHC region, none of the lupus-predisposing loci colocalized with diabetes loci. Thus, there is no evidence for common autoimmune–predisposing genes in NOD mice, although lupus- and diabetes-susceptibility genes shared by both NOD and BALB/c mice have not been ruled out (14).

Deficiency of Fcγ RIIb has been shown to enhance autoimmunity in several different disease models, including spontaneous lupus (74). Other complementation studies documented that the Fcγ RIIb knockout synergizes with the *Yaa* gene and, to a lesser extent, with the *Sle1* locus, but surprisingly not with the *Fas^{lpr}* defect (75). In terms of lupus, background genes are also critically important as evidenced by the fact that lack of Fcγ RIIb in B6, but not BALB/c, mice results in systemic autoimmunity. Genome-wide analysis to define the genetic basis for this difference in susceptibility revealed three regions, designated *sbb1–3* on chromosomes 9, 12, and 17, that were linked to ANA, spleen weight, and/or proteinuria (75) (Table 7.2). One of these loci, *sbb1*, was derived from the nonsusceptible BALB/c genome. Notably, none of the B6 loci identified in the (129xB6) cross (chromosomes 3 and 4) were found in this study, suggesting suggesting that the 129 gene segment may not contribute to lupus development in the Fcγ RIIb knockouts or may reflect differences in the BALB/c and 129 genomes (different sets of susceptibility genes that overlap with B6).

Specific “Lupus” Predisposing Genes

Spontaneous variants or mutations that predispose to autoantibody production or other lupus-like manifestations in mice have been documented for the major histocompatibility complex (MHC) class II genes, Fas, Fas ligand (*Fasl*), and hemopoietic cell phosphatase (*Hcph* or SHP-1) (Table 7-3). The Y accelerator of autoimmunity and lymphoproliferation (*Yaa*) “gene”, which is responsible for the male predisposition to lupus in BXSB mice, has recently been shown to be an expansion of the pseudoautosomal region of the Y-chromosome with duplication of several X-chromosome genes. Defects or polymorphisms in other immune-related genes have been postulated to predispose to lupus, but their roles have not been firmly established. These genes include the T cell receptor (TCR) (76 ,77 ,78 ,79 ,80 ,81), immunoglobulin (Ig) (82 ,83 ,84) and TNF-α (Tnf) (85 ,86 ,87 ,88), Fcγ RIIb (44), and CD22 (55). *Cr2* (33), C1q (49), SLAM family genes (31), and P2X7 receptor (89).

MHC Class II Genes

Predisposition to systemic autoimmunity is strongly linked to specific MHC (H-2 in mice) haplotypes in certain lupus backgrounds, particularly the BXSB (90) and BWF1 mice (91 ,92). For the BXSB strain, H-2^b is the lupus-predisposing haplotype (90 ,93), whereas the heterozygous H-$2^{d/z}$ is associated with the greatest susceptibility in BWF1 hybrids (reviewed in (63)). Although certain MHC haplotypes can promote susceptibility, additional predisposing genes are required for the development of SLE, since autoimmunity does not develop when the predisposing MHC haplotypes are on normal backgrounds or crosses (63 ,94). For example, (NZBxNZW)F1 mice (H-$2^{d/z}$) are highly susceptible to lupus, whereas F1 hybrids between NZB and PL/J or B10.PL (H-2^u, identical to H-2^z in the antigen-binding domains), or BALB/c mice congenic for H-2^z, are not susceptible (95 ,96 ,97). Other examples are the BXSB.*1l* and MRL-*Fas^{lpr}.1l* (*1l* for long-lived) substrains that have identical H-2 haplotypes, but are much less susceptible to disease because of possible single mutations in non-MHC genes (1 ,2 ,3).

The actual gene (or genes) within the MHC complex that promotes lupus susceptibility remains to be determined.

Polymorphic class II genes are strong candidates based on findings in H-2 congenic mice and their central roles in both repertoire shaping and antigen recognition (90 ,98 ,99). NZB mice congenic for H-2^{bm12}, but not the closely related H-2^{b}, develop accelerated disease despite the fact that these haplotypes are apparently similar except for the I-A molecule. This seems to implicate three amino acids in the I-A ß-chain at positions 67, 70, and 71, since the H-2^{bm12} and H-2^{b} sequences differ by these residues in the MHC peptide-binding groove. Strong linkage with lupus disease in NZ background mice is conferred the hemizygous H-2^{z} haplotype in combination with either the d, b, or v haplotypes (91 ,92 ,100 ,101 ,102). It has been proposed that in this instance, autoimmune-promoting MHC class II specificites are created by transcomplementation of the different α- and ß-chains with formation of novel hybrid molecules (103 ,104). The finding that other heterozygous H-2^{z} haplotypes, including H-$2^{v/z}$ (102) and H-$2^{b/z}$ (100 ,101), also increases susceptibility makes it less likely that novel class II hybrid molecules are responsible. Another possibility is that the two different H-2 haplotypes regulate the production of different sets of nephritogenic autoantibodies (105). In this case, heterozygous H-$2^{d/z}$ haplotype mice would produce both sets of pathogenic antibodies resulting in greater susceptibility to nephritis. Studies attempting to directly implicate class II molecules by expressing I-A^{z} or I-E^{z} transgenes in the NZB × C57BL/6 background, however, found no increased susceptibility with either transgene (106 ,107). Although the inference is that the class II molecules are not responsible, it is also possible that the transgene did not adequately recapitulate the expression patterns required to promote autoimmunity, since it is known that slight changes in class II expression result in substantial effects on disease susceptibility, e.g., homozygous versus heterozygous expression (108) or the presence or absence of I-E (109). In this regard, transgenic mice would not have the same levels of class II molecules as wild-type mice since they would have normal levels of class II in addition to the transgene.

Table 7-3: Spontaneous Mutations Associated with Lupus-Like Manifestations

Name	Gene	Chr*	Mb	Susceptible Allele	Alteration	Major Autoimmune Manifestations
Fas ligand	*Fasl*	1	85.0	*gld*(generalized lymphoproliferative disease)	T→C, 847 nt. (Phe→ Leu)	lymphoproliferation, DN T cells, autoAbs, GN
SHP-1, PTP-1C, hemopoietic cell phosphatase (protein tyrosine phosphatase, nonreceptor type 6)	*Ptpn6*	6	60.2	*me*(motheaten) me^{v}(viable motheaten)	C deletion, 228 nt. aberrant RNA splicing T→A 1076 nt. disrupts splice donor site	autoAbs (both me and mev mutations)
MHC complex	*H2*	17	32.0	depends on background strain	H-$2^{d/z}$(BWF1) H-2^{b}(BXSB) H-2^{bm12}(NZB) others.	enhanced autoimmunity, including autoAb, GN, lymphoproliferation (depending on MHC and background)
Fas, APO-1, CD95	*Fas*	19	33.6	*lpr* (lymphoproliferation)	ETn insertion with aberrant RNA splicing	lymphoproliferation, expansion of DN T cell subset, autoAbs, GN, arthritis depending on background (both *lpr* and lpr^{cg} mutations)
				lpr^{cg} (lymphoproliferation complementing *gld*)	T → A, 786 nt. (Ile → Asn) disrupts binding of Fas to Fasl	
Y accelerated autoimmunity and lymphoproliferation		Y		*Yaa*	4 Mb expansion due to duplication of X-chromosome pseudo autosomal region; includes TLR7 gene	accelerated autoimmunity, enhanced Ab responses to foreign and self-antigens

*Chr, chromosome; chromosomal locations are based on the Mouse Genome Informatics (http://www.informatics.jax.org/). See text for references.

Other studies have sought to explain why H-2^d (I-E^+) congenic BXSB mice (normally H-2^b (I-E^-)) do not develop lupus (99 ,109 ,110). Lack of susceptibility was not from I-E-dependent, endogenous superantigen-induced modifications of the TCR BV repertoire imposed by H-2d, because H-$2^{b/d}$ haplotype mice are susceptible to disease despite similar deletion of the appropriate BV bearing T cells (90). Interestingly, BXSB mice expressing a high copy number (~50) of an I-Eα^d transgene with approximately 20-fold higher Eα^d mRNA levels than H-$2^{b/d}$ mice were found resistant to the development of autoimmunity, similar to H-2^d BXSB mice (109). Protection by the I-Eα transgene has also been reported for I-E^+ haplotype lupus backgrounds (111 ,112). It is postulated that this resistance is a result of competitive inhibition of autoantigenic peptide binding to I-A^b by a large excess of transgenic I-Eα peptide. This, however, does not fully explain the lack of susceptibility of H-2^d BXSB mice that have normal copy number of I-Eα^d. Nonetheless, these findings suggest a novel mechanism wherein excess production of MHC-binding peptide from I-Eα or perhaps other proteins alters antigen presentation and, possibly, disease susceptibility. Whether the mechanism is due to inhibition of autoantigen presentation or repertoire shaping during central thymic selection or in the peripheral immune system is not known.

Other potential lupus-predisposing genes within the H-2 complex include a polymorphic NZW TNF-α gene that appears to promote lupus in BWF1 hybrids independently of the H-2 (85 ,86), complement components C2 and C4 (35), IEX-1 (113) and a recessive NZW locus (*Sles1*) that appears to suppress autoimmunity in NZW mice (19). The *Sles1* locus has been mapped to 956 kb, but still includes the H-2 complex (114).

Fas and Fas Ligand

Fas (APO-1 or CD95) is a 306-amino acid, 45 kDa, cell surface membrane protein related to the TNF receptor superfamily of type I membrane glycoproteins. Fas is expressed on actively proliferating cells in the thymus, liver, ovary hear, skin, and gut epithelium with particularly high levels on $CD4^+CD8^+$ thymocytes, activated T and B cells, and some neoplastic cells (115 ,116). Following binding to its ligand, Fas transduces signals leading primarily to apoptotic cell death (117 ,118). The ligand for Fas, FasL, is a 40 kDa type II transmembrane glycoprotein belonging to the TNF ligand family of proteins. It is expressed almost exclusively on T cell lineages, primarily upon activation (119), and in certain immunologically sequestered sites such as the testis, eye and placenta (119 ,120 ,121 ,122). In these areas, FasL may contribute to maintenance of immune privilege by inducing Fas-mediated apoptosis in invading inflammatory cells, reducing inflammatory responses (123) and, in the case of the retina, may also control the growth of new vision-damaging subretinal blood vessels (124). Further details on Fas/FasL-induced apoptosis can be found in Chapter 8 .

Two spontaneous loss-of-function mutations in Fas (*Fas^{lpr}* and *Fas^{lpr-cg}*) and one in FasL (*$Fasl^{gld}$*) result in similar autoimmune manifestations (Table 7.3). The *Fas^{lpr}* (lymphoproliferative) defect is caused by an early retroviral transposon (ETn) insertion within the second intron between exons 2 and 3, which causes aberrant RNA splicing, a frame shift, and premature termination of the mRNA at the long terminal repeat region of the ETn (125 ,126 ,127 ,128 ,129). Low levels (~10%) of wild-type Fas mRNA and surface protein, however, are detectable (127 ,128 ,129 ,130 ,131), and recombinant Fas knockout mice, but not *lpr* mice, develop liver hyperplasia in addition to lymphoproliferation (132). The *Fas^{lpr-cg}* mutation is caused by a single point mutation (T → A at nucleotide 786; isoleucine→asparagine; this residue is valine in humans) within the intracytoplasmic domain of Fas that modifies an amino acid in the so called “death domain” essential for signal transduction (126 ,133). Finally, the *$Fasl^{gld}$* allele is a point mutation in

the carboxy-terminal extracellular domain (T→C at nucleotide 847, phenylalanine→leucine) of FasL on chromosome 1, which abolishes the binding of Fas to the FasL (134 ,135 ,136 ,137).

Mice homozygous for these mutations have accumulation of CD4-CD8- (double negative, DN), $B220^+$, TCRab^+ T cells and the induction or acceleration of systemic autoimmunity (63 ,138). Both severity of the autoimmune disease and degree of lymphoproliferation, however, depend on other background genes (63). *Fas*^{lpr} does not complement *Fasl*^{gld}, whereas the *Fas*$^{lpr\text{-}cg}$ allele, as suggested by its name, can complement both the *Fas*^{lpr} allele and to a slightly lesser degree, the *Fasl*^{gld} allele. Furthermore, in contrast to the recessive *Fas*^{lpr} mutation, autoimmunity and lymphoproliferation is observed in heterozygous MRL-*Fas*$^{lpr\text{-}cg/+}$ mice, although less severe and without the characteristic expansion of DN T cells (139). In humans, defects in *Fas* have been identified as a cause of the rare autoimmune lymphoproliferative syndrome (ALPS or Canale-Smith syndrome) (140 ,141 ,142). This syndrome has also been described with loss-of-function mutations in caspase 10, a cysteine protease involved in the downstream apoptosis-promoting pathway of Fas (143). The majority of SLE patients, however, do not appear to have deficiencies or mutations in *Fas* or *FasL* (144 ,145 ,146 ,147).

As a result of the important role that Fas/FasL plays in apoptosis, defects in clonal deletion of T and B cells have been sought to explain the *lpr* phenotype. Clearly a T cell defect is evident by the accumulation of DN *lpr* cells. Most studies, however, have found no defects in central thymic deletion in Fas-deficient mice to exogenous and endogenous superantigens (148 ,149 ,150 ,151) and conventional class I and II presented antigens (152 ,153 ,154). In contrast, abnormalities in the receptor-mediated apoptosis of mature T cells (151 ,152 ,155 ,156) along with the early expansion of a distinct BV8.3/BD1.1/BJ1.1 T cell receptor in $CD4^+$ cells from MRL-*Fas*^{lpr}, but not *MRL*-$^{+/+}$ (157 ,158), have implicated defective peripheral T cell deletion as a possible mechanism contributing to autoimmunity. Thus, Fas/FasL interactions are important for activation-induced cell death (AICD) (159 ,160). AICD is initiated by antigen receptor engagement, which upregulates Fas and induces FasL in T cells, primarily in $CD8^+$ cells and the Th0 and Th1 $CD4^+$ subsets. Subsequent binding of Fas and FasL leads to RNA-dependent apoptosis (159 ,161 ,162 ,163). Fas-mediated killing is essential for $CD4^+$ Th1 T cell-mediated cytotoxicity and is one of at least two cytotoxic T lymphocyte (CTL) pathways for $CD8^+$ T cells (164 ,165 ,166 ,167 ,168). The Fas-dependent CTL pathway, however, in contrast to the perforin-dependent CTL pathway, cannot kill pathogen-infected targets (169), suggesting that Fas/FasL functions primarily in maintaining cell homeostasis, i.e., killing expanded clones that have outlived their immunologic function.

Evidence from the TCR BV repertoire of DN $B220^+$ T cells (150 ,158), DN cell cytolytic activity and positive perforin expression (170), anti-CD8 treatment and targeted gene knockouts of MHC class I (ß2-microglobulin) (171 ,172 ,173 ,174), CD8 (175), or CD4 (175) indicate that the majority of DN cells are derived from $CD8^+$ T cells. DN *lpr* cells are likely activated peripheral T cells that fail to undergo AICD, a finding consistent with their activated phenotype, which includes $CD44^{hi}$, expression and phosphorylation of $p57^{fyn}$, constitutive tyrosine phosphorylation of Vav and high expression of FasL in both *gld* and *lpr* mice (176 ,177). The elevated constitutive expression of FasL has been postulated to cause the unidirectional *lpr*-associated wasting syndrome (i.e., graft-versus-host disease-like abnormality) observed when *lpr* bone marrow is adoptively transferred to wild-type recipients (176 ,178 ,179 ,180).

An important role for T cells in *lpr* disease was suggested by reversal of both lymphoaccumulation and autoimmunity in MRL-*Fas*^{lpr} mice expressing a wild-type Fas transgene under the control of the CD2 promoter (181). However, the significance of this became less certain when subsequent studies of non-autoimmune mice expressing the same CD2-*Fas* transgene revealed a lack of thymic atrophy and other T cell characteristics normally associated with aging (182), thereby indicating alteration of normal T cell development. Subsequently, more specific transgenic expression of Fas in T cells, but not B cells (lck promoter), was found to block lymphoproliferation, but not autoimmunity (183), whereas expression in both B and T cell (lck distal promoter) reduced both lymphoproliferation and autoimmunity (184). This suggests that Fas expression in B cells may be a critical factor, as supported by other studies that clearly implicate B cells in the development of *lpr*-mediated autoimmunity.

Mixed bone marrow chimera studies have demonstrated that autoantibodies are produced from Fas-defective, but not wild-type, donor B cells (185). B cells from *lpr* and *gld* mice exhibit resistance to spontaneous apoptosis in vitro (186). Double-transgenic mice expressing the antibody to hen egg-white lysozyme (HEL) and either membrane-bound or soluble HEL were used to study the fate of autoreactive B cells in Fas-deficient *lpr* mice (187). Elimination of self-reactive *lpr* B cells recognizing membrane-bound HEL and functional inactivation of B cells to soluble HEL appeared to occur normally; however, an age-related breakdown of tolerance to soluble HEL was observed, suggesting a defect in the censoring of autoreactive B cells. Further studies indicated that anergic B cells normally are eliminated by $CD4^+$ T cells through the Fas/FasL pathway but, without Fas killing, B cells are triggered to proliferate (188 ,189). Consistent with this are findings that anti-dsDNA B cells in Fas-deficient mice are not developmentally arrested and excluded from splenic follicles as in nonautoimmune mice, but migrate into the follicles (190), and abnormal increases in IgG2a- and RF-secreting B cells in the T-rich zones of the PALS (191) Fas/FasL-mediated apoptosis also may further contribute to B cell homeostasis by eliminating bystander B cells that are activated by CD40L alone (192). More recent studies, however, using selective ablation of Fas in T cells, B cells, T and B cells, or all cells found that full development of lymphoproliferative disease required deletion of Fas in both lymphoid and nonlymphoid tissues (193).

Protein Tyrosine Phosphatase and Motheaten Mice

SHP-1 is a protein tyrosine phosphatase (PTP) ubiquitously expressed in hemopoietic lineage cells. Two recessive spontaneous mutations, motheaten (*me*) and motheaten viable (me^v), result in similar severe developmental and functional abnormalities of multiple hematopoietic cells lineages, leading to mortality of *me* and me^v mice around 3 and 9 weeks of age, respectively (194). Motheaten mice are immunodeficient, but paradoxically develop features of systemic autoimmunity such as hypergammaglobulinemia, antinuclear antibodies and immune complex deposits in multiple tissues (195). The hypergammaglobulinemia is produced by an expanded population of atypical plasmacytoid cells and a reduced B cell population composed primarily of $CD5^+$ B1 cells. The T cell compartment is normal at birth, but by 4 weeks of age there is involution, absent lymphoid follicles, impaired CTLs, and reduced responses to both mitogens and allo-antigens. Other hematopoietic alterations include defective natural killer (NK) cell differentiation and function, increased erythroid precursors, and enormous expansions of monocytes/macrophages and neutrophil populations. The term motheaten refers to a characteristic skin pattern of patchy inflammation and alopecia caused by large subcutaneous and dermal acccumulations of neutrophils that rupture and scar.

SHP-1 consists of an N-terminal catalytic domain and two in tandem SH2 (src homology 2) domains that are important for its function. The me mutation is a single cytidine residue deletion at position 228 within the N-terminal SH2 domain that converts the normal sequence to a donor splice-site consensus sequence. This results in aberrant splicing and deletion of the 101-bp portion of exon downstream from the newly created splice site. The me^v mutation disrupts a normal GT 5′ splice donor site (GT→GA, residue 1076), which also leads to aberrant splicing, this time within the SHP-1 catalytic domain, 15-bp upstream of the normal splice site (196 ,197). SHP-1 activity in the me mutant is absent, whereas a profound reduction is found in the me^v mutant (198); findings consistent with the greater severity of disease in me mice.

SHP-1 is recruited by negative regulatory molecules that contain immunoreceptor tyrosine-based inhibitory motifs (ITIM) (199), such as CD22, Fcγ RIIb, and CD72, to specific sites where it then inhibits molecular complexes that require tyrosine phosphorylation for activation, such as the BCR and TCR. Loss of this essential inhibitory function in me mice appears responsible for the exuberant activation and expansion of hematopoietic cell populations and the resulting disease manifestations. Although me mice have increased immunoglobulin levels and autoantibodies, the major clinical sequelae that lead to early mortality in these mice are not observed in spontaneous SLE, and the disease is not mediated by autoantibodies or T and B lymphocytes (196 ,197 ,200 ,201 ,202). Autoantibody production may be caused by the inability of CD22 and possibly Fcγ RIIb to negatively regulate the B cell antigen receptor when SHP-1 is absent (203). Nevertheless, heterozygous deficiency of SHP-1 can synergize with partial deficiencies of CD22 and lyn to promote loss of B cell tolerance (204).

Yaa Gene

BXSB mice have a marked male predilection to systemic autoimmunity, in contrast to the female predominance in humans and other susceptible mouse strains. This striking sexual dichotomy is not because of hormonal factors, but to a Y-chromosome alteration, designated *Yaa* (205,206,207a) that was recently identified as an approximately 4 Mb expansion containing at least 13 known and 4 unknown X-chromosome-specific genes (207b). Among the genes in this duplicated interval, TLR7, which is activated by single-strand RNA, appears to be a strong candidate. In support of this, Yaa^+ B cells have enhanced response to TLR7 (imiquimod), but not TLR9 (CpG) ligands. Other genes within the *Yaa* fragment, however, have not been excluded.

Transfer of the *Yaa* gene (Y-chromosome) to nonautoimmune and autoimmune mice has demonstrated that it contributes additively to lupus, but is dependent on other background genes. Nonautoimmune strains such as CBA/J or C57BL/6 are largely unaffected, whereas all lupus-susceptible strains examined, including BXSB, NZB, NZW, and MRL-Fas^{lpr}, show accelerated disease that generally maintains the clinical characteristics of the background strain (63 ,64 ,65 ,208). Mice with mild lupus appear affected more by the *Yaa* duplication than strains with already accelerated disease (90). This selective augmentation by *Yaa* contrasts with the induction of generalized autoimmunity by the *lpr* and *gld* mutations (208) and suggests different mechanisms, as further supported by the findings that *lpr* and *Yaa* congenic mice with identical backgrounds have different phenotypes (63), and that the *lpr* and *Yaa* are additive (209). Interestingly, DBA/1.*Yaa* (consomic for the BXSB Y-chromosome) were less susceptible to collagen-induced arthritis (CIA) than wild-type DBA/1 mice, suggesting that *Yaa* plays different roles in CIA and lupus (210).

Regarding the mechanisms responsible for the *Yaa* phenotype, double bone-marrow chimera experiments using a mixture of Yaa^+ and Yaa^- cells of different IgH allotypes revealed selective production of anti-DNA antibody and hypergammaglobulinemia by Yaa^+ B cells (211). The antibody-promoting effect of *Yaa* is applicable not only to self-antigens, but to foreign-antigens as well (212) and, analogous to the effects in lupus mice, enhancement was observed mainly for antigens eliciting low, T cell–dependent antibody responses and not for those eliciting high antibody responses (212). Accordingly, *Yaa* was postulated to increase the expression of an “intercellular adhesion molecule” on B cells, which promotes low-avidity, T-helper–B cell interactions (207a). Thus, nontolerant T-helper cells that are normally quiescent in Yaa^- animals because of insufficient antigen presentation become activated in Yaa^+ animals. Other mechanisms that increase antigen presentation, intracellular signaling, and coreceptor molecule expression are also possible. Interestingly, Yaa^+ mice of lupus

and nonautoimmune backgrounds have reduced numbers of marginal zone B cells, consistent with the notion that their B cells are more hyperreactive, and which argues against a major pathogenic role for MZ B cells in this model (213 ,214).

Similar mixed bone marrow chimera experiments, but with *Yaa*$^{+}$ and *Yaa*$^{-}$ T cells of different Thy-1 allotypes, revealed similar disease severity regardless of whether *Yaa*$^{+}$ cells were present or eliminated by anti-Thy-1 antibodies (112). Consistent with this, T cells from *Yaa*$^{+}$ mice do not have enhanced proliferative responses compared with T cells from *Yaa*$^{-}$ mice (214). Finally, no difference in disease severity was found when T cell-deficient (TCR α-chain knockout) BXSB male mice were reconstituted with either *Yaa*$^{+}$ or *Yaa*$^{-}$ T cells (215). Overall, these findings clearly indicate that expression of the *Yaa* defect in T cell is not required for autoimmunity.

Genetically Manipulated Genes That Promote SLE-Like Disease

Genetic manipulation of specific genes for other reasons in nonautoimmune strains has sometimes serendipitously yielded novel and informative models of SLE. Thus far, at least 50 different genes have been identified by this approach, although in some cases autoimmunity may be due to the use of the mixed (B6x129) background. Nevertheless, several common mechanistic pathways have been identified, including enhanced activation of B and T cells, defective apoptosis, defective clearance of self-antigens, enhanced antigen presentation, and cytokine-mediated activation (Tables 7-4 and 7-5).

Genes Related to B Cell Activation

The fate of B cells following antigen receptor (BCR) engagement is a complex process that involves direct or indirect interaction of the BCR with numerous molecules that can promote or inhibit cell activation. Among these are several tyrosine kinases (lyn, fyn, Btk, Blk, Syk), phosphatases (CD45, SHP-1, SHP-2, and SHIP) and accessory molecules (CD19, CD22, FcRγIIb, CD72). Genetic manipulation of many of these B cell regulatory molecules have resulted in mice with features similar to lupus.

Lyn is a nonreceptor Src-related tyrosine kinase involved in negative regulation of BCR signaling (216 ,217). Although lyn also plays a role in positive signaling, this function appears largely redundant since deficiency of lyn leads to hypersensitivity to BCR-mediated triggering. Mice with homozygous deletions of lyn have increased activation and higher turnover rates of B cells, splenomegaly, elevated IgM levels, autoantibodies, and GN. Homozygous deficiency of CD22, a B cell-specific cell surface sialoadhesin that specifically binds to asialoglycoproteins, results in a similar picture with hyperresponsiveness to BCR signaling, expansion of the peritoneal B1 cell population, and autoantibodies (218 ,219 ,220). Similarities between the lyn and CD22 knockouts stem from the fact that the inhibitory action of CD22 requires the recruitment and phosphorylation of lyn, which, in turn, recruits SHP-1, bringing it in proximity to the BCR where it can dephosphorylate the BCR and downregulate the response. Heterozygous deletions of CD22, lyn and SHP-1 were shown to have additive effects on B cell abnormalities consistent with the notion that they are limiting elements to a common pathway (204).

CD19, along with CD21 and Tapa-1 (CD81) on B cells, form the functional cell surface receptor complex for C3 fragments, which enhances BCR signal transduction and is crucial for B cell development and tolerance. Mice overexpressing a CD19 transgene develop hyperresponsive B cells to BCR cross-linking, marked expansion of the B1 cell population, hypergammaglobulinemia, and autoantibodies (221). Furthermore, expression of the CD19 transgene in anti-hen egg lysozyme Ig (HEL-Ig)/soluble HEL double-transgenic mice led to the appearance of anti-HEL antibodies, suggesting that a defect in anergy to certain soluble antigens might be the underlying mechanism (222).

As discussed previously, the Fcγ RIIb on B cells is an important inhibitor of B cell antigen receptor signaling. Gene knockout of FcγRII amplifies humoral and anaphylactic responses (223), and leads to the development of lupus-like disease (74) as well as increased severity of type II collagen-induced arthritis (224) and type IV collagen-induced Goodpasture syndrome (225). The lupus-like disease, however, only occurs in B6, but not BALB/c, mice indicating a significant role for background susceptibility or resistance (74). To what extent this is a result of the *Sle1b* SLAM/CD2 haplotype or to 129 background genes will need to be determined particularly since Fcγ RIIb is located on chromosome 1. In this regard, a more recent study found that autologous bone marrow reconstitution of Fcγ RIIb$^{-/-}$ mice with Fcγ RIIb-expressing retrovirus-transduced cells, not only increased expression of Fcγ RIIb on B cells, but also markedly reduced anti-dsDNA antibodies and GN (226). Although this supports the association of lupus with Fcγ RIIb deficiency, the same disease ameliorating outcomes were also observed in similarly-treated NZM2410 and BXSB mice indicating that increased Fcγ RIIb expression had more global effects on autoimmunity. Interestingly, increased expression of Fcγ RIIb by 40% in 40% of B cells was sufficient to inhibit autoimmune disease.

Aiolos is a zinc finger DNA-binding protein that shares a common GGGA core sequence binding motif with the closely homologous nuclear factor Ikaros (227). It is highly expressed in mature B cells and, to a lesser extent, in developing bone marrow B cells and thymocytes. In the nucleus, Aiolos is mainly localized to the 2 MD chromatin remodeling complex that also contains Ikaros, the Mi-2 ATPase, histone deacetylases and other components of the NURD (nucleosome remodeling histone deacetylase complex (227 ,228 ,229 ,230). NURD complexes have been suggested as participants in transcriptional repression by promoting DNA methylation and allow repressors to gain access to chromatin

(228 ,229 ,230). Ikaros was also found to interact with the mSin3 family of corepressors (231). These findings suggest that Ikaros family members regulate gene expression during lymphocyte development by recruiting certain histone deacetylase complexes to specific promoters (231). Mice with homozygous deletions of the Aiolos gene develop defects primarily in the B cell compartment, with hyperresponsiveness to BCR and CD40 stimulation, increased number of conventional B cells but a marked reduction in B1 cells, increased proportion of B cells with activated phenotype, hypergammaglobulinemia (particularly of IgE and IgG1), a three-fold reduction in IgM and positive ANAs in about half of animals by 16 weeks of age (232). Detected T cell abnormalities were limited to a slight increase in proliferative capacity of thymocytes and mature T cells. Thus, deficiency of Aiolos appears to facilitate B cell entry into cell cycle, maturation to germinal center lymphocytes, and a breakdown of B cell tolerance.

Table 7-4: Genetically-Manipulated Single Gene Alterations That Promote Lupus-Like Manifestations

Name	Gene	Chr*	Mb	Major Autoimmune Manifestations	Ref.
Knockouts/Mutations					
CTLA-4	*Cd152*	1	61.2	multiorgan lymphoproliferative dis., myocarditis, pancreatitis	(265,266,267)
PD-1 (programmed cell death 1)	*pdcd1*	1	93.7	prolif. arthritis, GN, glomerular IgG3 deposits.	(276)
mannoside acetyl glucosaminyltransferase 5	*Mgat5*	1	127.0	proliferative GN, enhanced EAE	(314)
CD45 (protein tyrosine phosphatase, receptor type C	*Ptprc*	1	137.9	lymphoproliferation, anti-dsDNA, splenomegaly, GN	(251)
Ro, SS-A (TROVE domain family, member 2)	*Trove2*	1	143.6	antiribosome and antichromatin autoAb, GN	(371)
roquin (RING CCCH (C3H) domains 1)	*Rc3h1*	1	160.8	autoAbs, lupus-like disease, incr. follicular T helper cells and GCs. M199R mutation.	(323)
FcgR2 (Fc receptor, IgG, low affinity IIb) serum amyloid P component	*Fcgr2b*	1	170.9	exacerbation of lupus-like disease in B6-Faslpr mice	(430)
	Apcs	1	172.8	anti-chromatin Ab, GN, female predom.	(357)
IL-2Rα	*Il2ra*	2	11.6	lymphoproliferation, hyperIgG, autoAb, anti-RBC Ab	(340)
Ras GRP1 (RAS guanyl releasing protein 1)	*Rasgrp1*	2	116.8	spont. recessive mutation prevents translation of Ras GRP1 protein, $CD4^+$ T cells resistant to AICD, lymphoprolif., autoAb	(353)
Nrf2 (nuclear, factor, erythroid derived 2, like 2	*Nfe2l2*	2	75.4	hyperIgG, anti-dsDNA Ab, GN, splenomegaly	(403)
TYRO3 protein tyrosine kinase 3 (Tyro 3 family)	*Tyro3*	2	119.3	triple knockout (Tyro3, Axl, Mer): lymphoproliferation, increased activated T and B cells, autoAb, GN	(364)
Bim (Bcl2 interacting mediator of cell death, Bcl2-like 11)	*Bcl2l11*	2	127.6	lymphoid/myeloid cell accumulation, autoAb, GN, vasculitis	(328)
c-mer proto-oncogene tyrosine kinase (Tyro 3 family)	*Mertk*	2	128.2	autoAb (Mertk knockout alone), (also see TYRO3 above)	(368)
IL-2	*Il2*	3	36.6	lymphoproliferation, hyperIgG, autoAb, anti-RBC Ab	(339)
GADD45 (growth arrest and DNA-damage-inducible 45 alpha)	*Gadd45a*	3	67.3	autoAb, GN, mortality, defective T cell death	(307)
TSAd (SH2 domain protein 2A)	*Sh2d2a*	3	87.6	hyperIgG, autoAbs, GN	(431)
lyn (Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	*Lyn*	4	3.6	enhanced B cell activation, splenomegaly, hyperIgM, autoAb, GN	(216, 217)
E2F2 (E2F transcription factor 2)	*E2f2*	4	135.1	late onset autoimmunity with widespread inflammatory infiltrates, GN, ANA, enhanced T cell activation with accumulation autoreactive memory/effector T cells.	(322)
C1q, α, ß, γ polypeptides (different genes)	*C1q a,b,c*	4	135.8	autoAb, GN	(50, 432)

Tgfß1	*Tgfb1*	7	20.9	multiorgan lymphocytic and monocytic infiltrates	(288)
Zfp-36 (tristetraprolin)	*Zfp36*	7	23.8	complex systemic disease: cachexia, dermatitis, arthritis	(388, 390, 433)
CD22	*Cd22*	7	26.3	enhanced B cell activation, autoAb	(218, 434)
MFG-E8 (milk fat globule-EGF factor 8)	*Mfge8*	7	72.9	splenomegaly, incr. GCs, autoAbs, GN. reduced engulfment of apoptotic cells.	(378)
LAT (linker for activation of T cells)	*Lat*	7	120.4	mutation inhibits T cell development, but induces lymphoproliferation	(310)
PCMT (protein-L-isoaspartate (D-aspartate) *O*-methyltransferase 1	*Pcmt1*	10	7.5	wild-type mice reconstituted with PCMT(-/-) BM develop high titer anti-DNA Ab and GN	(325)
fyn (+lyn)	*Fyn*	10	39.4	synergizes with the Lyn ko to accelerate disease; in MRL-Fas*lpr* mice suppresses autoimmune dis., but exacerbates lymphoprolif.	(435, 436)
Gadd45ß	*Gadd45b*	10	81.1	splenomegaly, glomerular immune complexes, no autoAbs; synergizes with Gadd45 $ß^{-/-}$ to produce marked splenomegaly, dsDNA/histone Abs, immune complex GN.	(308)
TACI (tumor necrosis factor receptor superfamily, member 13b)	*Tnfrsf13b*	11	60.9	fatal lymphoprolif., autoAb, GN	(244)
Aiolos (zinc finger protein, subfamily 1A, 3)	*Znfn1a3*	11	98.3	activated B cells, increased IgG, autoAb	(232)
PECAM-1, CD31 (platelet/endothelial cell adhesion molecule 1)	*pecam1*	11	106.5	ANA, immune complex GN with age	(259)
Stra13 (stimulated by retinoic acid 13)	*Stra13*	11	120.5	lymphoid organ hyperplasia, autoAb, IC GN	(354)
G2A (G protein-coupled receptor 132)	*Gpr132*	12	108.3	lymphoid hyperplasia, hyperIgG, autoAb, GN	(312)
Gadd45 γ	*Gadd45g*	13	50.4	synergizes with Gadd45 ß knockout (chr 10).	(308)
Protein kinase C δ	*Prkcd*	14	28.7	splenomegaly, lymphadenopathy, hyperIgM/IgG1/IgG2a, dsDNA, GN	(253)
IL-2Rß	*Il2rb*	15	78.5	lymphoproliferation, hyperIgG, autoAb, anti-RBC Ab	(341)
DNase1	*Dnase1*	16	3.7	ANA, immune complex GN	(359)
Cbl-b (Casitas B-lineage lymphoma b)	*Cblb*	16	50.9	multiorgan lymphoid infiltrates, anti-dsDNA Ab	(298)
p21 (cyclin-dependent kinase inhibitor 1A)	*Cdkn1a*	17	26.9	antichromatin Ab, GN, female predominance	(300)
Complement component 4	*C4*	17	32.4	impaired immune complex clearance, ANA, GN, female predom.	(356)
Emk (ELKL motif kinase, Par-1, MAP/microtubule affinity-regulating kinase)	*Mark2*	19	7.0	growth retardation, hypofertility, splenomegaly, lymphoid infiltrates, immune complex GN	(399)
Pten	*Pten*	19	32.1	lymphadenopathy, autoAb, GN, decr. survival, female predom. (+/- knockout mice)	(349)
Transgenics					
FLIP (retrovirus-mediated expression)	*Cflar*	1	59.0	hyperIgG, autoAbs, GN	(346)
Bcl-2 (B cell promoter)	*Bcl2*	1	106.5	lymphoid hyperplasia, hyperIgG, autoAb, GN	(329)
CD19	*Cd19*	7	120.4	increased B cell activation, B1 cell population, IgG, autoAb	(221)

BAFF (α1antitrypsin or β-actin-promoter)	*Tnfsf13b*	8	9.4	autoAb (RF, CIC, dsDNA Ab), GN	((242, 243))
Fli-1 (class I promoter)	*Fli1*	9	32.3	lymphoid hyperplasia, autoAb, GN	(256)
IFN-γ (keratin promoter)	*Ifng*	10	118.0	autoAb, GN, female predom.	(380)
IL-4 (class I promoter)	*Il4*	11	53.4	hyperIgG1/E, autoAb, GN	(381)
IEX-1 (Ig μ enhancer)	*Ier3*	17	33.5	lymphoproliferation, autoAb, GN, skin lesions, arthritis	(113)
LIGHT (lck promoter)	*Tnfsf14*	17	54.9	enlarged hyperactive periph. T cell compartment, lymphoid hyperplasia, autoAb, GN, lymphocytic infiltrates of tissues	(318)
CD154, CD40L (B cell- or epidermis-specific promoter)	*Cd40lg*	X	52.0	B cell promoter: late onset autoAb, GN epidermis promoter: dermatitis, lymphadenopathy, hyperIgG, dsDNA, GN)	(260) (379)

Genes are listed by chromosomal locations. Gene names and chromosomal locations are from the Mouse Genome Informatics (http://www.informatics.jax.org/) and Ensembl Mouse Genome Database (http://www.ensembl.org/Mus_musculus).
*ND, not determined.

Table 7-5: Mechanisms for Induction of Systemic Autoimmunity Defined by Single-Gene Alterations

Enhanced B Cell Activation*
Lyn knockoutCD22 knockout
SHP-1 loss-of-function mutations
Fcγ RII knockout
Aiolos knockout
CD19 transgenic
Tnfsf13b (BAFF, BlyS, TALL-1, or THANK) transgenic
TACI knockout
CD45 E613R knockin mutation
PKCδ knockout (tolerance pathway)
Fli-1 transgenic
PECAM-1 knockout
CD40L transgenic (B cell–specific expression)
Enchanced T Cell Activation
CTLA-4 knockout
PD-1 knockout (probably B cells as well)
TGF-ß deficiency (knockout/dominant negative)
Cbl-b knockout (possibly B cells as well)
$p21^{cip1/waf1}$ knockout (pancyclin kinase inhibitor, primarily T cells)
Gadd45α knockout
Gadd45ß knockout (with and without Gadd45γ knockout)
LAT Y136F knockin mutation
G Protein-Coupled Receptor G2A knockout
Mgat5 knockout (enhanced T cell activation)
LIGHT transgenic (with proximal Lck, but not CD2 promoter)
E2F2 knockout
Roquin M199R mutation
PCMT knockout
Defective Apoptosis
Fas or FasL mutations (*lpr* and *gld* mice, ALPS in humans)
Bim knockout
Bcl-2 transgenic
TSAd knockout (primarily T cells)
IL-2 or IL-2R knockout (primarily T cells)
Flip transgenic
Pten+/- knockout
IEX-1 transgenic
RasGRP1 mutation (loss-of-function RNA splicing defect)
Stra13 knockout
Defective Clearance of Proinflammatory/Immunostimulatory Apoptotic Material
C1q knockout
C4 knockout
DNase I knockout
Tyro3 family (Tyro3, Axl, Mer) triple-gene knockout
Ro (Trove2) knockout
MFG-E8 knockout
Enhanced Antigen Presentation
CD40L transgenic (keratin-14 promoter)
Cytokine-Mediated Activation
IL-4 transgenic
IFN-γ; transgenic
TTP (Zfp-36) deficiency (excessive TNFα)
TNFα transgenic
Other Mechanisms
α-mannosidase II knockout
Emk knockout
Nrf2 knockout (anti-oxidant)

*Genes are tentatively categorized by the most likely mechanism.

BLyS (BAFF, Tnfsf13b) is a member of the TNF ligand superfamily expressed primarily on cells of myeloid origin, such as monocytes and dendritic cells (233 ,234 ,235 ,236). Both the transmembrane protein and a secreted homotrimeric form, released by cleavage at a furin canonical motif in the stalk region, are active in the costimulation of B cells, the main cell type known to express its receptor. Three TNF family members, BR3, TACI, and BCMA, are the receptors for BLyS, of which TACI and BCMA are also receptors for APRIL (237 ,238 ,239). BLyS promotes peripheral B cell survival, particularly the transitional type 2 B cells; a subset of splenic immature B cells that are considered targets for negative selection (240 ,241). Overexpression of BLyS in the liver (α_1-antitrypsin promoter with the APO E enhancer) (242) or in multiple tissues (ß-actin promoter) (243) resulted in a similar picture, consisting of elevated numbers of B cells and, to a lesser extent, T cells, as well as increases in activated Bcl-2-expressing mature B cells, memory/effector phenotype T cells and syndecan-1-positive plasma cells. B cells from transgenic mice survived longer in culture than those from wild-type controls. Furthermore, transgenic mice developed a lupus-like disease characterized by elevated levels of all immunoglobulin subclasses, rheumatoid factor, anti-DNA antibodies, circulating immune complexes, and kidney immunoglobulin deposits with proteinuria and elevated blood urea nitrogen. Importantly, elevations in BLyS was also shown in both BWF1 and MRL-*Fas*^{lpr} mice, and treatment of BWF1 mice with a soluble TACI-Ig fusion protein, which blocks BLyS function, inhibited proteinuria and prolonged survival (237).

Deficiency of the BLyS and APRIL receptor, TACI (*Tnfrsf13b*) also leads to the development of anti-DNA autoantibodies, immune complex-mediated GN and early mortality (40% mortality at 18 months) (244). TACI$^{-/-}$ mice exhibit marked B cell hyperplasia along with enhanced antigen-specific antibody production, and in vitro activation of the TACI intracellular domain could inhibit B cell activation and, in the A20 B cell line, induce apoptosis (244 ,245 ,246). These studies suggest that TACI plays a significant negative regulatory role in B cells. Interestingly, loss of function mutations of TACI in humans is primarily associated with common variable immunodeficiency and IgA deficiency, and not systemic autoimmunity (247 ,248). The reason for this difference remains to be determined, but might be a result of species-specific differences or possibly background gene effects.

CD45 is a receptor protein tyrosine phosphatase expressed on all nucleated hematopoietic lineages, where it is required for antigen receptor signal transduction and functions to promote cell activation. Homodimerization of CD45 inhibits phosphatase activity by symmetrical interactions, wherein the catalytic site of one molecule is blocked by a structural wedge from the other (249 ,250). Replacement of the glutamine at residue 613 by an arginine (E613R) destroys the inhibitory capacity of the wedge, and knockin mice with this mutation were generated to examine the significance of dimerization (251). Although mice appeared normal at 4 weeks, they subsequently developed progressive lymphadenopathy and splenomegaly, increased number of activated T and B cells, anti-dsDNA autoantibodies, immune-complex GN and early mortality. Autoimmunity was dominantly transmitted. Expression of the E613R mutation in B cells, but not T cells, is critical for the development of autoimmunity and leads to B cell hyperresponsiveness (252).

Protein kinase Cδ (PKC-δ) is highly expressed in developing pro- and pre-B cells, and is involved in B cell receptor (BCR) signaling. Mice homozygous for the null mutation in the PKC-δ gene developed splenomegaly, lymphadenopathy, increased numbers of B2 cells, germinal center formation and IL-6 production, mild increases of IgM, IgG1, and Ig2a, antinuclear and IgG1 anti-DNA autoantibodies, as well as GN (253 ,254). Further studies in PKC-δ-deleted mice transgenic for the HEL Ig receptor exposed to soluble HEL (as artificial autoantigen) indicated that absence of PKC-δ prevented B cell tolerance, and allowed maturation and terminal differentiation of self-reactive B cells (253). Since deficiency of PKC-δ did not affect BCR-mediated B cell activation in vitro and in vivo, it was concluded that PKC-δ plays a selective and essential role in tolerogenic, but not immunogenic, B cell responses.

Fli-1 is s member of the Ets family of transcription factors that bind to DNA sequences containing the consensus GGA(A/T) core motif and regulate gene expression (255). Overexpression of a Fli-1 transgene, under the control of the MHC class I promoter, was found to result in lymphoid hyperplasia, hypergammaglobulinemia, elevated antinuclear antibodies, and severe immune complex GN (256). Fli-1 transgenic B cells were hyperresponsive to a variety of stimuli, showed resistance to AICD and had prolonged survival compared to nontransgenic B cells. Although Fli-1 expression is increased in murine SLE (257), the significance of this finding is difficult to assess because of nonphysiologic expression of Fli-1. Additional studies in heterozygous Fli-1$^{+/-}$ MRL-*Fas*^{lpr} showing reduced serum

IgG, autoantibodies, and GN as well as improved survival (257), however, suggests that Fli-1 plays an important role in lupus pathogenesis. Homozygous Fli-1-deficiency is lethal in utero and therefore Fli-1$^{+/-}$ mice, which are viable and express half the level of Fli-1 protein, were studied.

Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), a member of the immunoglobulin superfamily expressed on endothelial and hematopoietic cells, has two cytoplasmic ITIM domains suggesting a role in regulating cell activation. Nevertheless, initial studies of PECAM-1 knockout mice were focused on the role of PECAM-1 on adhesion, however, it was not found to play an essential role in either vascular development or leukocyte migration (258). On further examination, PECAM-1-deficient mice were found to develop antinuclear autoantibodies by 9 months of age and significant immune complex GN by 17 months (259). Several B cell alterations, including increased response to BCR engagement, LPS, and DNP-Ficoll, reduced B2, but increased B1a cells, and an apparent block in the transition of immature to mature B cells in the bone marrow were observed. These findings suggest that PECAM-1 may play a significant role in the regulation of B cells and autoimmunity.

Ectopic expression of a transgenic CD40L on B cells (VH promoter, IgH intron enhancer, and Ig 3′ enhancer) was shown to result in enhanced polyclonal IgG, anti-dsDNA and, in about half of the mice, the development of immune complex GN (260). CD40L is a member of the TNF ligand family, expressed mainly on activated T cells (261). It interacts with CD40 on B cells, and plays a pivotal role in promoting B cell proliferation and survival. Of interest, previous studied have reported ectopic expression of CD40L in patients with SLE (262 ,263) and in BXSB mice (264).

Genes Related to T Cell Activation

Systemic autoimmunity also develops after knockout deletion of certain genes that primarily alter T cell function. CTLA-4, a surface glycoprotein expressed exclusively on T cells, acts as an inhibitor of the CD28-B7.1/B7.2 costimulatory pathway in part by binding with higher affinity to B7.1 and B7.2. Consequently, mice with homozygous deletion of CTLA-4 develop a multi-organ lymphoproliferative disease associated with increased frequency of activated B and T cells, hypergammaglobulinemia and early mortality at 3 to 4 weeks of age with severe myocarditis and pancreatitis (265 ,266 ,267). The abnormal T cell expansion and disease manifestations are not a result of alteration in thymocyte development (268), but to a failure to maintain homeostasis of activated peripheral T cells, primarily the CD4+ subset (269 ,270). The precise mechanism through which this occurs has yet to be determined (271).

PD-1 is a 55 kDa ITIM-containing transmembrane cell surface glycoprotein expressed on activated T and B lymphocytes and monocytic cells that appears to play an important nonredundant role in maintaining the homeostasis of lymphocytes and myeloid cells following their activation (272 ,273). Engagement of PD-1 by its ligands, PD-L1 and PD-L2, has been shown to inhibit TCR-mediated lymphocyte proliferation and cytokine secretion (274). Mice deficient for PD-1 develop moderate hyperplasia of lymphoid and myeloid cells, increases in several Ig isotypes (particularly IgG3), enhanced responses to IgM cross-linking, and alterations in peritoneal B1 cells (275). Older (14-month-old) C57BL/6-PD-1$^{-/-}$ mice spontaneously develop GN and proliferative arthritis, but not elevated anti-dsDNA antibodies or rheumatoid factor (276). Further acceleration of GN and arthritis occurs when the PD-1 deletion is combined with the *Fas^{lpr}* mutation. Interestingly, manifestations appear highly dependent on background genes. For example, in contrast to the findings in the C57BL/6 strain, BALB/c mice-PD-1$^{-/-}$ mice develop a dilated cardiomyopathy as a result of anticardiac troponin I antibodies (277). Thus, PD-1 deficiency may accelerate background predisposition to autoimmunity, similar to the *Fas^{lpr}* and *Yaa* mutations. Lack of PD-1 also enhances EAE (278) and diabetes in NOD mice (279 ,280). While the mechanistic details not yet fully elucidated, it is evident that PD-1 plays an important nonredundant role in maintaining lymphocyte and myeloid cell homeostasis following their activation (273). Interestingly, human SLE has been linked in one population group to a regulatory polymorphism in the PD-1 gene (PDCD1) (281) and in other studies to other autoimmune diseases (282 ,283 ,284 ,285).

TGF-ß1 gene knockout mice rapidly develop massive necrotizing lymphocytic and monocytic infiltrates in multiple organs soon after birth, and succumb by around 3 weeks of age (286 ,287). Serum IgG autoantibodies to nuclear antigens as well as Ig glomerular deposits are detected, but appear to play a minor role in overall disease severity (288). Although deficiencies of either class II or class I (ß2m) molecules combined with TGF-ß1$^{-/-}$ reduced both tissue inflammation and autoimmunity, implicating both CD4^{+} and CD8^{+} T cells in these processes, in both instances there was only partial improvement in survival because of the remaining lethal myeloproliferative abnormalities (289 ,290). Direct inhibition of TGF-ß1 in T cells by a dominant negative TGFß receptor type II transgene under the control of a modified CD4 promoter specific for CD4^{+} and CD8^{+} T cells resulted in sickness, wasting, and diarrhea around 3 to 4 months of age, monocytic infiltrates in multiple tissues, enlarged peripheral lymphoid organs, increased percentage of memory/effector phenotype T cells, hypergammaglobulinemia, autoantibodies and glomerular immune complex deposits (291). Thus, TGF-ß is crucial for maintenance of T cell homeostasis and suppression of autoimmunity.

Cbl-b is member of the cbl family of adaptor proteins that predominantly function to inhibit receptor and nonreceptor tyrosine kinases (292 ,293). Two members, cbl-b and cbl, share a complex structure consisting of an amino-terminal phosphotyrosine-binding domain (PTB), a C3HC4 RING finger, a proline-rich region capable of binding proteins with SH3 domains, several phosphotyrosine residues for binding SH2 domains, a ubiquitin recognition

sequence and, at the carboxy-terminal, a putative leucine zipper. A smaller third member (cbl-c or cbl-3) was identified that contains the PTB, RING finger and a truncated proline-rich region, but is missing the rest of the carboxy portion (294 ,295). Cbl-b proteins appear to function as a negative regulator by inhibition of receptor clustering and raft aggregation in cell membranes (296). Cbl-b is expressed in normal and malignant mammary epithelial cells, a variety of normal tissues, and in hematopoietic tissues and cell lines. In accordance with their negative regulatory role, T cells from mice homozygous for the cbl-b gene knockout exhibit enhanced proliferation to antigen receptor signaling and do not require CD28 costimulation for IL-2 production or generation of T-dependent antibodies (297 ,298). Significantly, enhanced basal and activated levels of Vav, a guanine exchange factor for Rac-1/Rho/CDC42, was the only alteration in TCR signaling identified in these knockout mice. This was consistent with the previous findings that cbl-b binds to Vav and, when overexpressed, inhibits Vav stimulation of the c-Jun terminal kinase (299). Cbl-$b^{-/-}$ mice exhibit increased susceptibility to autoimmunity, both to experimental autoimmune encephalomyelitis (297) and to a spontaneous generalized autoimmune disease (298). The latter consisted of multiorgan lymphoaccumulation of polyclonal activated B and T cells with parenchymal damage, increased plasma cells, and antibodies to dsDNA by 6 months of age. Curiously, spontaneous autoimmunity occurred in only one (298) of the two Cbl-b knockout studies, suggesting that background strain, environment, or other factors are important.

Gene knockout of the cyclin inhibitor $p21^{cip1/waf1}$ in mixed background (C57BL/6 and × 129/Sv) also resulted in the development of systemic autoimmunity characterized by lymphoid hyperplasia, elevated IgG1, IgG antinuclear antibodies, GN, and early mortality (300). In vitro T cell proliferation was enhanced in these mice and there was an accumulation of effector/memory phenotype ($CD44^{high}$) $CD4^+$ T cells, although levels of other activation/effector/memory cell markers, such CD25, CD62L, CD69, and CD45RB, were similar to wild-type mice. An increased proportion of splenic B cells also expressed an activated (HSA^{low}, IgG^{low}) phenotype. Interestingly, females had more severe disease than males, similar to human SLE. Based on these findings, it was suggested that p21 negatively regulates T cell proliferation following long-term stimulation, as is presumably the situation for autoantigen-reactive $CD4^+$ T cells. In sharp contrast, other studies of mixed B6/129 or BXSB female p21-deficient mice were not able to confirm the development of lupus suggesting that background effects may have been responsible for the intial findings (301 ,302). Moreover, studies of lupus-prone male BXSB mice found an increase in p21 and other cyclin inhibitors in the expanded memory/effector $CD4^+$ T cells that are predominantly arrested in G1 (303) and, in fact, BXSB-$p21^{-/-}$ males had significantly reduced disease (304). This led to the opposite hypothesis, that the accumulating $CD4^+$ T cells, following successive rounds of division, become unable to enter into cell cycle and are resistant to apoptosis because of the build-up of cyclin inhibitors, a state similar to replicative senescence. Although no longer cycling, such cells may nonetheless secrete cytokines and activate B cells. Further studies will be needed to determine the precise role of p21 in spontaneous SLE.

The Gadd45 (growth arrest and DNA damage-inducible gene) family is composed of three members, α, ß, and γ, that play pivotal roles in replication, growth arrest, and apoptosis (305 ,306). Of these, Gadd45α is the only member regulated by p53, and is an important molecule in several processes, including maintenance of genomic stability, cell growth control, nucleotide excision repair, chromatic accessibility, and apoptosis. Several of these effects may be mediated by interactions of the Gadd45α with Cdc2, PCNA, and the MEKK4/MTK1 kinases (307). Studies in Gadd45α-deficient mice have shown that this molecule is a negative regulator of T cell proliferation induced by antigen receptor-mediated activation, and that deletion of the Gadd45α gene leads to the development of a lupus-like syndrome, particularly when coupled with deletion of the p21 cyclin-kinase inhibitor (307). While disease in Gadd45α-deficient mice was more severe in females than males, equal severity was seen when mice were deficient for both Gadd45α and p21. More recently, it was found that Gadd45ß deficiency is associated with enhanced T cell proliferation and resistance to AICD, splenomegay, and mild immune complex glomerular deposits (308). Gadd45$ß^{-/-}$ mice are also more susceptible to EAE. The related Gadd45γ also regulates proliferation and death of $CD4^+$ T cells, however, Gadd45γ-deficiency is not associated with significant autoimmunity (308). Nevertheless, the addition of the Gadd45γ knockout mutation to Gadd45$ß^{-/-}$ mice results in significant worsening of all lupus-like disease parameters suggests that Gadd45γ deficiency may be able to promote autoimmunity in certain susceptible backgrounds.

LAT (linker for activation of T cells) is a transmembrane scaffolding protein that, after TCR engagement, becomes tyrosine-phosphorylated and recruits multiple signaling molecules important for T cell activation (309). The distal four tyrosine residues of LAT (Y132, Y171, Y191, and Y226) are required for its activity. “Knock-in” mutation of LAT in mice in which the Tyr136 position was mutated to phenylalanine showed severe, but incomplete, block in T cell development associated with marked reduction in immature $CD4^+8^+$ and mature $CD4^+$ and $CD8^+$ thymocytes and splenocytes (310). However, by 4 weeks of age, homozygous mutant mice exhibited lymphadenopathy and splenomegaly with elevated markers of activated polyclonal $CD4^+$ T cells, B cells, macrophages and eosinophils, lymphocytic infiltrates in various organs, high production of IL-4, hypergammaglobulinemia (especially IgE and IgG1), and autoantibodies to nuclear antigens (310 ,311). These phenomena were attributed to defective PLCγ 1-calcium signaling pathway in early T cell development, leading to

inefficient negative selection of self-reactive T cells and their exportation to the periphery wherein activation of these cells may not depend on LAT or PLC-γ 1 (310).

G2A is an orphan G protein-coupled receptor (GPCR) expressed in various tissues, including lymphoid tissues (312). A variety of studies have suggested that G2A is a negative regulator of proliferation and integration of extracellular signals with cytoskeletal reorganization. Mice with targeted disruption of the gene encoding G2A showed a normal pattern of T and B lineage differentiation. As they aged, however, they developed secondary lymphoid organ enlargement, expansion of T and B cells, enhanced T cell proliferation responses and, when over 1 year of age, a progressive wasting syndrome, lymphocytic infiltration into various tissues, hypergammaglobulinemia, immune complex GN, and antinuclear autoantibodies (312). Although the molecular basis underlying the hyperresponsiveness of $G2A^{-/-}$ lymphocytes to TCR stimulation is presently unknown, the results reinforce the concept that lower thresholds for TCR activation can lead to overt autoimmune disease. Such a lower threshold for activation coupled with defective Fas-mediated apoptosis has also been considered to be a major contributor to the MRL-*lpr* lymphadenopathy and spontaneous lupus-like disease (313).

Mice deficient of the of the β1,6 N-acetylglucosaminyltransferase V (Mgat5), an enzyme in the N-glycosylation pathway develop late onset proliferative GN suggestive of an autoimmune–mediated pathogenesis, however, autoantibodies were not examined (314). Absence of this enzyme led to lowering of the T cell activation threshold by enhancing TCR clustering. Thus, T cells from deficient mice showed increased recruitment of TCRs to anti-CD3η antibody-coated polystyrene beads and required lower concentration of anti-CD3 plus anti-CD28 for efficient proliferation compared to control cells. Additional experiments showed that Mgat5 initiates GlcNAc β1,6 branching on N-glycans, thereby increasing N-acetylactosamine, the ligand for galectins, which are proteins known to modulate T cell proliferation and apoptosis (314 ,315 ,316). The findings indicate that a galectin-glycoprotein lattice strengthened by Mgat5-modified glycans restricts TCR recruitment to the site of antigen presentation, and therefore, dysregulation of Mgat5 increases T cell activation and susceptibility to autoimmunity.

LIGHT (*Tnfsf14*), a TNF superfamily member expressed transiently on activated T cells and on immature dendritic cells, binds to both the LTα1β2 heterotrimeric and HVEM (herpesvirus entry mediator) receptors and the DcR3 decoy receptor (317). LIGHT has been proposed to act as a costimulatory molecule for T cell activation (318). Transgenic expression of LIGHT on T cells by the proximal Lck promoter resulted in peripheral lymphoid organ hyperplasia, activation, and expansion of mature T cells, and increased T cell cytokine production, including IFN-γ and IL-4 (318). Transgenic mice also developed manifestations of lupus, including anti-DNA antibodies, RF, and immune complex GN, and also inflammation of the intestines and skin. In contrast, another LIGHT transgenic driven by the CD2 promoter had many of the same manifestations, but differed by the expansion of B cells in the lamina propria, the destruction of reproductive organs by bone marrow-derived cells, no increase in peripheral organ T cells, and, importantly, no lupus-like manifestations (319). These disparities have been ascribed to differences in the tissue-specific expression of the promoters (320).

E2F2 is a member of the E2F family of DNA binding heterodimers that are sequestered by Rb and released after Rb phosphorylation by activated cyclin/CDK kinases (321). Mice lacking E2F2 developed late-onset autoimmune features characterized by perivascular inflammatory infiltrates of multiple organs, splenomegaly, skin lesions, anti-dsDNA autoantibodies, and immune complex GN (322). Similar to spontaneous lupus, there were increased numbers of autoreactive effector/memory T cells with age. The most likely mechanism appeared to be lowering of the TCR activation threshold and more rapid entry of activated T cells into S phase in E2F2 deficiency. No defects in Fas- or dexamethasone-mediated apoptosis were detected. Further studies suggested that E2F2 might function as a transcription repressor of genes required for normal S phase entry, particularly E2F1 (322).

Roquin (*Rc3h1*) is a 1130 residue ubiquitously expressed member of the E3 ubiquitin ligase family based on the presence of a highly conserved amino terminal RING-1 zinc finger domain (323). Recently, a loss-of-function M199R mutation within the highly conserved ROQ domain of Roquin was generated by ENU mutagenesis (sanroque mouse strain) that resulted in the development of ANA, anti-dsDNA autoantibody, GN, necrotizing hepatitis, anemia, and immune thrombocytopenia (323). Lymphoid organ hyperplasia, polyclonal hypergammaglobulinemia, increased germinal centers, and expansion of memory/effector $CD4^{+}$ T cells, particularly follicular helper T cells, were evident, along with overexpression of the ICOS costimulatory receptor and IL-21. The presence of a CCCH zinc finger common to several RNA-binding proteins and localization of Roquin to cytosolic RNA granules suggested that Roquin regulates mRNA translation and stability, and likely acts as a repressor of ICOS.

Protein carboxyl methyltransferase (PCMT) is a highly conserved enzyme that repairs isomerized and racemized derivatives of L-asparaginyl and L-aspartyl residues produced in cells by spontaneous degradation (324). The critical importance of this function has been clearly demonstrated by $PCMT^{-/-}$ mice, which accumulate significant amounts of the aspartyl derivatives in brain, heart, liver, and RBCs, have growth retardation, and develop fatal seizures around 42 days of age (324). T cells from PCMT knockout mice are hyperresponsive to mitogen and antigen-receptor-mediated stimulation, while B cell responses are similar to wild-type animals (325). Despite the T cell defect, no significant alterations in T cell number or proportions of CD4, CD8, or effector/memory phenotypes,

were observed in young PCMT$^{-/-}$ mice. Mice reconstituted with PCMT-deficient bone marrow, however, developed positive ANA and dsDNA autoantibodies 7 to 9 months after transfer. At this time, kidney sections showed perivascular infiltrates, focal areas of necrosis and vasculitis, but no apparent immune complex GN. Overall, the effects of PCMT deficiency on bone marrow-derived cells is less severe than in other tissues, such as the brain, and appears limited to significant enhancement of T cell activation in vitro, and mild lupus-like disease in bone marrow chimeras.

Defective Apoptosis

Bim is a proapoptotic ligand of the Bcl-2 family that shares homology with other members in only the short (nine amino acid) BH3 motif (326). Through this domain, Bim binds to anti-apoptotic Bcl-2 molecules and blocks their function. Bim is largely bound to the cytoplasmic dynein light chain LC8 that is normally sequestered in the microtubule-associated dynein motor complex (327). Certain apoptotic signals release LC8, allowing LC8, together with Bim, to translocate to Bcl-2 and inhibit its function. Homozygous knockout of Bim resulted in an incompletely penetrant embryonic lethal phenotype apparently for nonimmunologic reasons. In the surviving offspring, however, alterations in the homeostasis of multiple hematopoietic cell lineages developed (328). As anticipated, Bim-deficient B and T lymphocytes were resistant to certain apoptosis-promoting signals, but not to FasL. The knockout mice were found to have lymphoid hyperplasia with increases in naive T and B cells, altered thymocyte subset composition, and increases in granulocytes and monocytes in the peripheral blood. With age, these mice developed systemic autoimmunity manifested by progressive lymphadenopathy and splenomegaly, dramatic expansion of plasma cells, hyper IgM, IgG, and IgA, antinuclear antibodies, immune complex GN, and vasculitis with a 55% survival at 1 year.

Similarly, transgenic expression of the Bcl-2 gene in B cells under the immunoglobulin enhancer resulted in lymphoid hyperplasia, hypergammaglobulinemia, high titers of antinuclear antibodies, and immune complex GN (329). Studies thus far have suggested that the constitutive Bcl-2 expression may promote autoimmunity by blocking apoptosis of autoantibody–producing B cells that normally arise spontaneously in germinal centers during the primary response to foreign antigens (330 ,331 ,332).

The T cell–specific adaptor protein (TSAd), encoded by the *Sh2d2a* gene and expressed in thymocytes and activated T cells, is an intracellular adaptor molecule whose specific role in T cell signaling transduction is not yet known (333 ,334). Nevertheless, TSAd-deficient mice develop elevated levels of IgG and IgM, autoantibodies to dsDNA, cardiolipin, and IgG (RF), immune complex GN, and lymphocytic infiltrates, particularly in the lung (318). TSAd$^{-/-}$ mice also exhibited greater susceptibility to pristane-induced autoimmunity. The development of autoimmunity may be related to the finding that TSAd-deficient T cells were more resistant to superantigen-induced cell death in vivo (similar to Bim knockout mice). In the case of TSAd, the impaired death response may be caused by reduced IL-2 synthesis (335). Although no studies have been reported for SLE, a GA repeat polymorphism within the SH2D2A promoter region associated with lower levels of TSAd has been linked to multiple sclerosis (336).

Gene knockouts of IL-2 (337 ,338 ,339) or either of its high (IL-2Rα) (340) or low (IL-2Rß) (341) affinity receptors result in a similar syndrome consisting of late immunosuppression with defective antibody and CTL responses, but also lymphoproliferation, expansion of memory/effector phenotype T cells, polyclonal hypergammaglobulinemia, autoantibodies, and immune-mediated hemolytic anemia. Inflammatory bowel disease similar to human ulcerative colitis occurs in mice lacking IL-2 (342) or IL-2Rα (340), but not IL-2ß (341). Autoantibody production depends on the expanded CD4^{+} T cells (341) and CD40/CD40L interaction (337). The accumulation of T lymphocytes appears to be a result of resistance of IL-2-deficient T cells to AICD, at least partly from decreased Fas-mediated apoptosis (343).

FLIP (gene name *Cflar*, for Caspase 8 and FADD-like apoptosis regulator) is a death-effector domain-containing protein similar in structure to the apoptosis-promoting CASP8 (FLICE), but devoid of a caspase domain. In contrast to FLICE, FLIP inhibits death receptor-induced apoptosis by blocking the recruitment and activation of CASP8 (344 ,345). Transplantation of bone marrow cells retrovirally transfected with FLIP resulted in the resistance of B and T cells to AICD, expansion of these cell populations and, in 4- to 6-month-old animals, the development of hypergammaglobulinemia, anti-dsDNA autoantibodies, and glomerular immunoglobulin deposits with histologic evidence of GN characterized by glomerular sclerosis and thickening of mesangium and basement membranes (346). Despite the fact that FLIP blocks Fas signaling of AICD, there were no accumulations of B220^{+} or CD4^{-}CD8^{-} DN T cells, or activated T cells. This suggests that Fas and/or FLIP may affect nonoverlapping pathways.

PTEN is a protein/lipid phosphatase initially identified as a tumor suppressor gene on chromosome 10q23, which is associated with a wide range of human malignancies (347 ,348). Germline mutations of PTEN also cause three autosomal dominant disorders: Cowden disease, Lhermitte-Duclos syndrome, and Bannayan-Zonana syndrome. Homozygous knockouts of PTEN are embryonic lethal, but heterozygous mice develop an autoimmune disorder characterized by severe polyclonal lymphadenopathy, diffuse inflammatory cell infiltrates of most organs, hypergammaglobulinemia, anti-DNA antibodies, immune complex GN, and decreased survival (349). Females were more severely affected, with survivals younger than 12 months of age compared with males older than 15 months. Defective Fas-mediated AICD of T and B lymphocytes was observed in PTEN-deficient mice as a result of impaired Fas signaling associated with increases in the survival factor, Akt. It was, therefore,

postulated that uninhibited increases in phosphatidylinositol (3 ,4 ,5)-triphosphate (PIP-3), the major substrate for PTEN (350), leads to the recruitment and activation of Akt and possibly other factors, which then inhibit Fas-mediated killing (349). PTEN-deficient mice, however, in contrast to *Fas^{lpr}* mice, did not have increases in either B220^{+} or CD4^{-}CD8^{-} DN T cell populations, and furthermore, PTEN heterozygous knockouts had more severe disease than nonautoimmune background Fas-deficient mice.

IEX-1 (Immediate Early response gene X-1, also named IER3, p22/PRG1, Dif-2, or mouse homology gly96) is a regulator of cell growth and apoptosis. Expression of this molecule is regulated by a variety of factors, including x-irradiation, ultraviolet radiation, steroids, growth factors, inflammatory stimuli, and various activators of the NF-κB/rel transcription factors (351). Mice transgenic for IEX-1 (under an H-2k^{b} promoter at the 5′ end and an Ig heavy chain (**(mu)**) enhancer at the 3′ end for specific expression in lymphocytes) exhibited decreased apoptosis of activated T cells, increased duration of an immune response effector phase, splenomegaly, lymphadenopathy, accumulation of activated T cells, increased polyclonal IgG2a and anti-dsDNA autoantibodies, alopecia of the skin, arthritis, and immune complex GN (113). These findings are particularly intriguing considering that the IEX-1 gene maps within the MHC locus of humans and mice.

RasGRP1 is a Ras guanine nucleotide exchange factor critical for the Ras-dependent maturation of thymocytes transitioning from the double-positive (CD4^{+}8^{+}) to single-positive (CD4^{+}CD8^{-}, CD4^{-}CD8^{+}) stages (352). Despite this, mice with a spontaneous RasGRP1 mutation (designated *lag* for lymphoproliferation-autoimmunity-glomerulonephritis), which blocked normal joining of exon 3 to exon 4 resulting in undetectable RasGRP1 protein levels, developed severe systemic autoimmunity by 5 to 8 months of age (353). Major manifestaions included enlarged spleens and LNs, hyperplastic germinal centers, hypergammaglobulinemia, ANAs, anti-dsDNA autoantibodies, diffuse proliferative GN with IgG and C3 deposits, and early mortality. In young *lag* mice, the number of peripheral T cells were reduced presumably from the block in thymocyte development. In contrast, older *lag* mice exhibited marked increases in CD4^{+} T cells that were predominantly of the memory/effector phenotype, consistent with a significant peripheral expansion of these cells. *Lag* CD4^{+} T cells had reduced responses to antigen and mitogens, but secreted significant amounts of IL-4 after activation. Importantly, CD4^{+} T cell from *lag* mice were resistant to AICD, which likely contributed to the lymphoproliferation and autoimmunity. B cells were also expanded and activated in lag mice, but, in contrast to T cells, they exhibited normal proliferation and apoptosis, suggesting that the B cell alterations were secondary to the T cell defects.

Stra13, a member of the basic helix-loop-helix family of transcriptional repressors, is expressed in lymphoid cells and activated CD4^{+} T cells (354 ,355). Although Stra13-deficient CD4^{+} T cells had reduced proliferation, as a result in part of impaired IL-2 secretion, Stra13 knockout mice developed manifestations of systemic autoimmunity by 4 to 5 months with lymphoid organ hyperplasia, increased number and activation of T and B cells, germinal center expansion, antinuclear antibodies, and immune complex GN (354). Activated CD4^{+} T cells in older knockout mice were polarized to the TH2 phenotype. The gradual accumulation of T and B cells appeared because of greater resistance of these cells to AICD from impaired differentiation of CD4^{+} T cells into effector cells and reduced expression of FasL following T cell activation. These studies indicate that Stra13 is a key regulator of T cell homeostasis and self-tolerance.

Defective Clearance of Proinflammatory/Immunostimulatory Self-Antigens

Deficiencies of early complement components (C1q-s, C2, or C4) have long been known to predispose to SLE, indicating an important regulatory role for the complement pathway in suppressing autoimmunity. Although inadequate clearance of immune complexes was postulated to be the most likely mechanism, this did not fully explain the loss of tolerance to nuclear antigens. Gene knockout mice for C1q and C4 were therefore generated to address this issue. Homozygous C1q-deficient mice recapitulated the human disorder with the development of typical, but mild, features of lupus, including autoantibodies and a 25% incidence of immune complex GN (50). A large number of apoptotic bodies were discovered in the glomeruli of these mice, suggesting that C1q plays an essential role in the clearance of apoptotic bodies.

In the case of C4 deficiency, accelerated lupus-like disease has been reported in both *Fas^{lpr}* (36) and normal background mice (356). Using a HEL Ig transgenic model, B cells from C4-deficient mice were shown to have a tolerance defect to soluble HEL (36). Thus, it was hypothesized that early complement components may be vital for maintaining self-tolerance by virtue of their role in presenting tolerizing antigens to B cells. Clearance of immune complexes is also impaired in C4 knockout mice by a mechanism independent of CR1/CR2 complement receptors (356).

A similar mechanism was also proposed to explain the unexpected development of lupus-like autoimmunity in mice with homozygous deficiency of serum amyloid P component (SAP), a highly conserved plasma protein named for its presence in amyloid deposits (357 ,358). Manifestations included female predominance, autoantibodies to chromatin and its components, but not to other nuclear, tissue, or organ antigens, immune complex GN, and low incidence of mortality. In contrast to C1q deficiency, no accumulation of apoptotic bodies was detected in glomeruli. SAP binds to DNA and chromatin, and can displace H1-histones, thereby increasing solubility and reducing the rate of degradation and clearance of chromatin (357). It was hypothesized that SAP promotes self-tolerance to chromatin and its subunits

by preventing immunogenic antigen-processing and/or by tolerizing chromatin-reactive lymphocytes. Subsequent studies, however, have strongly suggested that the association of SAP deficiency with lupus-like disease may have been entirely a result of the mixed 129xB6 background used in the initial study. SAP-deficient pure 129/Sv mice did not develop autoimmunity or produce significant autoantibodies following immunization with chromatin or apoptotic bodies, further backcrossing of the SAP knockout mutation to the B6 background resulted in reduced autoimmunity, and transgenic expression of human SAP in B6 SAP knockout mice did not correct the autoimmune phenotype (71). Moreover, comparison of (129xB6)F2 mice with and without the SAP knockout mutation, and comparison of the B6 SAP knockout with congenic B6 mice containing an introgressed chromsome 1 fragment that included the SAP *Apcs* (SAP) gene, indicated that essentially all of the traits previously ascribed to SAP deficiency could be accounted for by the 129 interval alone (32).

Dnase1, a 32–38 kDa protein, is the major nuclease present in the blood, urine, and secretions. Interestingly, knockout of the Dnase1 gene in nonautoimmune background mice was reported to increase the incidence of SLE manifestations, including positive ANA, anti-DNA antibodies, and immune complex GN (359). This finding is supported by recent observations that mammalian DNA in apoptotic material can stimulate B cells and dendritic cells via their TLR9 receptor (360 ,361 ,362). Interestingly, a loss-of-function mutation of DNASE1 has been associated with SLE in two patients (363).

Mutant triple knockout mice lacking a group of closely-related receptor tyrosine kinases, Tyro3, Axl and Mer (the Tyro3 family, TAM) have recently been reported to develop severe systemic autoimmunity characterized by splenomegaly, lymphadenopathy, increases in activated T and B cells, autoantibodies to phospholipids associated with thromboses and hemorrhage, autoantibodies to collagen and dsDNA, and deposition of immune complexes in tissues (364). The Tyro3 family of proteins are not expressed by quiescent lymphocytes, but are expressed by many other cell types, including APCs (macrophages, dendritic cells). Macrophages freshly isolated from TAM-deficient mice have increased expression of MHC class II molecules and produce elevated amounts of IL-2 and IFN-γ, suggesting their activation in vivo. Expression of these molecules and of CD86 (B7.2) was increased by these cells as well as by $CD11c^+$ cells subsequent to in vitro incubation with LPS. Moreover, CFSE-labeled B and T cells transferred to TAM-deficient mice hyperproliferated compared to cells transferred in the wild-type controls. Although Tyro-3 receptors are known to convey growth-promoting and prosurvival signals to cells (365), the findings with the mutant mice suggest that these receptors are also able to activate negative feedback loops that attenuate the positive signaling pathways that they have activated, and that the loss of negative signals from these receptors has more profound effects than the loss of the positive growth-promoting signals. Another means by which absence of these receptors may promote autoimmunity is by ineffective removal of apoptotic cells, which are increased in TAM-deficient mice (366). This latter possibility has been supported by the finding that mice with a cytoplasmic truncation of mer have macrophages deficient in the clearance of apoptotic thymocytes and develop a mild form of lupus-like disease with antibodies to chromatin (367 ,368).

Ro, a conserved RNA-binding protein encoded by the Trove2 gene, is a common target for autoantibodies in SLE, Sjogren syndrome, subacute cutaneous LE, neonatal lupus, and primary biliary cirrhosis (369). Ro binds to Y RNAs as well as other small cellular RNAs, such as 5S rRNA, and is thought to play a role in the stabilization and quality control of certain types of RNA (370). Interestingly, mice deficient in Ro were found to develop antinuclear autoantibodies and immune complex GN, and it was hypothesized that Ro may prevent autoantibody formation by sequestering defective and potentially autogenic ribonucleoproteins (371). Unsequestered RNA might also promote autoimmunity by activating B cells and dendritic cells through engagement of TLR7 (and also TLR8 in humans) or through TLR-independent pathways (360 ,372 ,373 ,374 ,375). However, other than slightly increased levels of IgG, IgM, and IgG2a, no other immunological alterations were observed. Furthermore, disease severity lessened as the knockout mutation was backcrossed to the B6 background, suggesting that 129xB6 background effects might account for some or all of the autoimmunity observed.

Milk fat globule EGF factor 8 (MFG-E8) protein, expressed in mammary glands, brain, spleen, and LN, functions as a bridging protein between phosphatidylserine on apoptotic cells to αvβ3 or αvβ5 integrins on phagocytic cells, thereby promoting the engulfment of apoptotic cells (376 ,377). In the spleen and LN, MFG-E8 is primarily expressed on tingible body macrophages ($CD68^+$) within germinal centers (378). Mice deficient for MFG-E8 developed splenomegaly with numerous germinal centers and increased numbers of B and T cells. Tingible macrophages in knockout animals were associated with increased numbers of unengulfed apoptotic cells. By 40 weeks, $MFG\text{-}E8^{-/-}$ mice had increased ANA and anti-dsDNA antibodies, as well as large glomerular deposits of IgG and hypercellular glomeruli. These findings suggest that inefficient phagocytosis of apoptotic B cells within germinal centers can lead to autoimmunity and is consistent with recent studies demonstrating that DNA and RNA in apoptotic material can activate B cells and dendritic cells through TLR9, TLR7, and TLR8 (360 ,361 ,362 ,372 ,373 ,374).

Enhanced Antigen Presentation

Targeted expression of CD40L to the basal keratinocytes of the epidermis of mice using the keratin-14 promoter was reported to lead to activation of resident tissue APCs (Langerhans cells) associated with dermatitis, lymphadenopathy, hypergammaglobulinemia,

anti-dsDNA autoantibodies, and immune complex GN (379). These findings are reminiscent of observations in mice transgenic for IFN-γ under the involurin promoter (380). Overall, they indicate that in situ activation of APCs in the skin can lead not only to local, but also systemic, autoimmune, and inflammatory responses, presumably a result of the migration of activated APC to the secondary lymphoid organs, and the management of pre-existing, previously quiescent, nontolerant self-reactive T cells.

Cytokine-Mediated Activation

Under certain circumstances, systemic autoimmunity also develops in mice transgenic for the major Th1 and Th2 cytokines, IFN-γ and IL-4, respectively. Expression of INF-γ in the suprabasal layer of the epidermis under the control of the involucrin promoter resulted in not only a severe inflammatory skin disorder, but also generation of autoantibodies to dsDNA and histone, and immune complex proliferative GN, particularly in females (380). This suggests that presentation of nuclear antigen by skin Langerhans cells and perhaps keratinocytes may be sufficient for the production of antinuclear autoantibodies, and provides a possible explanation for the UV sensitivity of SLE patients. Similarly, C3H mice transgenic for the IL-4 gene under the control of the MHC class I promoter also developed systemic autoimmunity characterized by elevated MHC class II molecules and CD23, enhanced responses to polyclonal stimuli in vitro, increased levels of IgG1 and IgE, anemia, antinuclear antibodies, and immune complex GN (381). These manifestations were likely a result of direct IL-4-induced polyclonal activation of B cells, since $CD4^+$ T cells were not required and there was no evidence for inefficient negative selection of B cells. The role of IL-4 in promoting lupus, however, is more complicated since autoimmunity was not observed in other transgenics expressing IL-4 on B cells or T cells (382 ,383 ,384 ,385), and expression of an IL-4 transgene in a spontaneous model of lupus did not exacerbate disease, but was protective (386). This would imply that a number of factors, such as the level and site of IL-4 production, and background genetic susceptibility, can modify the effects of IL-4 on systemic autoimmunity.

Tristetraprolin (*Zfp-36*) is a widely expressed zinc-binding protein initially thought to function as a transcription factor, particularly in lymphoid tissues, where high levels are found (387). Mice with homozygous knockout of tristetraprolin develop a complex syndrome consisting of cachexia, patchy alopecia, dermatitis, conjunctivitis, erosive arthritis, myeloid hyperplasia, glomerular mesangial thickening, and antinuclear antibodies (388). These manifestations are mainly caused by excessive TNF-α production by macrophages and are reversed almost entirely by treatment with anti-TNF-α antibody (388 ,389). Thus, tristetraprolin must function as a nonredundant negative regulator of TNF-α. The mechanism was later discovered to be due to the binding of tristetraprolin to an AU-rich element contained in the TNF-α mRNA, which destabilizes the mRNA (390). In contrast to the autoimmune–promoting effects of elevated levels of TNF-α, physiologic amounts may, under certain circumstances, play a role in suppressing systemic autoimmunity. In studies of *TNFR1* (p55) knockout mice, nonautoimmune mice did not develop defects in apoptosis or autoimmunity (391 ,392), yet the same knockout in lupus-prone C57BL/6-Fas^{lpr} mice resulted in accelerated lymphoproliferation and autoimmune disease (393). Since TNF can induce death of activated peripheral T cells (154), these findings might be attributable to TNF compensating for the lack of Fas in Fas^{lpr} mice.

Other Mechanisms

The α-mannosidase II enzyme is encoded by a single gene in mammals and resides in the Golgi apparatus, where it trims two mannose residues from hybrid N-linked oligosaccharides. This trimming of the mannose residues allows the subsequent addition of multiple glycan branches by glycosyltransferases required for generation of complex N-glycans found on mammalian cell surfaces (394 ,395). Mice rendered deficient in α-mannosidase II were reported to develop a lupus-like syndrome characterized by various autoantibodies, including anti-dsDNA, anti-Sm and antihistone, as well as hypergammaglobulinemia and immune complex GN (396). Nevertheless, lymphoid cell development, composition, and responses were normal. No clear explanation for appearance of systemic autoimmunity was evident, but alterations in N-glycan branching among some glycoproteins and tissues may lead to the formation of neoepitopes for which tolerance has not been established.

Emk (ELKL motif kinase) is a serine/threonine kinase with a conserved region of about 100 amino acids that terminates with the sequence glutamate-leucine-lysine/asparagine-leucine (a region referred to as the ELKL domain) (397). Emk has also been called MARK2, (microtubule affinity-regulating kinase 2), and mPar-1 based on homology with the Par-1 protein kinase of Caenorhabditis elegans (398). Evidence suggests Emk regulates cell polarity, cell cycle progression, and microtubule dynamics. It is expressed in several tissues, including the thymus and mature T and B cells (399). $Emk^{-/-}$ mice showed growth retardation and hypofertility (399 ,400). Additionally, as the deficient mice aged, they showed splenomegaly, lymphadenopathy, increased activated phenotype T cells ($CD44^{hi}CD62^{low}$, but no increases in IL-2R or CD69), increased responses to thymus-dependent antigens, lymphoid infiltrates in lungs, salivary glands and kidneys, and membranoproliferative GN with immune deposits and proteinuria (399).

The basic leucine zipper transcription factor Nrf2 (NF-E2-related factor 2) regulates an anti-oxidant response element (ARE) or EpRE (electrophile response element), whose consensus sequence is found in the 5′ regulatory region of a number of genes encoding detoxifying and anti-oxidant enzymes (401 ,402). Nrf-2-deficient female mice

over 5 mo of age reportedly developed severe GN with immune complex deposits together with higher serum IgG, anti-dsDNA antibody, and slight splenomegaly (403). In this regard, it is of interest that reactive oxygen species have been considered to be potential participants in the pathogenesis of lupus nephritis (404). Moreover, homozygosity for glutathione-S-transferase GSTM1 null alleles correlated with autoantibodies and lupus predisposition in humans (404) and mice deficient in heme-oxygenase-1 (a gene containing ARE) were noted to develop GN resembling that of the Nrf-2-deficient mice (405). In contrast, Nrf2 deficiency in MRL-*Fas*^{lpr} mice results in increased sensitivity to TNF-mediated apoptosis and disease suppression (406). Thus, the role of Nrf2 in lupus appears highly dependent on the underlying disease pathogenesis.

Conclusion

Considerable strides in defining the genetics of lupus have been made using spontaneous, induced, and genetically-manipulated mouse models. A clearer definition of the genetic landscape of lupus-predisposing loci/genes among lupus-predisposed and nonautoimmune strains has emerged, and progress in identifying susceptibility genes has advanced to cloning of candidates within finely mapped intervals. Studies of specific single-gene alterations that predispose to autoimmunity have suggested common mechanisms and identified unexpected pathways. Although complex, elucidation of the genetics of lupus should ultimately have a profound effect on our understanding of disease pathogenesis and therapy.

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Chapter 8

Apoptosis

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Apoptosis, Necrosis, and Autophagy

- *Apoptosis*: The modern understanding of apoptosis began with the electron microscopic descriptions of morphologic changes characterized by shrinkage of hepatocytes (i.e., shrinkage necrosis) after ischemic or toxic injury to the liver. The name *apoptosis* was coined by Kerr, Wyllie, and Currie in 1972 to describe the form of death that was "consistent with an active, inherently controlled phenomenon" characterized by cell shrinkage, nuclear condensation, and cell blebbing (Fig. 8.1) (1). This term also conveyed the concept of cell death that was similar to leaves falling from a tree (*apo* means "from" and *ptosis* "a fall" in Greek), implying a regulated "mechanism of cell deletion, which is complementary to mitosis" (1).

  Further developments in our understanding of apoptosis paralleled advances in molecular biology, genetics, and biochemistry. The detection of a nucleosomal ladder (2) was of considerable importance, because it defined a biochemical event (i.e., nucleosomal cleavage) and provided a simple electrophoretic test for detection of apoptotic cell death that remains a standard in the field. Studies in the 1980s demonstrated that the death of cells during nematode development was under strict genetic control. Remarkably, the death of these cells could be perturbed by mutation of a small number of genes called ced (for cell death abnormal) genes (3). Horvitz and colleagues determined that two ced genes, ced3 and ced4, encoded death effectors, whereas ced9 was an anti-apoptotic gene. Most of the remaining ced genes were responsible for engulfment and removal of the "corpses." This simple model in which CED-3 is the main death protease that is activated by CED-4 and inhibited by CED-9 has served as a paradigm for defining apoptotic pathways in mammalian cells. In 2002, Robert Horwitz was awarded the Nobel Prize for "discoveries concerning the genetic regulation of … programmed cell death" (http://www.nobel.se/medicine/laureates/2002/index.html).

  Mammalian cells are more complex and, as will be discussed in detail, have multiple defined pathways that follow the basic *Caenorhabditis elegans* model. The molecules within these pathways, the downstream effectors of apoptosis, the caspases (cysteine aspartate proteases), and the proteins implicated in the clearance of apoptotic cells are discussed in detail below. The control of cell death is of seminal importance in a number of diseases, including cancers, autoimmune diseases, and degenerative disorders (4 ,5). Regulation of apoptosis and handling of dying cells is especially relevant to SLE as discussed below.

- *Necrosis*: Necrosis has traditionally been viewed as a passive form of cell death resulting from toxic or physical insults leading to adenosine triphosphate (ATP) depletion. Morphologically, necrosis is notable for plasma cell membrane disruption leading to cellular swelling and cytoplasmic vacuolation (Fig. 8-1). In contradistinction to the genetically controlled program of apoptosis, necrotic death induces inflammation around the dying cell due to the release of various intracellular components (Fig. 8-1) (6). Although chromatin degradation is evident in necrotic cells, the condensation and organized DNA fragmentation seen in apoptotic cells is usually absent. Notably, the same inducers (e.g., ischemia, hydrogen peroxide) may produce apoptosis or necrosis, depending on the severity of the injury and the rapidity of cell death (7). The cell's fate is determined in part by cellular energy reserves such as ATP (8). Some inducers may initially cause apoptosis followed by necrosis (postapoptotic necrosis). This will be evident when removal of the apoptotic cells is delayed. The transition from apoptotic to necrotic cells is likely to be important in the context of autoimmunity (see below).
- *Autophagy*: Autophagy is an alternative, nonapoptotic form of programmed cell death that has gained much attention over the past several years. Autophagy means literally, to eat oneself, and in this process cells switch to a catabolic program in which cellular constituents are degraded for energy production as a survival mechanism during periods of nutrient stress. In cells undergoing autophagy a double membrane vesicle forms, and encapsulates whole organelles leading to degradation following fusion with lysosomes (Fig. 8-1). The ATG gene family, including ATG7 and beclin1, that is highly conserved from yeast through humans, is involved in autophagic cell death (9). Interestingly beclin1 is monoallelically deleted in a variety of human cancers, and may function in cell-growth control and tumor suppression (10). Autophagy shares a number of morphologic features with necrotic cell death and may also be seen in neuronal cell death associated with polyglutamine repeats. It has also been observed that autophagy may play a role in the survival of lymphocytes following growth factor withdrawal (11). The reader is directed to reviews of autophagy for more details of this process (12 ,13).

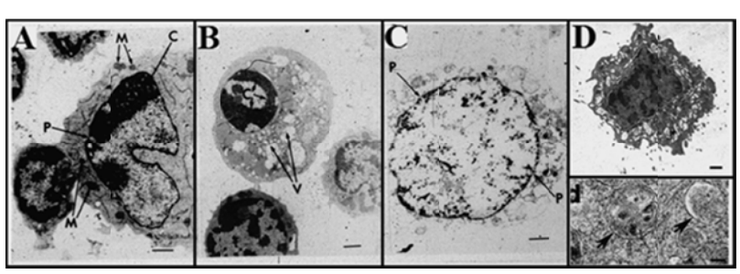

Figure 8-1. Electron microscopic morphology of cell death. A, A cytotoxic T cell (lower left) conjugated to its target, P815, a murine mast cell before the initiation of cell death. *B,* Induction of apoptotic changes in P815. Note the reduction in target cell size, nuclear condensation, and vacuoles with the relative preservation of organelles. *C,* Osmotic lysis and necrosis in P815 induced by antibody and complement. Note the increased size of the nucleus and apparently random fragmentation of the chromatin. Organelles are severely disrupted. *D,* Autophagic cell death of L929 cells treated 12h with caspase inhibitor (zVAD). Arrows show membrane-bound vacuoles characteristic of autophagosomes. Autophagy image courtesy of Dr. Mike Lenardo. C, dense chromatin; M, mitochondria; P, nuclear pore; V, vacuoles. (Adapted from

Russell JH, Masakowski V, Rucinsky T, et al. Mechanisms of immune lysis III characterization of the nature and kinetics of the cytotoxic T lymphocyte induced nuclear lesion in the target. *Immunol J* 1982:128:2087 with permission.)

Biochemistry of Apoptosis

Figures 8-2 and 8-3 show schematic overviews of the cell death program. A brief outline of each major functional component within the program, from the signals for death to removal of the apoptotic cells, is discussed here, but space limitations preclude a detailed analysis of the layers of regulation at each step of the pathway. For a more comprehensive discussion of the biochemical pathways controlling apoptosis the reader is referred to recent excellent reviews (5 ,14).

Numerous proteins involved in apoptosis, including receptors, adaptors, effectors, and inhibitors, contain modules/domains that are structurally similar and evolutionarily conserved. Interestingly these motifs are predominantly involved in promoting homotypic protein-protein interactions (Fig. 8-3). Furthermore, as will be discussed below, these domains occur in proteins involved in apoptosis as well as inflammation. It has been suggested that death domain (DD), death effector domain (DED), caspase recruitment domain (CARD), and Pyrin domains all evolved from the prototypic DD–fold corresponding to an antiparallel six helix bundle (15). Because of the central role for caspases, DNases and the bcl-2 family of proteins, we will briefly describe each in greater detail below prior to discussing how these families of proteins interact.

The cell death process can be divided into a number of stages: inductive stimulus, signal transduction, activation of caspases, activation of nuclease(s) with nuclear condensation, redistribution of the cellular contents into apoptotic bodies, and removal of the dying cells (see Figs. 8-2 and 8-3). Since the nature of the inductive stimulus dictates the initial biochemical pathways engaged, we will separately discuss extrinsic (“death receptor”) and intrinsic (“stress-induced”) pathways.

Caspases

The cysteine protease family of caspases plays a central role in apoptosis and the orthologs in *C. elegans* and Drosophila demonstrate evolutionary conservation. Caspases are produced as catalytically inactive zymogens and function as homodimers, with each monomer comprised of a large and small subunit. Caspases can be divided into two categories reflecting both structural and functional differences; “initiator” and “effector” caspases (Table 8-1) (for a comprehensive review see (16 ,17)).

The “initiator” caspases, which, in mammals, include caspase-2, -8, -9, and -10, have extended N-terminal prodomains that allow for clustering and autoactivation of the zymogens. The clustering of the initiator caspases depends on adaptor proteins that utilize homophilic interactions between conserved nonenzymatic domains present on both the adaptor and the caspase. These adaptors include Fas-associating protein with death domain (FADD), which recruits caspase-8 via a homophilic interactions between death effector domains in both proteins (DED, see below and Figs. 8-2 and 8-3), and Apaf-1 that recruits caspase-9 through a homophilic interaction through the aptly named CARD. Although the recruitment and activation of various initiator caspases occurs in response to unique sets of stimuli and in separate cellular compartments (see below), the structural basis of the homotypic recruitment has a remarkable degree of similarity between different interactive motifs (death domains, DEDs, CARD) (18). Autoactivation of initiator caspases likely occurs through oligomerization (17 ,19 ,20), ultimately leading to the activation of downstream effector caspases (caspases 3, 6, and 7). As discussed below, caspase-8, considered a pro-apoptotic protease, functions both in Fas-induced apoptosis (21) as well as lymphocyte activation and protective immunity (22 ,23).

The effector caspases are necessary for the execution of apoptosis. They cleave specific substrates such as the structural proteins fodrin, gelsolin, and lamins, key intracellular enzymes involved in DNA repair (e.g., poly (ADP)ribose polymerase, DNA-PK). These changes facilitate inactivation of synthetic functions of the cell, dissolution of the nuclear membrane, and packaging of cellular proteins into apoptotic blebs on the cell surface. Caspases also cleave regulatory proteins such as Bcl family members and the inhibitor of caspase-activated DNase (ICAD). Cleavage of ICAD leads to the release of active CAD, which enters the nucleus and cleaves nucleosomes at the linker region, yielding the characteristic “DNA ladder”(24 ,25).

Not all caspases are involved in the execution of apoptosis. Human caspases 1, 4, 5, and mouse caspase 11 and 12 are most likely involved in inflammation. Caspase 1, was originally defined as the enzyme that cleaves IL-1

(interleukin-1-converting enzyme (ICE)) into its active form. It has been shown that caspase 1 and caspase 5 interact and form a multiprotein complex that has been called the inflammasome (analogous to the apoptosome) (26). Caspase 1 and 5 bind to the adaptor proteins, ASC (PYCARD) and NALP1 (DECAP), respectively by their CARD domains. The protein, pyrin, that is in mutated in familial mediterranean fever (FMF), binds to ASC. Mutation of pyrin therefore most likely leads to inflammation through uncontrolled activation of caspase 1 and IL-1 and IL-18 (27 ,28). Furthermore, mutations in NALP3, a homologue of NALP1, are responsible for three genetic autoinflammatory syndromes (Muckle-Wells syndrome, familial cold autoinflammatory syndrome (FCIS), and chronic infantile neurological cutaneous and articular syndrome (CINCA). Macrophages from Muckle-Wells patients spontaneously secrete active IL-1ß as a result of increased activity of the inflammasome (29).

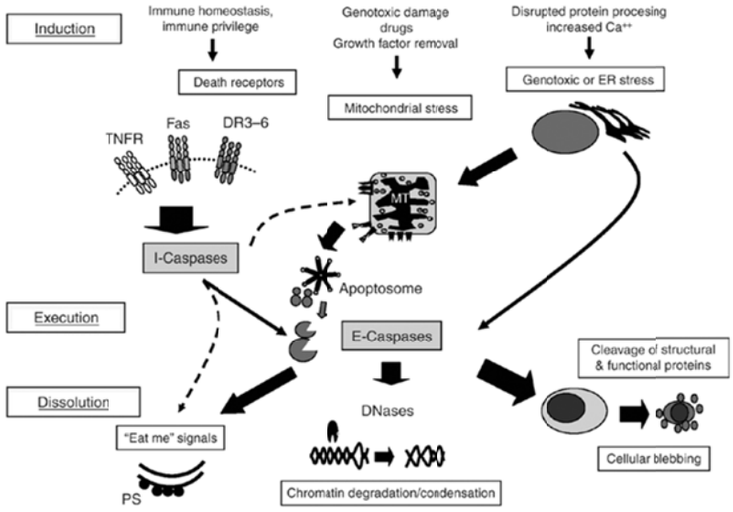

Figure 8-2. Mammalian apoptotic pathways. Cell death can be initiated by multiple pathways, including an extrinsic (left panel) and intrinsic pathways (middle and right panel). Apoptosis occurs in discrete stages, with induction stimuli leading to further execution and dissolution steps. Examples of stimuli that can induce each of these pathways are shown and discussed in further detail in the text. These various death pathways differ in the upstream initiator caspases (I-Caspases) activated but converge to cleave the effector caspases (E-Caspases) such as caspase 3 during execution of apoptosis. Central to the apoptotic program is the formation of the “apoptosome” which functions to amplify the death signal and leads to activation of the effector caspases 3, 5, and 7. The alterations that occur during dissolution of the cell are too numerous to mention but a few are highlighted in view of their potential relevance to autoimmunity (see text). Exposure of phosphatidylserine (PS) on the cell surface (left lower) may be relevant to the generation of antiphospholipid autoantibodies and coagulation disorders in vivo. Cleavage products of chromatin by various DNases (lower middle) as well as proteins, such as lamins and DNA-PK may be antigenic.

Inhibition of Caspases—Intracellular Inhibitors of Apoptosis (IAPs)

IAPs are a family of anti-apoptotic proteins that are highly conserved through evolution. The neuronal apoptosis inhibitory protein (NAIP) was discovered through the association of NAIP mutations in patients with the severe form of spinal muscular atrophy. Four additional members of the family (i.e., c-IAP-1, -2, X-IAP, and survivin) that share a baculovirus IAP repeat domain have subsequently been identified. The main function of IAPs appears to be inhibition of caspase 3 and 9 (30). IAPs block apoptosis induced by a variety of stimuli, including

Fas, tumor necrosis factor-α (TNF-α), ultraviolet irradiation, and serum withdrawal, and survivin is overexpressed in certain cancers and in the rheumatoid arthritis synovium (31).

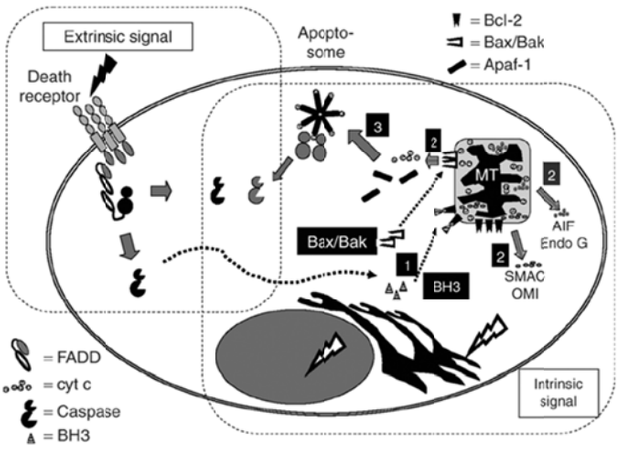

Figure 8-3. Overview of extrinsic and intrinsic apoptotic pathway. Death receptor extrinsic signaling is shown on the left as modeled by Fas-induced apoptosis. Fas aggregation leads to formation of the "death inducing signaling complex" (DISC), which contains the bifunctional adaptor FADD and the initiator caspase 8. High local caspase concentrations lead to the dimerization, activation and subsequent release of caspase 8 from the cell membrane. The intrinsic apoptotic signaling pathway is schematized on the right (see text for specifics of various pro- and anti-apoptotic proteins). Signals from cellular stress lead to the translocation of the pro-apoptotic Bax/Bak and BH3-only proteins to the mitochondria (shown as 1). BH3-only proteins antagonize the protective effects of Bcl-2 on cellular viability. Bax/Bak in turn aggregate and form large oligomers in the mitochondrial membrane leading to the release of cytochrome c into the cytosol (shown as 2). Apaf-1 forms a large scaffold for caspase activation known as the apoptosome in the presence of cytochrome C (shown as 3). Both pathways lead to the activation of effector caspases and subsequent cleavage of a multiplicity of cellular substrates.

IAPs themselves are inhibited by two mitochondrial proteins named Smac/Diablo and HtrA2/Omi, which are released into the cytosol during the intrinsic and some extrinsic apoptotic programs (Fig. 8-3). Mutations in the IAP inhibitor, HtrA2/Omi, have been implicated in a lethal neuromuscular disorder in mice, ostensibly due to increased sensitivity of embryonic fibroblasts to apoptosis (32).

In addition to the IAPs, various viral proteins inhibit caspases, which probably function to enhance viral fitness. The baculoviral protein p35 is a pan-caspase inhibitor that functions through the covalent linkage of the viral protein to an active site catalytic residue. The poxvirus apoptosis inhibitory protein, CrmA, likely functions similarly to p35. To date, no mammalian orthologues of p35 or CrmA have been identified. As discussed below, both an endogenous cellular protein (cFLIP) as well as a virus encoded protein, (vFLIP), inhibit Caspase 8 signal activation from the Fas/CD95 death receptor (21).

DNases and Degradation of Apoptotic DNA

One of the hallmarks of programmed cell death is nuclear condensation and subsequent DNA fragmentation. Numerous mammalian DNases have been implicated in the degradation of DNA, but the precise role of each DNase has not been completely elucidated. DNases in mammalian cells that are clearly implicated during apoptosis include the caspase activated DNase (CAD/DFF40), and the caspase independent DNAses including DNase II (located in lysosomes) and apoptosis-inducing factor (AIF)/endonuclease G (located in mitochondria) (Table 8-1).

Initially, degradation of chromatin into large DNA fragments to produce large (50–200kb) fragments occurs through co-operative interaction between Endonuclease G and AIF. Subsequently CAD/DFF40 cleaves DNA into internucleosomal units producing a characteristic 180 bp ladder. Mice deficient in CAD/DFF40 or ICAD (required for functional CAD) have defects in thymocyte DNA degradation, confirming an absolute requirement for CAD/DFF40 in cell-autonomous DNA fragmentation (24).

Table 8-1: Families of Intracellular Proteins Involved in Apoptosis

Caspases	Pro-domain	Function
Initiator		
Caspase 8, 10	long, with DED	Extrinsic (death receptor)
Caspase 9	long, with CARD	Intrinsic pathway
Caspase 2	long, with CARD	Both Extrinsic (death receptor) and Intrinsic (chemotherapy) pathways
Executioner		
Caspase 3/6/7	short	Cleavage of apoptotic substrates
Caspase inhibitors		
IAPs	—	Active-site blockage of caspases
p35, CrmA	—	Pan-caspase inhibitors
cFLIP	DED	Cell inhibitor of DISC formation
Bcl-2 members		
	Prototype	**Others**
Anti-apoptosis		
Bcl-2 sub-family	Bcl-2	Bcl-xL, Bcl-w, Mcl-1
Pro-apoptosis		
Bax sub-family	Bax	Bak, Bok
BH3-only sub-family	Bid	Bim, Bad, Puma, Noxa, Bik
DNases		
	Activation	**DNA degradation**
CAD	Caspase	Cell autonomous, generates nucleosomes
AIF + Endo G	Mitochondrial	Cell autonomous, cleaves chromatin
DNase II	Low pH	Ingested DNA in phagocytes
DNase I	?	Extracellular DNA

DED, death effector domain; CARD, caspase recruitment domain; IAP, inhibitor of apoptosis; cFLIP, cellular FLICE-inhibitory protein; DISC, death-inducing signaling complex; CAD, caspase-activated DNase; AIF, apoptosis-inducing factor, EndoG, endonuclease G.

The discovery that CAD/DFF40-null mice have terminal transferase-mediated dUTP-biotin nick-end labeling (TUNEL)-positive cells engulfed by macrophages led to a search for a cooperative DNase. The function of the lysosomal DNase, DNase II, was established by gene targeting as mice lacking DNase II accumulate undigested DNA-containing bodies in phagocytes (33 ,34). These findings emphasize the importance of non-cell autonomous DNA degradation that occurs following phagocytic ingestion of apoptotic cells. Intriguingly, mice deficient in both CAD/DFF40 and DNase II have additional defects in thymic development associated with inflammatory cytokines, including type I IFNs (35), further supporting a model in which DNA degradation during apoptosis is a sequential process that requires initiation in a cell autonomous way and is then fully executed by the phagocyte.

DNase I, the predominant serum DNase, is crucial for the degradation of DNA released from dead and dying cells into the bloodstream. Mice deficient in DNase I, at least on some strain backgrounds, develop a lupus-like phenotype including glomerulonephritis and antinuclear antibodies (ANA), ostensibly a result of the elevated levels of undigested DNA (36). It is unclear precisely how endogenous DNA becomes immunogenic under these circumstances.

The Bcl-2 Family: Central Regulators of Apoptosis

Bcl-2, the first protein described in this family, was originally discovered in the context of promoting B cell lymphomas through an ability to block apoptosis (37 ,38). There are at least 20 known Bcl-2 family members in mammals that have diverse cellular localization and function, and are broadly divided into three interacting groups (Table 8-1 and reviewed in (39)).

All Bcl-2 family members possess one or more BH (Bcl-2 Homology) domains, which allow for interaction with a variety of pro- or anti-apoptotic proteins. Multidomain prosurvival members Bcl-2, and closely related Bcl-xl, Mcl-1, and A1, can protect cells from a wide range of potentially death-inducing stimuli, including irradiation (UV and gamma), growth factor withdrawal, and chemotherapy. The pro-apoptotic multidomain group that includes Bax, Bak, and Bok likely functions by altering the permeability or conductance of mitochondrial and other membranes resulting in the release of additional pro-apoptotic mediators (see below). Finally, the “BH3-only” group of pro-apoptotic proteins contains at least eight members and functions primarily by

antagonizing the protective effects of the Bcl-2 family. Some BH3-only proteins may sensitize cells for apoptosis while others likely function more directly in activating apoptosis (5). The BH3-only members act as sentinels in various organelles, integrating proximal death or survival signals, and ultimately facilitating Bax/Bak induced apoptosis. A possible mechanism is to compete with Bax and Bak for binding to anti-apoptotic Bcl-2 members thereby releasing Bax and Bak to permeabilize organelle membranes (40).

The cellular localization of the Bcl-2 family members is diverse and likely reflects the complexity of networks that sense cellular damage and govern life or death. The anti-apoptotic Bcl-2 sub-family is associated with membranes, including the cytoplasmic face of the mitochondrial membrane. While Bcl-2 itself inserts into membranes in healthy cells, other related proteins must undergo allosteric changes prior to membrane association, allowing for the unique response to cytotoxic stressors. Similarly, the pro-apoptotic Bax family has both cytosolic (e.g., Bax) and membrane associated (e.g., Bak) members in healthy cells that translocate to the outer mitochondrial membrane after appropriate stimuli. The BH3-only family adds to the complexity of apoptosis regulation as individual members are expressed in a cell-type specific manner, and may allow for the response to organelle specific signals (see below). Constitutively expressed BH3-only members remain latent until released by a variety of stimuli. Examples include Bad (sequestered to 14-3-3 scaffold proteins) and Bid (undergoes cleavage). Finally, some BH3-only members are transcriptionally regulated so that certain forms of apoptosis resulting from cellular stress increase the expression of pro-apoptotic proteins (40).

Abnormalities in the Expression of Bcl-2 Family Members Cause Lupus-Like Autoimmunity in Mice

Mice that overexpress the anti-apoptotic protein, Bcl-2, or are deficient in the pro-apoptotic protein, Bim, develop a lupus-like disease on certain strain backgrounds (41 ,42). Bcl-2 is not a critical player in positive or negative thymic selection but may promote autoimmunity by enhancing cell survival in peripheral lymphocytes. In contrast, loss of Bim does impact thymic selection as evidenced in a Bim-deficient T cell receptor transgenic model where T cells targeted against the male antigen, HY, have impaired deletion of autoreactive $CD4^+8^+$ thymocytes in male mice (43). Bim may also regulate B cell survival.

Table 8-2: Death Receptor Family Members

Receptor	Ligand	Main Functions
Fas/CD95	FasL	Activation induced cell death, T-cell proliferation
TNF-R1	TNF	Immune activation and cell survival, apoptosis
TNF-R2	TNF	Immune activation and cell survival
DR3	TL1a (TWEAK)	T-cell costimulation
DcR3*	TL1a, FasL	Suppress T-cell responses
TRAIL-R1	TRAIL	For all TRAIL family, cell death of certain transformed cells and in some cells activation of NF-κB
TRAIL-R2	TRAIL	
TRAIL-R3*	TRAIL	
TRAIL-R4*	TRAIL	
OPG	TRAIL, RANKL	
DR6	unknown	Suppress T- and B-cell responses

*decoy receptors

Initiation and Pathways of Apoptosis

Extrinsic Signaling through Death Receptors

Our understanding of the molecular basis for apoptosis has been guided by the dissection of the signal transduction pathways downstream of Fas and TNF. The “death receptor” family consists of six members all of which contain an eighty amino acid cytoplasmic tail known is the “death domain” and which is required for apoptosis (Table 8-2 , Fig. 8-4). In addition to receptors for signaling apoptosis, there are a number of “decoy receptors” (DcRs) that also bind the same ligands of the TNF superfamily. These receptors either lack functional intracellular death domains (e.g., DcR3) or exist as soluble receptors (e.g., OPG) and are therefore unable to transmit an intracellular signal. By sequestration of death ligands, decoy receptors may prevent signal transduction from death receptors.

Fas/CD95 is a member of the TNFR-superfamily and plays a central role in T cell homeostasis (44). Restimulation of activated human T cells through the TCR results in Fas translocation into lipid raft microdomains and renders the cells sensitive to apoptosis by uncrosslinked FasL, most likely due to increased proximity and pre-association of receptor subunits (45) (Fig. 8-4). The localization of Fas to lipid rafts is likely cell-type specific as murine thymocytes have constitutive localization of Fas to lipid rafts.

Upon binding by Fas ligand, Fas further oligomerizes, and a number of apoptosis-related proteins are recruited via homotypic interactions and collectively form the death-inducing

signaling complex (DISC). Fas itself binds to the adaptor protein FADD via death-domain interactions, and FADD binds to caspase 8 through DEDs (Fig. 8-4). The recruitment and activation of caspase 8 requires “induced proximity” (20 ,46), and likely proceeds through dimerization of monomeric zymogens. Signaling through other death receptors, including TNF, DR3, and others, depends on the FADD as well as other adapter-induced recruitment of caspases. Activation of initiator caspases by death receptors leads to initiation of the “effector” caspase cascade as described in greater detail below (*intrinsic death pathways*).

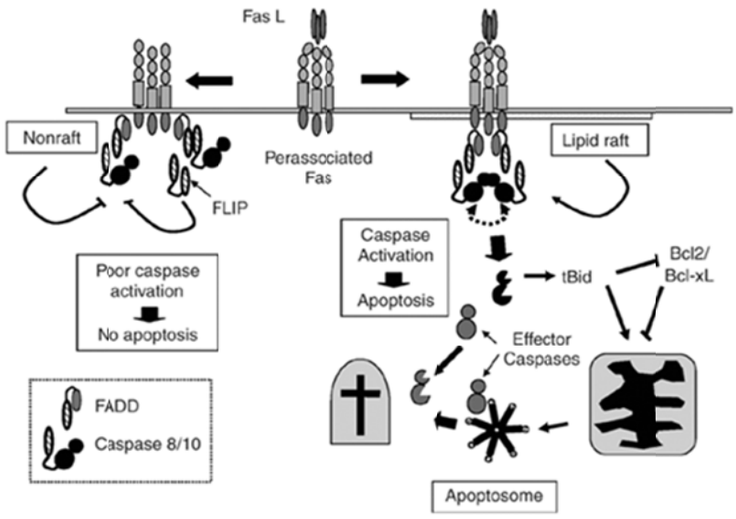

Figure 8-4. Death Receptor Signaling through Fas. Fas recruits the death-domain containing adaptor FADD and caspase-8 following engagement by FasL. In order to efficiently signal for apoptosis, Fas needs to translocate into lipid rafts which allows for caspase-8 autoactivation. Caspase-8 also cleaves tBid which functions to inhibit Bcl-2/Bcl-xl and promote the formation of the “apoptosome” consisting of Cytochrome C and Apaf-1 as described in the text.

Although the six DD-containing receptors initiate cell death in certain contexts, all may signal cell survival/proliferation in different cell types and/or in different contexts. The ability to signal an opposite cell fate, seems to depend on the recruitment of proteins such as the tumor necrosis factor receptor-associated factors (TRAFs) that activate nuclear-factor-kappa ß (NF-κB), thereby promoting cell survival (see below). The increasingly complex roles of “death” receptors and caspase 8 are illustrated by impaired T cell activation coupled with lymphocyte accumulation in patients with caspase 8 deficiency (22). Caspase 8 can also mediate NF-κB signaling in response to signals in response to antigen-, Fc-, or Toll-like receptor 4 in lymphocytes and natural killer (NK) cells (23).

TNF receptor 1 (TNFR 1) can trigger cellular activation via NF-κB or apoptosis via activation of apical caspases. Under most conditions TNFR1 signaling results in NF-κB activation, however when protein synthesis is blocked or NF-κB is specifically inhibited cell death can be triggered. In vivo, when NF-κB activation is blocked, endogenous TNF signaling through TNFR1 can lead to embryonic lethality because of massive liver cell death during development (48).

Similar to Fas, TNFR1 signals through FADD and caspase 8, and this signaling depends on the adaptor molecule TRADD, which has a region homologous to the FADD death domain. TNFR1 signaling involves assembly of two molecularly and spatially distinct signaling complexes that sequentially activate NF-κB and caspases (45 ,49). Early after TNF binding, RIP1, TRADD, TRAF2, and cIAP1 are recruited to TNFR1 to form complex I, leading to NF-κB translocation, which protects cells from apoptosis (50 ,51). At later time points, RIP1, TRADD, and TRAF2 dissociate from TNFR1 and recruit FADD and caspase-8 to form complex II. In the absence of NF-κB activity from complex I, complex II can initiate caspase-8 activation and cell death (49).

Regulation of Death Receptors

In most resting cell types that express Fas on their cell surface, the receptor does not appear to signal apoptosis and in lymphocytes may actually promote proliferation (52). Resistance to death is explained both by low levels of expression of the receptor, by physical separation of Fas from lipid rafts (45), and by active inhibition by a protein

called FLIP. FLIP resembles the structure of FADD, binds to Fas and prevents the adaptor protein, FADD, from initiating apoptosis. When lymphocytes become activated, FLIP is usually degraded allowing Fas signal transduction to occur unimpeded (Fig. 8-4) (21). FLIP can affect sensitivity to both Fas and TNFR signaling by competing with caspase 8 for FADD. Although numerous studies suggest that FLIP is a negative apoptotic regulator, the exact role of FLIP in Fas signaling is controversial, explained in part by the existence of different isoforms (21). The first FLIP described was from Herpesvirus (53) and likely contributes to viral persistence by activation of NF-κB pathways leading to both protection from cell death as well as growth and proliferation (54).

Function in Immune Regulation

Although the role of Fas and FasL interactions in the thymus remains controversial (55), this pathway is involved in the maintenance of immune privilege in the eye and the testis, in the pathogenesis of graft-versus-host disease, and in immune evasion by tumors (56). The major physiologic function of Fas and FasL in the immune system is the preservation of peripheral tolerance. This is achieved by the phenomenon of activation-induced cell death (AICD) whereby CD8 T cells, Th1 CD4 T cells, and possibly NK cells induce apoptosis of activated T cells, B cells, and macrophages. The deletion of activated immune cells removes the source of proinflammatory molecules, prevents the continued presentation of self peptides by primed (high levels of costimulatory molecules) antigen-presenting cells and eliminates B cells that have mutated to self-specificity in the germinal centers (57). Neutrophil homeostasis is also maintained by the Fas/FasL system. The Foxo3a forkhead transcription factor maintains neutrophil viability during inflammation by suppressing FasL, and blockade of Fas-FasL rendered Foxo3a$^{-/-}$ mice susceptible to two models of neutrophilic inflammation (immune complex-mediated arthritis and thioglycollate-induced peritonitis) (58).

It should be noted that, whereas TRAIL signals apoptosis through DR4 and DR5 predominantly in tumor cells, TRAIL may play a role in negative selection of thymocytes (59). Similarly, DR3 (the receptor for TWEAK) has also been implicated in negative selection (60). Finally, DR6, plays a role in immunologic homeostasis as evidenced by enhanced T- and B cell proliferation in DR6 deficient mice (61).

Deficiencies in Death Receptor Signalling Lead to Systemic Autoimmunity

Death receptors have been clearly implicated in both murine and human autoimmune diseases (62). The first clue that regulation of apoptosis was relevant to autoimmunity came with the identification of mutations in Fas and Fas ligand in the lpr and gld mouse models of lupus (reviewed in (56)). Since that time numerous spontaneous and induced genetic alterations that effect apoptosis have been shown to predispose to systemic autoimmunity (63). The autoimmune lymphoproliferative syndrome (ALPS) is an extremely rare disease usually detected during childhood (64 ,65 ,66). ALPS patients present clinically with a nonmalignant accumulation of lymphocytes in lymphoid organs, hypergammaglobulinemia, cytopenias, autoantibodies, and occasionally, glomerulonephritis or arthritis. The diagnosis of ALPS requires the aforesaid clinical features coupled with resistance of lymphocytes towards FasL-mediated apoptosis/AICD (67) and increased numbers of CD4-CD8- circulating T cells. The vast majority of patients have mutations in Fas, FasL or caspase 10. Either environmental and/or other genetic modifiers are necessary for disease onset, as not all humans and mice (lpr) with Fas mutations develop disease.

Mutations in the p55/TNFR1/CD120a receptor in humans results in a periodic autoinflammatory syndrome called tumor necrosis factor receptor-associated periodic syndrome (TRAPS). Mutations predominantly occur in the first two CRD of the receptor, which may in some cases result in reduced shedding of the extracellular domain of the receptor and reduced neutralization of circulating TNF-α (27).

Intrinsic Death Pathways from Cellular Stress

Cells depend on a variety of signals for active maintenance of survival including those relating to overall nutritional and bioenergetic status. Loss of signals from neighboring cells (68) or withdrawal of growth factors or cytokines results in initiation of a cell death program. Damage or stress to intracellular organelles may be induced from outside or within the cell, and these pathways depend on the dynamic interplay of Bcl-2 family of proteins and other regulators (14).

The Mitochondria as an Integrator of Cell Metabolism and Apoptosis

Mitochondria are cytoplasmic organelles that contain their own 16-kb genome encased by inner and outer membranes with a number of proteins, including cytochrome-c, situated between these membranes. Mitochondria help to maintain redox potential and are the energy powerhouse of the cell through the generation of ATP by oxidative phosphorylation. The discovery that many bcl-2 family members constitutively or inducibly localize to the mitochondrion, illuminated a central role for this organelle in orchestrating apoptosis.

The mitochondria contains numerous proteins that are crucial to the apoptotic machinery. Cytochrome c, an indispensable cofactor with Apaf-1 in the activation of caspase-9, is found only in the inner mitochondrial membrane. Additionally, the inner mitochondrial membrane contains inhibitors of anti-apoptotic proteins, Smac/Diablo and HtrA2/Omi, as well as inactive endonucleases (Endo G, AIF), which become active following release from the mitochondria (Fig. 8-3). The pro-apoptotic BAX and BAK proteins are essential for mitochondrial permeabilization, and mice doubly deficient in both BAX and BAK are resistant to multiple forms of apoptosis (69).

These proteins undergo changes allowing oligomerization and permeabilization of the mitochondrial outer membrane. This permeabilization in turn promotes release of pro-apoptotic proteins and formation of a complex including Apaf-1, cytochrome-c (Apaf-2) and caspase 9 (Apaf-3). This multiprotein complex, aptly termed the “apoptosome” (70), amplifies the death signal and leads to activation of the effector caspase-3, 5 and 7 (Figs. 8-3 and 8-4). The mechanism whereby BAX/BAK promotes release of these proteins is controversial, and may involve the formation of pores (71) or by alteration of intrinsic mitochondrial proteins triggering permeability transition. Once the mitochondrial membrane has been disrupted, multiple pro-apoptotic molecules are released, including cytochrome c, the IAP inhibitors Smac/Diablo and HtrA2/Omi as well as the proteins involved in chromatin degradation (Endo G, AIF).

Metabolic Stress

Withdrawal of either growth factors or nutritional sources leads to metabolic changes including lower oxygen consumption and a reduction in both ATP levels and protein production. In many cell types, these conditions lead to a form of apoptosis that can be blocked by overexpression of either Bcl-2 or Bcl-xL (14). A link between proteins involved in sensing bioenergetic status of a cell with those that control apoptosis has been identified. The pro-apoptotic BH3-only protein, BAD, forms part of a multiprotein complex that includes glucokinase and that regulates glucose-driven mitochondrial respiration (72). In response to withdrawal of survival factors, BAD is phosphorylated and orchestrates cell death. BAD additionally serves to help regulate blood glucose levels, and mice deficient in BAD have defective glucokinase activity that manifests as diabetes.

Another example connecting the apoptotic machinery with mitochondrial function is the interaction between a mitochondrial voltage-dependent anion channel (VDAC) and Bcl-2 family members (73). The Akt kinase, which promotes cell growth and inhibits apoptosis, also facilitates localization of hexokinase to the mitochondrial membrane (74). Hexokinase in turn, associates with VDAC and prevent Bax toxicity (75). Interestingly, Akt requires glucose to regulate hexokinase (and hence to protect against apoptosis), demonstrating a connection between the protein machinery regulating energy stores and the promotion of survival with the interface at the mitochondrial membrane.

Genotoxic Stress

Mutations occur frequently in mammalian DNA and are usually promptly repaired. However, if repair fails or DNA is severely damaged by radiation or drugs, the transcription factor, p53, is upregulated and phosphorylated by DNA damage sensors such as ATM. Activated p53 induces a cell cycle arrest through induction of the cyclin-dependent kinase inhibitor, p21. If the DNA damage is repaired, cell cycle arrest is abrogated, whereas if the injury cannot be repaired, the cell undergoes apoptosis. The critical importance of p53 as a tumor suppressor is illustrated by the high frequency of p53 mutations in cancers (76). p53 induces apoptosis, in part, by transcription of death effectors such as Bax that cause mitochondrial stress as well as two BH3-only bcl-2 family members Puma and Noxa (77).

Additional proteins, including both p53 and histone H1.2 may have a direct role in apoptosis following DNA damage. Following translocation to the mitochondria (78), p53 can directly activate the pro-apoptotic protein, Bax, to permeabilize mitochondria and engage the apoptotic program (79). p53 also functions in the release of Histone H1.2 from the nucleus into the cytoplasm (80). Once released into the cytoplasm, H1.2 can induce cytochrome c release from mitochondria in a Bak-dependent manner. These examples illustrate how apoptotic signals that originate in the nucleus are transmitted to the mitochondria.

Endoplasmic Reticulum Stress

The endoplasmic reticulum (ER) is now recognized as an important organelle that regulates the intrinsic apoptotic pathway. The ER is the major intracellular store of calcium, and in addition, functions to ensure proper protein folding. Disruptions in protein folding can lead to the “unfolded protein response” (UPR) and trigger cell death (81). One ER-stress response in mice has been shown to depend upon caspase 12 (82). Both Huntington disease and Alzheimer disease have been implicated in ER stress-induced apoptosis due to misfolded or mutant proteins. The signal for apoptosis due to ER stress may depend on calcium release, although the mechanism remains uncertain. The calcium binding protein Annexin V has been shown to be required for ER stress induced apoptosis (83).

Bcl-2 and Bax/Bak also function in ER stress-induced apoptosis in opposing ways. Bcl-2 blocks transmission of a stress signal from the ER to the mitochondria (84). Mice doubly deficient in Bax and Bak have markedly reduced ER calcium concentrations and defects in ER stress-induced apoptosis that could be corrected with overexpression of calcium pumps [sarcoplasmic/endoplasmic reticulum calcium ATPases (SERCA)] (85). These studies highlight the role of calcium dynamics in apoptosis and the functional interaction between the ER and mitochondria.

Removal of Apoptotic Cells

Receptors and Ligands

Within the immune system alone, more than 10^{12} apoptotic cells are removed from the body each day. These apoptotic cells are generated in vast numbers in the central lymphoid organs such as the thymus and bone marrow by out of frame rearrangements of antigen receptors, negative selection, or simple “neglect.” A significant load of apoptotic cells is produced in the peripheral immune system because of the relatively short life span of lymphocytes and myeloid cells and secondary selection of high-affinity B cells in germinal centers.

The specialized sites of selection (e.g., thymus, bone marrow, lymphoid follicles) have remarkably efficient phagocytes that rapidly remove the dying cells.

An early event in apoptosis is the appearance of phosphatidylserine (PS) on the cell surface membrane (Fig. 8-5). This membrane asymmetry (PS is usually located on the inner surface of the membrane) is caused by the reduced function of a translocase and possibly by activation of a lipid scramblase (86). PS is an important ligand for phagocytosis of apoptotic cells (87) Similarly, sugars such as N-acetyl-glucosamine and N-acetyl-galactosamine may be selectively exposed on the apoptotic membrane, triggering their recognition by phagocytes (88).

Despite the detection of only limited chemical alterations on the apoptotic cell membrane, blockade of a large and diverse number of receptors on phagocytes can impair the uptake of apoptotic cells (reviewed in (89)) (Fig. 8-5). This diversity may in part be explained by the different cells, activation states, and conditions used for phagocytic assays, but it undoubtedly also reflects the overlapping and partially redundant function of each individual receptor. All of the receptors identified have other functions, perhaps reflecting an evolution from receptors designed to remove apoptotic cells during development to pattern recognition receptors useful for host defense (90). Many of the receptors are integrins comprising the vitronectin receptor, $\alpha_v\beta_3$, $\alpha_v\beta^5$, complement receptors 3 (CD11b/CD18) and 4 (CD11c/CD18), and class A and B scavenger receptors. Nonintegrin receptors include the ATP-binding cassette transporter (ABC1) and CD14 and CD91.

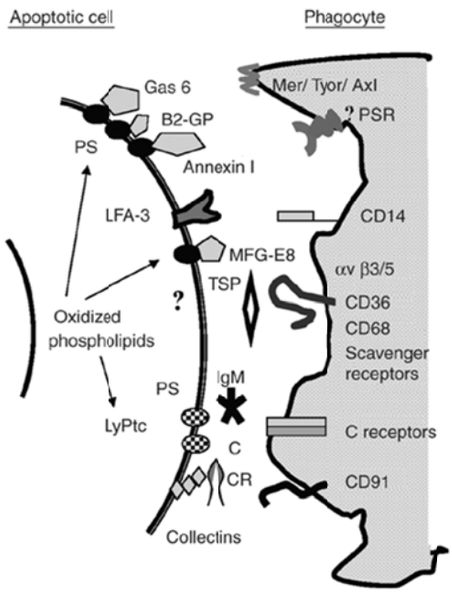

Figure 8-5. Receptors and ligands implicated in recognition or phagocytosis of apoptotic cells. A number of different “eat me” signals (ligands) are expressed on apoptotic, but not live, cells. In fact, live cells may express repulsive signals, such as CD47, that prevents them from being engulfed. The multiplicity of signals reflect different contexts and kinetics and the fact that certain ligands promote (‘tethering’) and others engulfment (‘tickling’) (135). Note that many ligands are actually serum proteins that coat exposed cell surface molecules and serve as opsonins for phagocytosis. It remains to be determined whether a unique phosphatidylserine receptor exists or whether PS is coated by protein opsonins. PS, phosphatidylserine; TSP, Thrombospondin; LyPtC, lysophosphatidylcholine; C, complement; CR, calreticulin.

In some cases, phagocytosis of apoptotic cells requires serum factors such as thrombospondin to bridge the $\alpha_v\beta_3$ and CD36 receptors (91) as well as serum complement (92) that is amplified on apoptotic cell membranes by natural IgM antibodies (93), mannose binding protein and acute phase proteins such as C-reactive protein (CRP). Other proteins such as MFG-E8, Gas6, β2-microglobulin, Annexin I (bind to PS or oxidized PS) or calreticulin (binds to collectins), have been implicated in promoting the phagocytosis of apoptotic cells in certain contexts (Fig. 8-5) Finally, engagement of the closely related Tyro 3 family receptor tyrosine kinases, MER, TYRO, and Axl by their ligands such as Gas6, protein S appear to be required to prevent production of IL-12 or TNF-α from macrophages that have ingested apoptotic cells (94).

Function in Immune Regulation

Apoptotic cell death is an integral part of development as well as normal tissue homeostasis so it is necessary that the immune consequence of removal of these cells is absence of inflammation. Removal may occur through professional (macrophages and dendritic cells) or nonprofessional (epithelial cell, fibroblasts) cells. Macrophages are the most abundant professional phagocyte and both in vitro and in vivo studies support the concept that when macrophages engulf apoptotic cells, activation is suppressed by the release of multiple inhibitory cytokines including IL-10 and TGF-β, (95 ,96). Although dendritic cells (DC) are less abundant, they are much more potent than macrophages at T cell activation and appear to be pivotal to the maintenance of T cell tolerance via the presentation of self-antigen derived from apoptotic cells in the absence of costimulation (“steady state” condition) (97). Apoptotic cells suppress DC activation of T cells, in part, through suppression of IL-12 (98), which is caused by activation of a transcriptional repressor, GC binding protein (GC-BP) (99). The interaction of apoptotic cells with phagocytes therefore appears to be necessary for tolerance to self.

In contrast, DCs exposed to necrotic or tumor cells undergo maturation and activate both CD4 and CD8 T cells (100). Necrotic cells release pro-inflammatory constituents including heat shock proteins (HSPs), HMGB-1, and possibly nucleoproteins themselves (6 ,101). Maturation of DCs has clearly emerged as a critical switch to stimulate effector T cell development and administration of DCs loaded with apoptotic cells can accelerate lupus in NZB/W F1 mice (102) and mature DCs can induce autoantibodies to nucleoproteins in normal mice (103).

Defective Clearance of Apoptotic Cells Predispose to Lupus-Like Disease in Mice

Deficiencies of a number of proteins implicated in the removal of apoptotic cells have been reported to cause lupus-like diseases in mice. These include deficiencies of receptors such as mer as well as serum opsonins such as natural IgM antibodies, C1q, SAP, and MFG-E8 (reviewed in (104)). The mechanisms involved differ; mer deficiency results in APC receiving a pro- rather than anti-inflammatory signal upon ingestion of apoptotic cells. Defective clearance of apoptotic cells in mice deficient (knockout) in sIgM, C1q, SAP, and MFG-E8 may predispose to lupus through slow clearance of apoptotic cells (105 ,106) leading to postapoptotic necrosis and/or through lack of engagement with specific inhibitory receptors on the phagocyte. Interestingly, the sites at which defective apoptosis manifests differs—in C1q deficient mice apoptotic cells accumulate in the kidney whereas in MFG-E8 knockout mice—apoptotic cells accumulate in germinal centers.

Apoptosis Abnormalities in Human SLE

A large number of abnormalities in the apoptotic process or handling of apoptotic cells has been reported in SLE patients. A brief outline of the observations and possible mechanisms are discussed (see Fig. 8-6).

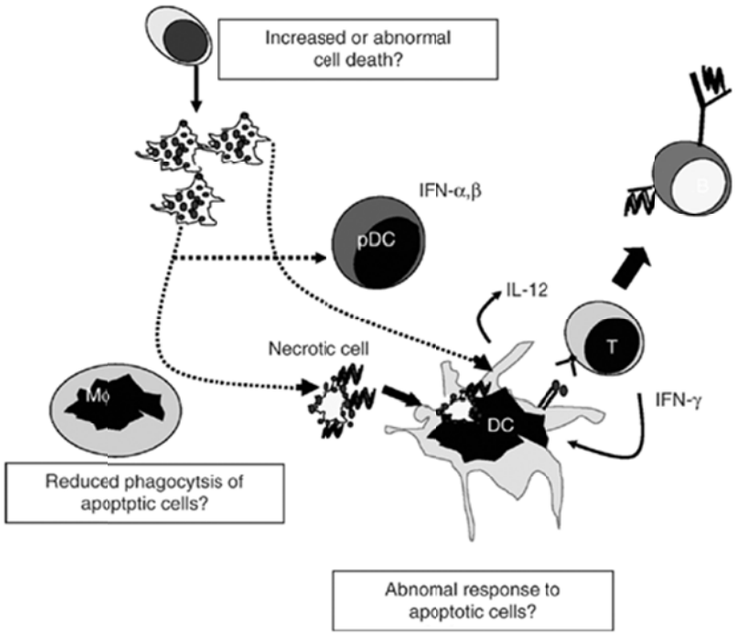

Figure 8-6. Role of apoptotic cells in the generation of autoantibodies to self antigen in SLE. Autoantibodies to cellular antigens in SLE may be generated by abnormalities in one or more of the pathways shown. See text for details.

Is the Process of Cell Death Normal in SLE?

As discussed above, although mutations in Fas and Fas ligand predispose to the ALPS syndrome in children, Fas/FasL mutations are exceptionally rare in SLE. However, the generation of autoimmunity to blood cells in ALPS as well as positive ANA in patients with caspase 10 mutations, indicate that the extrinsic pathway remains informative with regard to understanding loss of tolerance to cellular antigens.

Lymphopenia is a prominent feature in SLE and indicates that either cell death is excessive or that lymphocyte homeostasis is abnormal. An increase in apoptosis of SLE peripheral blood mononuclear cells has been observed in vitro (107 ,108). This may result from the increased number of activated lymphocytes in SLE or be an effect of elevated cytokines such as IL-10 (109) or IFN-α (see below). Although alterations in FasL or Bcl-2 have been reported, there is currently no compelling evidence for an intrinsic defect in apoptosis regulators in SLE.

Why the immune system targets a select subset of self antigens in each disease has never been satisfactorily explained. Of interest, it was observed that during apoptosis autoantigens, including those normally found in the nucleus, cluster and concentrate in surfaces blebs (110). As noted above, apoptosis leads to the controlled activation of multiple intracellular nucleases and proteases which, in turn, leads to the cleavage of a numerous cellular molecules; one consequence of this autodigestion is the generation of

“neoepitopes” (111). Some of these antigens undergo modification, including cleavage, phosphorylation and oxidation. However, it is expected that under normal conditions, these “neoepitopes” are also generated in the thymus and bone marrow leading to tolerance. On the other hand, inflammatory changes in the peripheral immune system that might occur secondary to UV light, oxidation, or cleavage by granzyme B delivered by cytotoxic T cells may qualitatively alter self antigens released by dying cells to stimulate immune responses. Good examples of alerted self in apoptosis are the lipid antigens, PS, and lysophosphatidyl choline (LPC), both of which may become oxidized and are recognized by certain anticardiolipin or natural anti-LPC antibodies (112 ,113 ,114).

Additional evidence implicating the products of dying cells in the immunization of SLE patients includes the strong focus of the autoimmune response on nucleosomes. Nucleosomes are detected in the circulation of SLE patients with active disease (115). Autoantibodies to nucleosomes precede those to DNA and histones, (116 ,117) and nucleosomes are more strongly antigenic for T cells and B cells than DNA or histones alone (118). Nucleosomes, but not isolated DNA or histones, deposit in the glomeruli, suggesting that it is the in situ fixation of nucleosomes, rather than DNA/anti-DNA immune complexes, that causes lupus nephritis (119 ,120).

Is the Response to Dying Cells Abnormal?

Type I interferons (IFNs) have emerged as critical cytokines in SLE (121 ,122). Studies of both SLE patients and lupus-prone mice suggest that activation of type I IFNs likely plays an important role in disease pathogenesis. It has long been known that serum levels of IFN-α are elevated in many SLE patients, and gene expression profiling revealed that patients demonstrate upregulation of IFN-responsive genes in their peripheral blood mononuclear cells (PBMC) (122 ,123). Of note, Ronnblom et al. reported that IgG antinucleic acid autoantibodies in combination with apoptotic cells stimulate a strong IFN response by SLE PBMC (124). A possible mechanism for IFN production under these circumstances has been identified. Mammalian DNA, similar to exogenous (e.g., bacterial or viral DNA), can act as an adjuvant when ingested by B cells or DCs in the form of immune complexes (125 ,126 ,127). DNA-stimulated pro-inflammatory signaling in these systems utilized a combination of an innate signaling receptor (TLR9 for DNA) with a second receptor (BCR or Fc-receptors) that facilitated uptake of the immune complexes. Together, these results strongly implicate endogenous DNA or nucleoproteins from dead or dying cells as immunostimulants for B cells and dendritic cells. While these very important experiments support a role whereby IFN production can by amplified by circulating immune complexes to perpetuate disease, the stimulus driving the original loss of tolerance remains unclear (Fig. 8-6).

Do SLE Patients Have Reduced Clearance of Apoptotic Cells?

Like several spontaneous lupus strains of mice, lupus patients are reported to have reduced clearance of apoptotic cells (128), although there are additional interpretations of these results (92). Inherited C1q deficiency in humans is sufficient to cause lupus in >90% of individuals in whom this mutation has been observed, and inherited C2 or C4 deficiency also predispose to SLE in 10% and 75% of patients respectively (reviewed in (129)). These observations suggest that deficiencies of the classical complement components offer a clue of fundamental importance in the pathogenesis of SLE. Although other interpretations are possible, both in vitro and in vivo experiments strongly support the idea that the early complement components are required for the clearance of apoptotic cells (92 ,130).

Another relevant serum abnormality observed in SLE is low serum levels of CRP that is associated with a polymorphism in the CRP promoter (131). Since CRP is potent scavenger of intact apoptotic cells (132) as well as snRNP and chromatin (133), reduced levels of CRP are likely to impair efficient removal of apoptotic cells and their debris.

Conclusions

Our understanding of the apoptotic program has grown exponentially over the past decade. Numerous human diseases have been directly linked to genetic defects in the apoptotic pathways, including cancer, neurodegenerative disorders and autoimmune diseases. Caspases initiate and amplify a variety of death signals allowing for selective and ordered cellular demolition. The fine balance between pro- and anti-apoptotic Bcl-2 family members regulate the cell fate in response to many (but not all) stress or signaling pathways. Recent discoveries highlight the complex integration of signals from various organelles that determine cell fate, and the multiple functions of central players in the apoptotic process. It is likely that the knowledge obtained in a relatively short space of time will translate into better diagnostics and therapies to enhance or retard cell death or to facilitate the removal of cell debris in the appropriate clinical circumstances.

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Chapter 9

The Interaction of T Cells with Cells of the Innate Immune System and B Cells in the Pathogenesis of SLE

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Systemic lupus erythematosus (SLE) is a systemic disease that is characterized by generalized autoimmunity. Unlike organ-specific autoimmune diseases such as insulin-dependent diabetes mellitus or myasthenia gravis, SLE is characterized by autoantibodies against nuclear, cytoplasmic, or cell surface molecules that transcend organ-specific boundaries. It is the inflammatory responses triggered by local formation and/or deposition of antigen antibody immune complexes that are responsible for the clinical manifestations of vasculitis and multi-organ system disease.

SLE is a T Cell-Dependent Disease

The production of pathogenic autoantibodies in SLE is T cell–dependent (1 ,2 ,3 ,4 ,5 ,6). In murine models of SLE, depletion of $CD4^+$ T cells blocks disease onset (7), and athymic mice do not develop SLE (8 ,9). In humans, the effects of human immunodeficiency virus (HIV) infection on $CD4^+$ lymphocytes may ameliorate SLE activity and spur remission (10).

Although numerous abnormalities of T cell function have been described in this autoimmune disease, investigators have failed to find a common abnormality expressed by all patients. There is no evidence that autoantigen–specific T cells in SLE patients reflect a defect in the generation of the T cell repertoire. Instead, the T cell response to self antigens reflects a breakdown of tolerance to these structures.

It is now appreciated that T cell recognition of foreign and self-antigens is similar and self-reactivity to histocompatibility molecules is not only physiologic, but is indispensable to the generation of normal immune responses. Moreover, since a T cell receptor can recognize multiple peptide antigens, one might ask how a limited number of highly cross-reactive T cells can respond to the large number of foreign peptides and yet remain tolerant, or nonresponsive to self peptides? In fact, thymic deletion of T cells that are capable of reacting against certain self-peptides is incomplete and autoreactive T cells capable of initiating SLE exist in healthy individuals (11) and have the potential to cause lupus (12). Immunoregulatory mechanisms normally render these cells nonresponsive or tolerant to self peptides. In SLE, however, various intrinsic and acquired cell T cell defects that will be summarized result in a breakdown of tolerance and development of disease. This is because B cells with the potential to make antinuclear autoantibodies also are not deleted during ontogeny (13). Once T cells capable of providing help for these B cells are activated, a circuit is initiated that ultimately results in the production of pathogenic IgG-DNA and other autoantibodies (14 ,15 ,16). Moreover, ineffective attempts by the immune system to downregulate the ongoing autoimmunity may lead to further T cell abnormalities that perpetuate the disease.

In this chapter we will consider the general properties of T cells, their functional development, and how they are regulated. Since it has become apparent that cells of the innate immune system greatly affect T cell functional properties, this topic will also be discussed. A link between the innate and adaptive immune systems has been established in the development of SLE. This link explains the appearance of autoantibodies reactive with nucleic acid-protein particles (17).

General Properties of T Cells

T cells are thymus-derived lymphocytes that develop effector or regulatory functions depending upon how they are stimulated by antigen and professional antigen-presenting cells (APCs) called dendritic cells (DC). Unlike B cells, T cells are unable to bind antigen directly; rather, they recognize antigen complexed to major histocompatibility complex (MHC) molecules. $CD4^+$ T cells recognize peptides complexed to MHC class II (HLA-DR, -DP, -DQ) $CD4^+$ typically recognize microbial peptides after phagocytosis and processing by DC, $CD8^+$ cells recognize peptides complexed to MHC class I (HLA-A, -B, -C). These cells generally recognize peptide fragments of newly synthesized intracellular proteins, and therefore can eliminate viral infected cells. The T cell antigen receptor (TCR) expressed on the surface consists of a two-chain, heterodimer that is noncovalently complexed to the signal-transducing CD3 structure. Most T cells display antigen receptors (TCRs), which are αß chain heterodimers.

They are positively selected by self histocompatibility antigens in the thymus and following release, they recirculate continuously between blood and peripheral lymph nodes. Although most $CD4^+$ cells have helper functions and $CD8^+$ cells become killer cells (18), each can develop the opposite function in SLE (Table 9-1).

T cells are called naive or virgin when they leave the thymus. Because naive $CD4^+$ cells lack receptors for adhesion molecules that are expressed by memory or previously stimulated cells, they require antigen presentation by mature DC under optimal conditions for activation. Once they acquire characteristic adhesion receptors, they can be restimulated under much less stringent conditions (19 ,20 ,21 ,22). Such $CD4^+$ cells respond well to soluble antigens and support B cell differentiation. Corresponding memory $CD8^+$ cells, which are the precursors of antigen-specific killer cells, also are generated (23 ,24). In humans, naive and memory T cells can be identified by reciprocal expression of CD45 isoforms on their cell surfaces (CD45 is a membrane tyrosine phosphatase that has a vital role in signal transduction). Naive T cells display the high-molecular-weight CD45RA isoform, and memory T cells express the low-molecular-weight CD45RO isoform (19 ,20 ,21 ,22).

The distinction between naive and memory T cells is especially important in the development of autoimmunity, because potentially harmful autoreactive T cells are in the immature, naive state. Because of clonal anergy or T cell suppression, these cells will be maintained in the resting state. Once tolerance is broken, however, and these autoreactive T lymphocytes become memory cells, autoimmunity is easily sustained.

Table 9-1: Human T and Natural Killer (NK) Cell Populations

Population	TCR Structure	Comment
$CD4^+$	αß chains	Recognize peptide antigens complexed to self MHC class II structures (HLA-DR, -DP, -DQ)
$CD8^+$	αß chains	Recognize peptide antigens complexed to self MHC class I structures (HLA-A, -B, -C). Generally develop cytotoxic activity, but a subset of CD28-cells can develop cytokine-dependent suppressive activity in mice.
$CD4^+$ $CD25^+$	αß chains	Heterogeneous populations of natural, thymus-derived cells and peripheral $CD4^+$ cells that have been induced by IL-2 and TGF-ß to become suppressor cells. Express the transcription factor FoxP3 Control the proliferation, migration and effector functions of other T cells. Prevent autoimmunity
γδ $CD3^+$ $CD4^-$ $CD8^-$	Restricted expression of γδ chains	Minor population in blood, abundant in intestine; directly recognize phosphorylated and stress-induced proteins; important immunoregulatory functions
NKT, express CD3 and NK markers (CD56); generally $CD4^-$, $CD8^-$	Restricted expression of αß chains	Minor population in blood, but present in skin and mucosal tissues such as intestine, liver, lungs; recognize CD1, glycolipid antigens
Natural killer (NK) $CD56^+$, $CD16^+$, $CD11b^+$ $CD3^-$, $CD4^-$, $CD8^{+/-}$	Germline	Found in blood, spleen, and mucosal tissues including female reproductive organs; rare in peripheral lymph nodes; generally produce IFN-γ, but can produce other immunoregulatory cytokines such as TGF-ß

HLA, human leukocyte antigen; IFN, interferon; MHC, major histocompatibility complex; TCR, T-cell receptor; TGF, transforming growth factor.

T cells can be divided into T effector cells and regulatory cells that control the activation and functional properties of effector cells. T effector cells proliferate in response to antigen stimulation, produce cytokines and become helper cells for antibody production or killer cells. Regulatory cells generally do not proliferate in response to antigen and block other T cells from becoming activated or block their effector functions. Effector T cells can be divided into T helper 1 (Th1) or T helper 2 (TH2) cells depending upon the cytokines they produce. Th1 cells produce IL-2 and IFN-γ, cytokines that favor eliminating microbial invaders, and Th2 cells produce IL-4 and IL-13 that are associated with allergy and helminthic infections. Regulatory cells control the activity of both Th1 and Th2 cells.

Regulatory T Cells

Regulatory or suppressor T cells are heterogenous populations that consist of subsets of $CD4^+$, $CD8^+$ and NKT cells (see below). They can originate in the thymus or develop from T cells in the periphery.
While previously interest was focused on $CD8^+$ suppressor cells, in recent years $CD4^+$ cells that constitutively express CD25, the α chain of IL-2 receptor, have received considerable attention.

$CD4^+CD25^+$ cells can be divided into a natural subset that develops in the thymus (25 ,26 ,27), and a peripheral subset that develops from $CD4^+CD25^-$ cells in the periphery (28 ,29). Both natural and peripheral $CD4^+CD25^+$ cells express the forkhead family transcription factor, FoxP3 that controls their development (30). In the periphery IL-2 and TGF-ß induce $CD4^+CD25^-$ cells to express CD25 and become suppressor cells (29 ,31 ,32). Natural $CD4^+CD25^+$ cells have a TGF-ß independent origin. The mechanism of action of both of these subsets is contact-dependent and probably depends upon TGF-ß (33). The principal function of natural $CD4^+CD25^+$ cells regulatory cells is to prevent organ-specific autoimmune diseases such as gastritis, thyroiditis, or sialadenitis (25 ,26). In mice, separation of regulatory T cells from effector cells leads to organ-specific autoimmune diseases. Thus, so called “ignorant” self-reactive T cells that are not eliminated by the thymus cause disease because they are held in check by regulatory T cells.

Natural $CD4^+CD25^+$ cells probably have limited effects in SLE. Depletion of these cells in lupus-prone mice does enhance the onset of autoimmunity, but does not alter the course of the disease (34). Although regulatory T cell function appears to be intact in young lupus prone mice, they become deficient following the onset of disease. This is probably because production of IL-2 and TGF-ß, the two principal cytokines required for the generation of peripheral $CD4^+CD25^+$ regulatory cells (35) is decreased in SLE (36 ,37 ,38).

IL-2 and TGF-ß also induce $CD8^+$ cells to develop potent suppressive activity that have a cytokine-dependent mechanism of action (39 ,40). In addition, tolerogenic peptides can also induce $CD8^+$ suppressor cells that express FoxP3 and have a TGF-ß dependent origin and mechanism of action (41 ,42). The adoptive transfer of $CD8^+$ suppressor cells in lupus-prone (NZB × NZW)F1 mice can markedly prolong their survival (43). $CD8^+$ suppressor cells could either complement the effects of $CD4^+CD25^+$ suppressor cells, or enhance their development. TGF-ß induces $CD8^+$ cells to develop suppressive activity within 2 days (39). The TGF-ß produced by these cells could then induce $CD4^+$ cells to become $CD4^+CD25^+$ cells. A combination of $CD4^+$ and $CD8^+$ regulatory T cells generated with IL-2 and TGF-ß have long term protective effects in animal models in vivo (see below). $CD4^+CD25^+$ regulatory T cells can induce other $CD4^+$ cells to develop suppressive activity by a phenomenon called infectious tolerance (31 ,44).

Table 9-2: Effects of Triggering Toll-Like Receptors on Immune Cells

Cells	TLR	Ligand	Effects
Myeloid dendritic cells	TLR3, TLR8	viral nucleic acid acids	Induce IFN-alpha and IL-12
	TLR4	Bacterial lps	Induce IL-6 that confers resistance to Tregs
Plasmacytoid dendritic cells	TLR9	Bacterial DNA (CpG)	Increased maturation and antigen-presenting properties
B cells	TLR 9	CpG	Enhancement of antigen-presenting properties and expansion.
CD4+CD25+ regulatory T cells	TLR2	Bacterial Lipoprotein	Expands regulatory cells
	TLR-4	Bacterial lps	Enhances survival and proliferation
	TLR5	Flagellin	Enhances suppressive activity

lps, lipopolysaccaride

$CD4^+$ cells repeatedly exposed to IL-10 or immature antigen-presenting cells can become IL-10 producing regulatory cells called Tr1 cells (45). Although IL-10 production is elevated in SLE, Tr1 cells have not been described in this disease. Regulatory cells can be generated in vivo indirectly by the administration of certain combinations of immunosuppressive drugs (46 ,47), by blocking costimulatory CD40/CD40L interactions (48), or with anti-CD3 monoclonal antibodies administered in vivo (49).

Dendritic Cells

Cells of the innate immune system have pattern-recognition receptors on their cell surface that enable them to recognize microbial invaders. Before the evolution of T cells and adaptive immunity, infectious agents were eliminated by phagocytic cells and natural killer (NK) cells. The phagocytic cells have evolved into specialized antigen-presenting cells for T cells that are called dendritic cells. Like their primitive precursors, they bear Toll-like receptors (TLRs), capable of binding microbial products. These cells also express receptors for the Fc portion of IgG and complement receptors capable of clearing immune complexes. A subfamily of TLR consisting of TLR3, TLR7, TLR8, and TLR9 specifically bind nucleic acid motifs expressed by various infectious agents (50). Unlike Fc and complement receptors, these TLRs are not expressed on the cell membrane. Triggering of TLRs greatly affect the functional properties peripheral T cell develop (Table 9-2).

Antigen-presenting DC can be divided into two principal subsets, those derived from myeloid and those from lymphoid precursors. Their principal difference is the TLRs they express. Myeloid DC migrate to the skin and other peripheral

tissues. Once they phagocytose and process antigens, they migrate to lymphoid organs where they mature and trigger an immune response. Human myeloid DC characteristically express TLR3 and TLR8. Plasmacytoid or pDC that are derived from lymphoid precursors characteristically express TLR7, and TLR9 that binds bacterial DNA (51). Importantly, the triggering of TLR induces the cells to produce large amounts of type 1 interferons, cytokines now believed to be important in the pathogenesis of SLE (52 ,53).

DC in peripheral tissues and lymphoid organs are mostly immature and lack the co-stimulatory molecules to induce T cells to become effector cells. Therefore, presentation of self-peptides to potentially dangerous autoreactive T cells by immature DC renders them nonresponsive or tolerant to these antigens. However, once viruses or other infectious agents trigger TLRs on these DC, they mature and become capable of activating autoreactive T cells. These cells now express costimulatory molecules such as CD40, B7.1 (CD80), and B7.2 (CD86) that can induce T cell activation. They also produce cytokines such as IL-12 and type 1 interferons that promote T helper cell 1 (Th1) differentiation. Fortunately, self-reactive T cells that had been rendered tolerant generally cannot respond to mature dendritic cells that express these costimulatory molecules. Moreover, certain mature DC can also induce T cells to become regulatory cells (54). Thus, it is an oversimplification to consider immature DC as inducers of tolerance and mature DC inducers of effector cells.

It has been reported that $CD4^+CD25^+$ regulatory T cells express TLR-4, -5, -7, and -8 and that exposure of these cells to the TLR-4 ligand lipopolysaccharide (LPS) induces upregulation of several activation markers and enhances their survival/proliferation (55). On the other hand, LPS triggering of TLR-4 on DC induces them to produce IL-6, a cytokine that increases the resistance of T cells to the suppressive effects of $CD4^+CD25^+$ cells (56).

Some interactions with TLR can induce immunosuppression instead of activation and some organisms are able to promote their survival by ligating these TLR. An example of this is the response of DC exposed to *Candida albicans*. Ligation of TLR2 by this organism results in increased production of IL-10. Mice deficient in TLR2 exhibit decreased IL-10 production and, interestingly, a decrease in T regulatory cells (57). A similar link between TRL2 ligation and IL-10 production with associated T regulatory activity was found upon stimulation with schistosomal lysophosphatidylserine (58).

NK Cells

Natural killer (NK) cells were named for their ability to lyse particular tumor target cells in the absence of any obvious activating stimulus (59 ,60 ,61) and can also kill certain cells that are infected by intracellular organisms (62). NK recognize classical class I HLA molecules by killer cell inhibitory receptors (KIRs) and bind the nonclassical class I molecule, HLA-E to a heterodimer receptor formed by the association of CD94 with various members of the NKG2 proteins (63). The monoclonal antibodies to CD11b, CD16, and CD56 react predominantly with NK cells (64 ,65 ,66 ,67). Both CD11b and CD56 identify virtually identical lymphocyte populations (68). All NK cells express CD11b, but those that express CD56hi produce cytokines rather than kill by contact (51). An important feature that distinguishes NK cells from T and B cells is their ability to respond directly to IL-2 and IFN-γ (69 ,70). Resting T and B cells generally require a first signal through their antigen receptors to respond to these cytokines.

NK cells can spontaneously lyse certain tumor target cells and can kill IgG coated cells by a mechanism called antibody-dependent cellular cytotoxicity (ADCC). In addition to their cytotoxic properties, NK cells can enhance or suppress antibody production (71 ,72 ,73). IL-2 activated NK cells express CD40 ligand (74) and can induce resting B cells to become antibody-producing cells (75). Depending upon the subset and the way they are stimulated, NK cells can produce IFN-γ, TNF-αγ, IL-10, or TGF-ß (75). When NK cells are added to $CD4^+$ cells and B cells they enhance antibody production. However, when $CD8^+$ cells are also present, IgG production is markedly inhibited. The interaction of activated NK cells and $CD8^+$ cells results in NK release of active TGF-ß and this cytokine induces $CD8^+$ T cells to suppress antibody production (39). NK cells can constitutively produce active TGF-ß and are the principal immediate lymphocyte source of this cytokine (75). NK cells produce large amounts of IFN-γ that is induced by viral induce triggering of TLR2, 3, and 4 (76). NK cells can synergize with or eliminate DC depending upon their numbers and the way they were stimulated (51).

NKT Cells

NKT cells may be considered an intermediate between the innate and adaptive immune system. They may be derived from the thymus or the liver from a common precursor of T and NK cells (77 ,78). They express markers common to both of these lymphocyte populations. Two thirds of them express TCRs that are comprised of α and ß chains and one third express γ and δ chains. Unlike conventional cells that respond to peptide antigens, NKT cells respond to glycolipid antigens such as CD1. Also unlike conventional T cells that require two signals for activation, NKT cells require only a single signal (79). While rare in peripheral lymphoid tissue, these cells are present in mucosal tissues and have cytotoxic or immunoregulatory effects (79 ,80). Activated NKT cells in the lupus prone NZB/W mice produce IFN-γ that accelerates the progression of the disease (81).

Interplay Between T Cells and Cells of the Innate Immune System

There is constant cross-talk between DC, NK cells, NKT cells, and T cells that determines whether a given immune response can ultimately trigger an inflammatory response, or will suppress inflammation. Infectious agents activate DC through TLRs that induce NK cells to produce IFN-γ

and TNF-α. These cytokines enhance DC maturation, which, in turn, promotes the T cell–dependent B cell responses of the adaptive immune system (51).

On the other hand, DC, NK cells, NKT cells, and T cells all have the capacity to inhibit immune reactivity. These cells can produce TGF-ß, and if the response to antigen favors TGF-ß production, the paracrine effects of this cytokine can induce T cells and other cells to also produce TGF-ß (82). Moreover, TGF-ß inhibits NK and T cells from producing IFN-ß (83). The result will normally be the generation of T suppressor cells instead of T helper cells.

Development of Immunity, Autoimmunity, or Tolerance

The outcome of T cell response to antigen is multifactorial and includes the strength of the peptide/MHC complex binding to the T cell receptor, costimulation by antigen-presenting cells, and the cytokine environment. As a result, the T cell can become a Th1 or Th2 effector cell, a regulatory cell, or nonresponsive (Fig. 9-1). Strong persistent stimulation results in T helper cell differentiation whereas weak or brief T cell stimulation results in nonresponsiveness or tolerance. As stated above, the triggering of dendritic cell TLRs induces them to become mature antigen-presenting cells that favor T effector cell development. By contrast, antigen-presentation by immature cells favor anergy. The cytokines produced by DC also influence the T cell response. For example, the IL-10 and TGF-ß produced by DC of the mucosal immune system favor regulatory T cell development (84).

As an immune response proceeds and availability of antigen becomes exhausted, certain cell surface molecules expressed by activated T cells now induce “activation-induced apoptosis,” and most of the expanded T cell populations are eliminated. Late in an immune response production of IL-10 and TGF-ß increases and these cytokines induce regulatory T cells that also contribute to the termination of the immune response.

The presence of aggressive, self-reactive T cells in a lymphoid microenvironment where a strong immune response is progressing has the potential to trigger an autoimmune disease. Here mature antigen-presenting cells could break down self-antigens to cryptic peptides that are recognized by pathogenic T cells that have escaped thymic deletion. Other T cells could recognize foreign antigen determinants that cross-react with self (molecular mimicry). Generally, however, pathogenic T cells are inhibited by regulatory T cells that block their activation, their migration to inflammatory sites, or their functional activity. Even if these auto-aggressive T cells became activated, these cells should undergo activation-induced apoptosis like most T cells. Thus, persistent antigen-stimulation, a failure of activation-induced apoptosis, or defective T regulatory cell function can each favor naive disease-causing T cells to proliferate and convert them to pathogenic memory cells that could cause lupus.

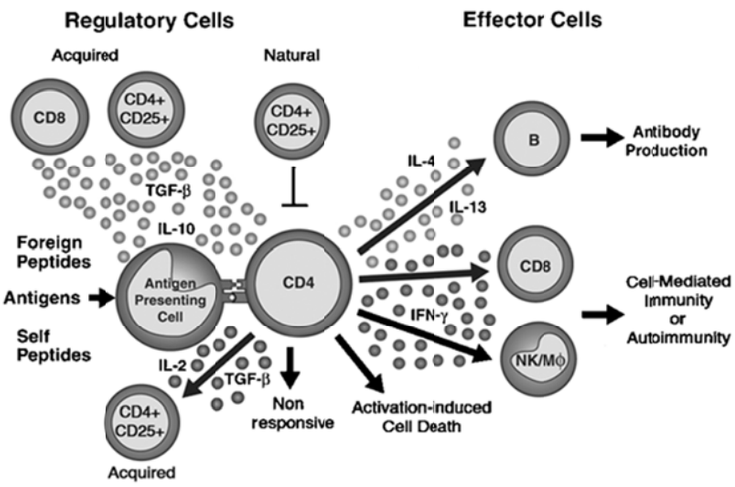

Figure 9-1. Development of immunity, autoimmunity, or tolerance. T cells respond to antigen by either becoming activated or anergic (nonresponsive). Immature antigen-presenting (dendritic cells) lack costimulatory molecules and induce anergy. Infectious agents trigger Toll-like receptors (TLRs) expressed by immature dendritic cells (not shown) and drive then maturity. These antigen-presenting cells activate T cells to provide help for antibody production or to induce cell-mediated immunity. An immune response is terminated as the availability of antigen decreases and most of the T cells are eliminated by activation-induced apoptosis. T-cell activation is also controlled by CD4+ and CD8+ regulatory T cells that maintain tolerance to self antigens. These cells occur naturally, or can be induced by IL-2 and TGF-ß. Thus, antigen-activated T cells can be induced to become either helper or suppressor cells depending upon how they are stimulated and the cytokine microenvironment.

In SLE the products of apoptotic cells trigger TLR expressed by dendritic cells and B cells and enhance their ability to process and present nuclear autoantigens to self-reactive T cells. These activated cells expand because of a lack of T-cell regulation and because they are resistant to activation-induced apoptosis. Thus, tolerance to nuclear autoantigens is broken and autoimmunity is initiated.

The Properties of T Cells and NK Cells in Sle

Percentage and Absolute Numbers of T Cell Subsets

Decreased numbers of T, B, and NK cells is a common manifestation of active SLE (85 ,86 ,87 ,88 ,89 ,90 ,91). Most patients with active SLE have total lymphocyte counts of less than 1000 cells/mm^3. Because of the relative decrease of lymphocytes in SLE in comparison to monocytes, the percentage of $CD3^+$ T cells often is decreased in mononuclear cell preparations. The apparent percentage of T cells may be further decreased by the contamination of immature granulocytes that copurify with mononuclear cells in patients with active disease.

Within the context of T cell lymphopenia, certain T cell subsets may be affected more than others in SLE. Early reports indicated a relative decrease in $CD8^+$ cells in SLE (92 ,93). One group indicated that patients with sicca syndrome, central nervous system (CNS) disease, lung disease, and muscle disease exhibit increased CD4-to-CD8 ratios (94). Other studies

have indicated that the relative percentage of $CD8^+$ cells is either normal or increased, and that $CD4^+$ cells often are decreased in active SLE (95 ,96). In such cases, the decrease in $CD4^+$ cells results in an abnormally low CD4-to-CD8 ratio, a finding that frequently is observed in patients with severe lupus nephritis (94 ,97). Corticosteroid therapy preferentially decreases $CD4^+$ cells and thus decreases the CD4-to-CD8 ratio (95). Decreased $CD4^+$ cells correlate with antilymphocyte antibodies (ALAs) that are specifically reactive against this T cell subset (98 ,99), and a strong relationship exists between high titers of ALAs, lymphopenia, and disease activity (85 ,98 ,100). Within the $CD4^+$ and $CD8^+$ T cell subsets, $CD28^+$ cells are decreased in SLE (101 ,102).

In addition to alterations in the number or percentage of $CD4^+$ cells, Stohl and coworkers reported an association between SLE and expression of a genetically determined variant of the $CD4^+$ molecule in Jamaican black individuals (103 ,104). Whether expression of this variant CD4 molecule truly predisposes certain individuals to develop SLE or simply acts as a marker for some other SLE-predisposing factor remains unknown at present.

NK Cells

Both the percentage and the absolute numbers of NK cells generally are decreased in patients with active SLE. The percentages of $CD16^+$ cells are decreased (105 ,106 ,107 ,108), and the percentages of CD3-$CD11b^+$ and $CD16/CD56^+$ lymphocytes also are decreased (91 ,107). In patients with inactive SLE, values for NK cells usually are normal. In comparison to normal donors, patients with SLE have decreased NK cytotoxic activity; the decrease tends to be more pronounced in more active patients (109 ,110 ,111 ,112 ,113 ,114). Decreased NK activity may be explained by decreased percentages of NK cells. However, since the numbers of lymphocytes binding NK-sensitive targets are comparable between normal donors and patients with SLE (111), it is more likely that NK cells in patients with active SLE have an impaired capacity to lyse target cells. A detailed analysis using purified NK cells is supportive of a defective NK lytic activity in a significant percentage of SLE patients. In fact there was significant inverse correlation between NK activity and global SLE disease activity score (115). Antibody-dependent cellular cytotoxicity mediated by CD16 is also decreased in SLE (115 ,116 ,117 ,118).

The mechanism(s) responsible for decreased cytotoxic activity of NK cells in SLE are not well understood. Serum factors such as immune complexes or ALAs could possibly account for this defect, at least in part. Serum from lupus patients can contain high levels of both immune complexes and ALAs. Immune complexes have been shown to suppress or decrease NK cytotoxic activity (110 ,114 ,119). Several groups have reported that incubation with serum from patients with SLE followed by treatment with complement greatly decreased NK cytotoxic activity (109 ,113 ,114). The inability of serum alone to decrease NK activity suggests that immune complexes, by themselves, were not sufficient. Whether certain ALAs are specific for NK cells is unknown. One study demonstrated that serum from patients with SLE plus complement did kill a substantial number of NK cells (113). In this study, preincubation of the serum with T lymphocytes removed the cytotoxic activity, suggesting that the antibodies were not NK cell specific. However, another study described the presence of autoantibodies that were specific for CD16 (120). There are ALAs in SLE that react with a variety of cell surface structures, some of which may have the potential to react with NK cells specifically or to prevent mediation of full functional activity. A study comparing NK activity of patients with that of relatives found a moderate reduction in activity of relatives (115).

As stated above, the combination of NK cells and $CD8^+$ cells suppresses T cell–dependent antibody production in healthy individuals (39 ,40). In SLE, however, the effect is the opposite. NK cells and $CD8^+$ cells added separately together enhance IgG production (121). This is probably because of defective production of TGF-ß by NK cells in SLE (36).

The significance of the NK cells' role in the suppression of antibody production has been demonstrated in several murine models of SLE. With certain strain combinations, the injection of parental spleen cells into F1 hybrid mice can result in the development of a lupus-like disease with autoantibody production. An inverse correlation was found between the levels of anti-dsDNA antibody and both the number and function of NK cells (122). The significance of this observation was shown by the reduction of autoimmunity through procedures that elevate NK activity and the exacerbation of symptoms by depleting the animals of NK cells. Similarly, it has been reported that development of autoimmunity in C56BL/6 lpr mice correlated with the disappearance of NK cells (123). In a more recent study of C57BL/6 lpr mice, the onset of autoimmunity correlated with the disappearance of a distinct population of cells that expressed the phenotype of NK cells but that also expressed TCRs (124). Taken together, these studies suggest that NK cells have an important role in the regulation of antibody production in vivo.

NKT Cells and CD4-CD8- (Double Negative) γδ Cells

In addition to almost all $CD4^+$ and $CD8^+$ T cells, which are restricted to protein MHC class I and II molecules (see above), a small percentage of T cells recognize glycolipid determinants such as CD1. These cells express the NK cell marker NKR-P1 and are called NKT cells. Most but not all of these cells lack CD4 and CD8 and had been called double negative cells. Increased percentages of DN T cells have been reported in SLE. Moreover, cloning these cells revealed that some αß and γδ cells could provide help for autoantibody production (3 ,19). These observations have been confirmed and extended. Three groups reported an increase in the DN subset in Caucasian or Japanese populations (125 ,126) although another could not find a significant

increase in Chinese patients with SLE. One group reported an increase in the γδ subset (127).

$CD3^+$ CD4 CD8 double negative cells that predominantly express αß TCR chains have been cloned and, like NKT cells, these T cells are restricted by CD1d (126). The DN T cells were stimulated by CD1c on human B cells and could provide help for IgG production. While in healthy subjects, they produced only IFN-γ; in SLE they produced both IFN-γ and IL-4. Interestingly, in active SLE, they produced high levels of IFN-γ. Another group reported that DN T cells in SLE produced IL-4.

Besides helper activity, certain subsets of DN or NKT cells possess potent suppressive effects. A small subset that bears an invariant Vα24JαQ TCR is one example. These T cells are decreased in the affected sibling of identical twins discordant for autoimmune diabetes and marked changes in gene expression have been reported in T cell clones from these twin pairs (128). There is one report of decreased Vα24JαQ positive T cells in a Japanese SLE population (129). Decreased percentages of the NKT γδ subset also been reported with an inverse correlation with disease activity (130). γδ T cells have been proposed to have an important role in mediating self tolerance. Thus, although NK T cells are present in only small numbers, different subsets may have important opposite effects in the pathogenesis of SLE.

Naive and Memory T Lymphocyte Subsets

$CD4^+$ T cells with the naive phenotype (i.e., $CD45RA^+$) were reported to be decreased in SLE (131 ,132 ,133). Because serum from patients with SLE contains autoantibodies against CD45RA, it was suggested that this subset was deleted (134 ,135). However, decreased $CD45RA^+$ cells and a proportionate increase $CD45R0^+$ cells could reflect T cell activation in vivo with the expected increase in the activated state.

T Cell and Antigen-Presenting Cell Function in SLE

In the early 1970s, workers from several laboratories reported that patients with active SLE responded poorly to intradermal injected skin test antigens (136 ,137 ,138 ,139). Thus, SLE was not a disease of immunologic overreactivity, but an imbalance between the cellular and humoral arms of the immune system. Many defects of T cell function were described in parallel with evidence of B cell hyperactivity. Table 9-3 presents a partial summary of T cell functional defects. The significance of these T cell defects was difficult to understand because B cells generally need T cell help to become antibody-producing cells. Moreover, other workers reported evidence of T cell hyperactivity in SLE.

It is now possible to reconcile apparently conflicting reports of both T cell hyperactivity and hypoactivity in SLE. In both human lupus and mouse models of lupus there is evidence of spontaneous and persistent T cell activation. Studies of lupus prone mice have revealed inherited intrinsic defects in the T cells of these animals that result in T cell hyperactivity and increased resistance to cell death. These self-reactive T cells of these mice have a lower threshold of activation to autoantigens, and following their response to these antigens, their survival is increased (140). It is also known that activated T cells are hyporesponsive to further antigen stimulation. Thus, chronic, persistent, T cell activation could explain, at least in part, T cell hypoactivity in SLE documented in in vitro studies.

Table 9-3: T Lymphocyte Functional Activities in Vitro

Function	Activity
Proliferation	
Mitogenic lectins	Decreased or normal
Anti-CD3	Decreased or normal*
Anti-CD2	Decreased
Soluble antigens	Decreased
Allogeneic mixed lymphocyte reaction	Decreased
Autologous mixed lymphocyte reaction	Markedly decreased
Response to IL-2	Decreased or normal
Helper cell activity	
Nonspecific	Decreased or normal
Antigen specific	Decreased
Suppressor cell activity	
ConA induced	Decreased or normal
Antigen specific	Decreased
Spontaneous inhibitors of IL-2 production	Increased
Cytotoxic cell activity	
In response to allogeneic or xenogeneic antigens	Decreased
In response to hapten-modified antigens	Increased
In response to anti-CD3	Decreased
In response to IL-2	Decreased

*When isolated T cells are used instead of peripheral blood mononuclear cells (PBMCs), the response to anti-CD3 is normal or increased.
ConA, concanavalin A; IL-2, interleukin-2.

Increased Spontaneous T Cell Activation In Vivo

Blood lymphocytes isolated from patients with active SLE exhibit numerous signs of activation in vivo (Table 9-4). Increased numbers of circulating T cells from these patients proliferate spontaneously (141) and express cyclin, a marker associated with proliferation (142). Increased numbers of T cells have undergone somatic mutation (143 ,144), or are undergoing apoptosis (145 ,146 ,147 ,148). There is increased T cell expression of MHC class II molecules (149 ,150 ,151), protein tyrosine kinase activity and c-rel [nuclear factor (NF)-κB] mRNA (152 ,153), release of soluble IL-2 receptors (154 ,155 ,156 ,157 ,158), TNF

receptors (159 ,160 ,161 ,162 ,163), and CD40 ligand (164) into the serum is also increased (152). A study of recent thymic emigrants into the peripheral T cell pool has also suggests increased T cell proliferation in SLE (165). Moreover, blood lymphocytes of patients with active SLE express IL-2mRNA, a finding that reflects persistent antigen stimulation (166). Finally, in mouse models of lupus there is polyclonal $CD4^+$ T cell activation (167 ,168).

Table 9-4: Evidence of In Vivo T-Cell Activation in Subjects with Active SLE

Increased numbers of spontaneously proliferating T cells
Increased frequency of circulating T cells bearing somatic mutations
Increased cell surface expression in unstimulated T cells of MHC class II (HLA-DR, -DP) structures, Fas, Fas ligand, CTLA-4, and a modest increase in interleukin-2 receptors
Increased serum levels of soluble interleukin-2 receptors, tumor necrosis factor-α receptors (p55 and p75), soluble Fas, and soluble CD40L
Increased levels of mRNA transcripts for interleukin-2 and c-rel (NF-kappa B) in unstimulated T cells
Increased protein tyrosine kinase activity
High levels of circulating early apoptotic blood mononuclear cells

CTLA, cytotoxic T-lymphocyte associated; HLA, human leukocyte antigen; MHC, major histocompatibility complex; mRNA, messenger RNA; NF, nuclear factor.

T Cell Hypoactivity In Vitro

In contrast to studies of freshly prepared T cells from patients with SLE, the proliferative response of these cells to mitogenic lectins (i.e., phytohemagglitinin [PHA], concanavalin A [Con A] and pokeweed mitogen [PWM] and soluble antigens) is decreased in SLE (139 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179), with an occasional exception (180). T cell responsiveness to both allogeneic and autologous lymphocytes was decreased in SLE (181 ,182 ,183 ,184 ,185 ,186). The principal reason for these findings is defective co-stimulation by accessory cells, or humoral inhibitory factors (see below).

Pairs of receptor/coreceptor pairs on T cells and accessory cells that are crucial to T cell costimulation include CD2/CD58 (LFA-3), CD11a (LFA-1)/CD54 (ICAM-1), CD40/CD40L, and CD28 or CD152 (CTLA-4)/CD80 (B7–1) or CD86 (B7–2). Blockade with specific mAb or soluble fusion proteins of the receptor/coreceptor interactions can inhibit T cell proliferation, T cell–dependent B cell differentiation, and/or induction of T cell cytolytic activity (187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195). Expression of CD80 and CD86 following activation is decreased in SLE (196 ,197). The inhibitory CTLA-4 receptor is normally expressed on SLE T cells, but one study suggested that its inhibitory effect is decreased in this disease (198). This could be a result of decreased expression of CD80.

While accessory cell–dependent, CD3/TCR-mediated proliferation in unfractionated PBMC cultures was often abnormally low in SLE (172 ,174 ,178), the response of purified T cells in SLE was normal-to-enhanced (199 ,200 ,201). This is because immobilized anti-CD3 antibodies that bind the CD3/TCR do not require a second costimulatory signal to induce proliferation. The proliferative response to anti-CD2 also was depressed because of multiple defects (102 ,202). As was the case for anti-CD3, an accessory cell defect explained this result in the majority of patients, because the defect disappeared following depletion of these cells. Most important, the addition of anti-CD28 also reversed the defect (102). Others have found that the costimulatory effect of anti-CD28 markedly enhanced the capacity of patients with active SLE lymphocytes to produce IL-2. (101) A defective proliferative response to anti-CD2 in SLE may be important because signaling through this pathway selectively triggers a TGF-ß-dependent suppressor-cell pathway (40).

Costimulatory Markers Associated with T Cell Hyperactivity

Increased expression of CD11a and CD54 in SLE (203 ,204 ,205) has been associated with development of autoreactivity (203). Other T cell surface antigens that are upregulated during the course of an immune response that play vital roles in T cell helper function and T cell cytolytic function respectively are CD154 (CD40 ligand) and CD95L CD178 (Fas ligand) by interacting with CD40 on the surface of B cells, can deliver a differentiation signal as well as either rescuing B cells from or priming B cells for apoptosis (206 ,207 ,208 ,209 ,210 ,211 ,212). CD154 is transiently expressed by $CD4^+$ T cells soon after activation and is quickly downregulated, especially in the presence of B cells (213 ,214). This tight regulation of CD40 ligand expression presumably protects the host against induction of indiscriminate polyclonal B cell differentiation by activated T helper cells. CD154 is, in fact, hyperexpressed by both $CD4^+$ and $CD8^+$ T cells in SLE (215 ,216 ,217), and biologically active circulating soluble CD154 is increased in SLE (218).

T Cell Cytolytic Activity in SLE

The ability of $CD8^+$ cells to develop cytolytic activity in SLE is also decreased. Abnormalities in T cell–dependent or T cell–mediated cytolytic activity include impaired generation of cytotoxic T cells against allogeneic or xenogeneic targets (219 ,220), and impaired anti-CD3-driven cytolytic activity (201 ,221). In contrast to these reports, others have reported that $CD4^+$ cells in SLE kill autologous monocytes (222). Another group reported $CD8^+$ cells from patients with disease flares express higher levels of perforin- and/or granzyme B. In this study lupus serum promoted the development of DC. These dendritic cells in turn, enabled $CD8^+$ cells to become cytolytic cells that generated high amounts of soluble nucleosomes and autoantigen fragments (223).

The defect in cytolytic activities of $CD8^+$ cells may not be a result of an intrinsic abnormality, but because of inadequate costimulatory effects of accessory, non-T cells. Although the cytotoxic effects of SLE T cells was markedly reduced in cultures containing blood mononuclear cells, when purified T cells were assessed, the responses of SLE $CD8^+$ cells were only modestly and insignificantly less than $CD8^+$ cells from normal controls (224). Moreover, twin studies revealed that substitution of “healthy” non-T cells for SLE non-T cells in four of the nine monozygotic pairs appreciably enhanced cytolytic responses, and substitution of SLE non-T cells for “healthy” non-T cells in five of the seven twin-pairs tested appreciably diminished cytolytic responses (224 ,225).

T cell defects may explain the abnormally high levels of Epstein-Barr virus (EBV) loads in patients with SLE. EBV viral loads were inversely correlated with the frequency of EBV-specific $CD69^+$ $CD4^+$ T cells producing IFN-γ, and were positively correlated with the frequencies of $CD69^+$ $CD8^+$ T cells producing IFN-γ, and with EBV-specific, HLA-A2 tetramer-positive $CD8^+$ T cells (226).

Regulatory T Cell Activity

It is now evident that antibody production is principally controlled by regulatory/suppressor T cells. In the past, numerous investigators reported that $CD8^+$ cells had impaired suppressive activity in SLE (227 ,228 ,229 ,230 ,231 ,232 ,233 ,234 ,235 ,236 ,237 ,238 ,239 ,240). This function was generally measured by the ability of $CD8^+$ T cells to inhibit the proliferation of other T cells to mitogen. In SLE, one group analyzed DNA-induced antibody synthesis in a pair of identical twins who were discordant for SLE; only B cells from the SLE cotwin could produce anti-DNA antibodies. Addition of the SLE cotwin's T cells to her own B cells promoted anti-DNA antibody production induced by calf thymus DNA. On the other hand, T cells from the unaffected cotwin did not promote anti-DNA antibody synthesis unless $CD8^+$ cells were depleted. This finding suggested that the healthy cotwin had anti-DNA specific T helper cells, but that they were kept nonfunctional by $CD8^+$ suppressor cells (15). More recent studies support impaired functional activity of SLE $CD8^+$ cells (241).

There is considerable evidence that regulatory $CD4^+$ $CD25^+$ cells prevent the activation of autoreactive T cells (242). Both natural and $CD4^+CD25^+$ induced from $CD4^+CD25^-$ cells in the periphery with IL-2 and TGF-β express FoxP3 (see above), and each may participate in controlling autoimmunity in SLE. The principal effects of natural $CD4^+CD25^+$ cells, however, are to prevent organ-specific autoimmune disease (25 ,26). Natural $CD4^+CD25^+$ cells that lack the capacity to produce TGF-β or IL-10 are present in mice with genetic deletions of TGF-β1 and CTLA-4: these mice die from overwhelming autoimmunity within several weeks after birth (243 ,244). Thus it is likely that TGF-β and the peripheral Tregs they induce have a central role in the prevention of SLE.

A combination of $CD4^+$ and $CD8^+$ regulatory T cells generated ex vivo with IL-2 and TGF-β have long term effects in vivo in mice. They can prevent a lupus like syndrome and a single injection will double the survival of animals with established disease (245). These long-lasting effects could be because of the ability of $CD4^+CD25^+$ cells to educate other $CD4^+$ cells to develop suppressive activity (31 ,44). Immunogenic peptides that accelerate the development of lupus in mice have protective effects instead if injected intravenously. Instead of inducing self-reactive $CD4^+$ cells to become aggressive, tolerogenic approaches induce $CD4^+$ and $CD8^+$ regulatory T cells. One group has demonstrated that lupus prone mice tolerized by very low doses of a pathogenic lupus peptide injected intravenously developed $CD4^+CD25^+$and $CD8^+$ suppressor cells that produced and required TGF-β for its mechanism of action (42). Another group found that tolerization with another immunogenic peptide resulted in the appearance of $CD4^+CD25^+$ suppressor cells with a TGF-β–dependent mechanism of action (41). This group reported that $CD8^+$ suppressor cells only developed following immunization of mice resistant to the development of lupus (246). Recently, this group has observed that the transfer of TGF-β producing $CD8^+$ Tregs to lupus-prone mice could markedly increase survival (247). The antigen specificities of these tolerogenic peptides will be described below.

Another group prevented lupus in mice with intranasal injections of a histone peptide. In these mice the numbers of $CD4^+CD25^+$ T cells were normal until just before they developed lupus and were markedly decreased thereafter (248). Other workers have documented $CD4^+$ TR-1 like cells following high doses of a peptide derived from the Sm protein (249).

It has been reported that the T cells from lupus-prone mice are resistant to the suppressive effects of $CD4^+CD25^+$ cells (250). In humans there is only limited information about $CD4^+CD25^+$ regulatory T cells in lupus. Unlike mice, only the brightly staining $CD25^+$ cells have the phenotype and properties of natural $CD4^+$ regulatory cells. Two groups have reported decreased numbers of these cells in SLE (251 ,252).

The Effect of Serum Factors on T Cell Function

Factors extrinsic to the cells such as autoantibodies or nonantibody serum components can inhibit the T cell proliferative response. SLE sera inhibit lymphocyte proliferation in response to mitogenic lectins (253 ,254 ,255), soluble antigens (98), and allogeneic (256 ,257 ,258) as well as autologous MHC antigens (176 ,259 ,260 ,261). These sera also block the generation of cytotoxic T cells (220) and interfere with antigen presentation by macrophages (262). Much of this inhibitory capacity can be ascribed to IgM and IgG antibodies, which react with various lymphocyte cell surface molecules. SLE ALAs react with activated lymphocytes more strongly than resting lymphocytes (98 ,263 ,264). IgG ALAs inhibit suppressor cell generation and activity (228 ,265 ,266). Additionally, IgG ALAs inhibit mitogen- and

mixed lymphocyte reaction (MLR)-induced proliferation (228 ,253 ,254 ,256 ,257 ,260 ,264) and preferentially inhibit the T cell response to soluble antigens (98).

Autoantibodies against the CD3/TCR have been identified in SLE. One group reported autoantibodies directed against “public” idiotopes present in the first complementarity determining region (CDR1) and the third framework (FR3) of the Vbeta gene products. They suggested that these autoantibodies are generated in response to overproduction of autodestructive T cells bearing particular Vbeta gene products and function to modulate (downregulate) the expression of these T cells (267). Consistent with this suggestion, another group has recently reported anti-TCR/CD3 antibodies in SLE that can account for decreased IL-2 production in SLE (268).

Although initially reported only to react with T suppressor cells (228 ,265 ,266), ALAs react with both $CD4^+$ and $CD8^+$ cells (269 ,270) and may react preferentially with $CD4^+$ cells (135 ,271 ,272). Such autoantibodies may result in altered CD4/CD8 ratios, leading to altered immune function. Additionally, IgM autoantibodies that are reactive with the membrane tyrosine phosphatase CD45 molecule have been described in SLE (135 ,272). These antibodies preferentially react with the high molecular weight CD45RA isoform that is expressed on naive T cells (19). Such autoantibodies may interfere with T cell signal transduction. Autoantibodies against the MHC class I associated ß2-microglobulin (273) and MHC class II molecules (260) also have been described. Such autoantibodies could inhibit T cell function by blocking cell-to-cell interactions.

In addition to ALAs, which were described in the 1980s, it was reported more recently that hyperactive B cells secrete TGF-ß complexed to IgG, and these complexes inhibit macrophage and neutrophil function (274 ,275). Increased levels of IgG-TGF-ß complexes have been found in lupus sera (275) and can explain, at least in part, some of the inhibitory effects of lupus sera on T cell function.

Altered Cytokine Homeostasis

Although patients with active SLE have increased numbers of circulating IL-2 producing T cells, the production of this cytokine and many others is decreased in response to antigen or mitogen stimulation (37 ,38). Lymphocyte production of both latent and active TGF-ß is decreased in SLE (36). The defect in total TGF-ß production was related to disease activity (276). Defective production of IL-2 and TGF-ß in SLE may be important in the pathogenesis of this disease. These two cytokines are required for peripheral $CD4^+$ cells and $CD8^+$ cells to become suppressor cells (28 ,29 ,35 ,39) SLE is characterized by B cell hyperactivity and autoantibody production. Patients with SLE have increased numbers of circulating B cells that secrete IgG and autoantibodies. A brief exposure of blood mononuclear cells from these patients to IL-2 and TGF-ß markedly decreased spontaneous IgG and antinuclear autobody production (277). It is likely that these cytokines induced certain lupus T cells to regain suppressive activity and shut down B cell activity.

One consequence of active SLE, and a product of continuous immune stimulation, is elevated IL-10 production (278 ,279). IL-10 inhibits T cell function by blocking the effects of APCs on T cell function. Among the cytokines that are downregulated by IL-10 are IL-2, TNF-α, and IFN-ß (83 ,280 ,281 ,282 ,283). Each of these cytokines has been considered to have an important role in the generation of cytotoxic T cells and those that downregulate antibody production. Even though under certain conditions IL-10 can be stimulatory (284), high levels of this cytokine strongly inhibit the expression of costimulatory molecules by antigen-presenting cells and thereby interfere with T cell function. IL-10 also makes T cells more sensitive to activation induced cell death (146).

In conclusion, the apparently contradictory functional defects described in SLE may be due to the multifactorial abnormalities in SLE that include persistent T cell activation, accessory cell defects, humoral factors, and ineffective feedback regulatory mechanisms. As a result of the failure to shut down autoimmune T cell reactivity, the reactivity of other T cells is impaired.

Antigen Recognition by T Cells in SLE

Tolerance Induction

In SLE tolerance to antinuclear antigens and many cytoplasmic antigens has broken down with the production of a wide variety of autoantibodies. This loss of tolerance is not a result of a general immune defect. Studies of mouse models of SLE suggest that ability to delete autoreactive T cells with high affinity for self is generally intact. Clonal deletion of self reactive T cells is normal in two models of SLE (285 ,286 ,287 ,288). Deletion of T cells stimulated with superantigen is also intact (287). T cell tolerance induction in B/W mice carrying a transgene encoding beef insulin was also normal (289). Rather, the breakdown in tolerance to nuclear autoantigens and autoantibody production is because of multiple inherited cellular abnormalities. These include abnormalities of antigen-presentation, clearance of apoptotic bodies and immune complexes, T cell activation and death, T cell regulation, and B cell defects. Increased apoptosis of mononuclear cells and a failure to clear them, results in persistent T cell exposure to nucleosomal antigens (290 ,291). Defects in T cell activation result in a decreased threshold for T cells to respond to antigen (217). DC and B cells have a heightened capacity to present nucleosomal autoantigens (292). Increased expression of B7.1 (CD86) on B cells (293) and CD40L on T cells (215 ,216) contributes to this increased reactivity. In autoimmune mice breaking of self-tolerance requires signaling via both the CD28/B7 and CD40/CD40L pathways (294).

In human SLE, short term T cell lines were resistant to anergy induction. This resistance was associated with increased expression of CD40L and persistent activation of the mitogen-activated protein kinase ERK. (295), abnormalities that result in upregulation of cyclooxgenase 2 (296, see below). These multiple factors result in the activation and clonal proliferation

of T cells with low avidities for nuclear autoantigens. These cells are not deleted by central mechanisms in the thymus.

Persistent Nuclear Autoantigen Availability Because of Increased Numbers and Decreased Clearance of Apoptotic Cells

There is increased apoptosis of T cells, neutrophils, monocytes, and even endothelial cells in SLE patients (145 ,146 ,148 ,179 ,297 ,298 ,299 ,300 ,301 ,302). Activated cells are more susceptible than resting cells to the induction of apoptosis (303 ,304), and evidence has been reviewed earlier indicating increased numbers of activated T cells in patients with SLE. Lymphoblasts from patients with other inflammatory rheumatic diseases and infections are also more susceptible to apoptosis, but not to the same extent of SLE. Increased apoptosis in SLE may be related to disease activity (145 ,297 ,301). Apoptosis of lymphoblasts from lupus patients with high erythrocyte sedimentation rates and elevated levels of Th1 cytokines was especially pronounced (298). Others however, have reported increased apoptosis unrelated to disease activity (148).

Apoptotic cells are the principal source for the nuclear autoantigens that are immunogenic in SLE. These products are derived from chromatin and small nuclear ribonucleoproteins (snRNPs). As cells die many of these autoantigens are redistributed to the cytoplasm and accumulate in membrane blebs. Nucleosomes, which are complexes of DNA and histone proteins from chromatin, are the major products of apoptosis. In the early process of apoptosis, there is extranuclear accumulation of the nucleosomal histones H2A, H2B, H3, and H4 (305) elements which are immunogenic in SLE (306). Nucleosomal particles in the serum of SLE patients correlate with disease activity (307).

While apoptotic cells are normally cleared rapidly, this function is defective in SLE (308 ,309 ,310). Mononuclear phagocytes in SLE have decreased expression of Fc receptors II and III, and have a decreased capacity to clear IgG-sensitized erythrocytes (311). In SLE, monocytes that should be clearing apoptotic bodies are killed by autoreactive $CD4^+$ cells. These T cells express increased levels of pro-apoptotic ligands, tumor necrosis factor family proteins FasL, TWEAK, TRAIL (312).

Hyperactive Antigen-Presenting DC and B Cells in SLE

Apoptotic cells that are not cleared become necrotic, and when taken up by antigen-presenting cells in this inflammatory form, these cells become highly immunogenic. Activation of the innate immune system by apoptotic and necrotic cells and complexes of nucleic acids and IgG result in the production of large amount of IFN-α. Products of DNA and RNA released from apoptotic cells complex with lupus IgG and bind TLR and FcR receptors expressed by monocyte-derived plasmacytoid dendritic cells (313 ,314). The triggering of these receptors results in strong DC activation and the production of IFN-α and other cytokines. TLR9 dependent and independent mechanisms of activation have been reported (315). Immune complexes with other proteins were less effective in activating DC. Serum from lupus patients and IFN-γ isolated from lupus serum can induce monocytes to become autoreactive DC that express high levels of B7 and other costimulatory molecules (316). The splenic dendritic cells DCs from the lupus prone NZB/W F1 mice spontaneously stimulate nucleosome-specific T cells to a much greater degree than both DCs from normal mice (317).

In addition to their role as the precursors of antibody-forming cells, activated autoreactive B cells present cognate antigen to T cells (318). B cells have been postulated to break tolerance to ignorant or anergic T cells. Alternatively, they can further stimulate T cells that have been activated by dendritic cells (292). Interestingly, lupus-prone MRL/Faslpr mice deficient in CD80 or CD86 develop lupus similar to B7 sufficient mice (319).

Antigen Specificities of Autoreactive T Cells

Although SLE is characterized by anti-DNA antibodies, native mammalian DNA is a poor immunogen. Even purified denatured DNA that is complexed to an immunogenic carrier protein does not induce antibodies to native DNA (320). Bacterial DNA, however, differs from mammalian DNA in the frequency and methylation of CpG sequences (321). These CpG sequences have adjuvant effects that can alter and enhance the immogenicity of native DNA. Moreover, the apoptotic nucleosomes have abnormally methylated GC-rich regions (322). As stated above, CpG-dinucleotides that bind to intracellular TLR9 in DC would markedly enhance the antigen-presenting properties of these cells. In this manner, DNA-protein complexes can trigger pathogenic anti-DNA antibodies in SLE. DNA that is bound to histone or complexed to other proteins is, in fact, a common target of autoantibodies in SLE (322 ,323).

It is now evident that nucleosomes are a major immunogen for autoantibodies that cause lupus nephritis (306 ,324 ,325 ,326). Conformational epitopes on native chromatin and the (H2A-H2B)2-DNA subnucleosome induce specific antibodies, which then spread in a stepwise manner to include IgG antihistone and anti-native DNA antibodies (5 ,326), which may be complement fixing. Nucleosomes typically express cationic residues that bind to complementary-charged domains of TCR that are expressed by autoreactive T cells (327 ,328). Thus, the epitopes that T cells recognize are peptide fragments of the proteins that are complexed with DNA or histones in nucleosomes.

Five major auto-epitopes from the core histones of the nucleosome particle that stimulate autoreactive helper T cells in human SLE have been identified (306). $CD4^+$ T cells from lupus patients recognized peptides from histone regions H2B, H4, and H3. At least two peptides from the H2A and H4 were recurrently recognized by autoreactive T cells from different lupus patients (329). Since lupus T helper cells do not respond to free histones, the immunogenic peptides are generally not processed and presented by antigen-presenting cells. To identify these “cryptic” peptides, 154 candidate peptides spanning the

entire length of core histone were tested. The peptides that stimulate human helper cells overlapped with the major epitopes for the T helper cells that induce anti-DNA antibodies in lupus-prone mice (329). Another group also found that histone peptides could induce autoreactive Th clones (330).

Nonnucleosomal peptides can also stimulate the autoreactive lupus T cells that provide help for pathogenic anti-DNA antibodies. Peptides from a pathogenic anti-DNA antibody VH region also have this property. Immunization of NZB/W F1 mice with either of three specific VH peptides increased anti-DNA levels, accelerated nephritis, and decreased survival. T cells that are immunized with these peptides produced either a TH1 or TH2 profile of cytokines, but adoptive transfer of either of these T cells accelerated disease (331). These peptides stimulated autoreactive T cells to provide help for a variety of B cells displaying a cross-reactive version of the original immunogen, a form of determinant spreading. This reciprocal T-B cell stimulation spreads until large cohorts of T and B cells have expanded. Presumably, similar spreading occurs in human SLE.

Whether nucleosomal or peptides derived from the VH region of several murine anti-dsDNA antibodies are pathogenic or protective is determined by the route of immunization. It is now evident that both nucleosomal and VH region peptides are tolerogenic when administered intravenously. Strikingly, the protective effects are due to the generation of $CD4^+CD25^+$ and $CD8^+$ regulatory T cells. The mechanism of action of both $CD4^+$ and $CD8^+$ suppressor cells was dependent upon secreted or contact dependent TGF-ß (41 ,42). Thus, lupus-specific autoepitopes can accelerate or ameliorate disease.

As discussed, T cell recognition of and response to both foreign and self-antigens is determined by the class I and II MHC gene products of the host. T cell cytokine production and subsequent helper activity therefore is influenced by the expression of MHC alleles on antigen-presenting cells. For example, expression of HLA-DR3 is associated with decreased production of IL-1 and IL-2 (332); expression of HLA-DR2 and DQw1 correlates with elevated anti-dsDNA autoantibody titers, decreased production of TNF-α in vitro, and lupus nephritis (333); and expression of both HLA-DR3 and DQw2 correlates with detectable anti-Ro/SSA and anti-La/SSB antibodies (334 ,335).

Drugs also can induce lupus syndromes by causing DNA damage and releasing altered nucleosomes (203 ,327 ,329 ,336), in addition to inhibiting DNA methylation (94 ,95 ,96). Studies of apoptotic keratinocytes have revealed blebs containing nucleosomes and spliceosomes, which bear most of the predominant SLE autoantigens (336 ,337 ,338). These structures are subject to oxidative modification. Following phagocytosis and processing by antigen-presenting cells, the altered epitopes may be rendered strongly immunogenic for nontolerant $CD4^+$ T cells.

T Cell Lines and Clones

Unlike most peptide antigens, the nucleosomal epitopes do not obey the rule of MHC restriction. They can be presented and recognized in the context of diverse MHC alleles. As stated above, the immunogenic peptides and the lupus TCRs bear reciprocally charged residues which result in high-affinity interactions between the α chain of lupus TCRs and the APC. Approximately one half of cloned human lupus T helper cells responded to nucleosomal antigens that contained cationic residues (326). The CDR3 loops of TCR α chains contained a recurrent motif of anionic residues, whereas the TCR ß chains contained both anionic and cationic residues in their CDR3, suggesting that these pathogenic clones probably recognize autoantigens with epitopes of mixed charges (328).

Cytokines produced by autoreactive SLE T helper cell lines or clones stimulated with immunogenic histone peptides have been identified. Some peptides preferentially induced a strong Th1 (IFN-γ) response, while others favored Th2 (IL-10 and/or IL-4) production (329). Another group found most Th clones secreted IL-2, IFN-γ and IL-4, whereas others produced predominantly IL-2 and IFN-γ (330). This group also reported that the histone peptides could induce T cells from healthy subjects to become similar autoreactive clones.

Human T cell clones generated from the peripheral blood mononuclear cells (PBMCs) of patients with SLE or mixed connective tissue disease (MCTD) were found to react against uridine-rich, RNA-snRNP antigen. As with the histone peptides, these snRNP clones could be produced from MHC genotype-matched normal controls. These were oligoclonal $CD4^+$ memory cells (339). Further studies have revealed that these autoreactive T cells can provide help for autoantibody production (340). This group has also described immunogenic epitopes derived from the Smith antigen (341). A proliferative response to a ribosomal P fusion protein has been reported (342), notwithstanding the impaired response of SLE T cells to soluble antigens.

The Response of SLE T Cells to Antigen

SLE T Cells Have a Low Threshold for Activation

T cells with low avidity receptors for self-antigens are not deleted by the thymus and are present in the periphery. Their persistence throughout life suggests that they are continuously being stimulated by self antigens (343) but at subthreshold levels for activation. In normal animals, these low levels may be sufficient to induce them to become suppressor cells (344). In SLE, however, these cells are activated and become pathogenic.

There is evidence of polyclonal T cell activation in SLE. Increased numbers of activated T cells are present in both human and mouse models of SLE (168 ,345 ,346). One strain of lupus prone mice bear a genetic locus on chromosome 7 that contributes to a lower threshold of T cell activation and resistance to activation-induced cell death (347). Several inherent defects of T cell receptor signaling in SLE result in abnormal signaling (see below). Using TCR transgenic

animals, one group demonstrated that T cells derived from an SLE background proliferated faster and made more IL-2 in response to peptide than nonautoimmune controls (167). Finally, lupus T cells express higher levels of costimulatory CD40L than normal T cells (216 ,217 ,218 ,295). Thus, lupus mice develop an accelerated disease when stimulated by pathogenic peptides, whereas in normal mice autoantibodies appear only transiently (348).

Increased Resistance to Activation-Induced Cell Death

SLE T cells are resistant to activation-induced apoptosis, a homeostatic mechanism that should eliminate self-reactive T cells that may have been stimulated by cross-reactive antigens. One group repeatedly stimulated normal and SLE T cells, and after resting these cells attempted to induce them to undergo apoptosis. The lupus T cells, however, were resistant to activation-induced cell death. To explain this result, these workers reported decreased phophorylation of Cbl-b, an adapter protein that downregulates critical kinases in T cells signaling pathways that include Zap70, Lck, phospholipase 1, protein kinase C, and the p85 subunit of phosphatidlinositol 3-kinase (217 ,295). Cbl-b is an ubiquitin kinase that results in the proteosomal degradation of targeted proteins (349). Lupus T cells with impaired Cbl-b function are resistant to anergy because of persistent activation of the extracellular-regulated kinase (ERK) (295). In turn ERK may stabilize CD40L mRNA and result in hyperexpression of this costimulatory molecule. The result of decreased levels of Cbl-b would be sustained IL-2 production, a finding reported in both human and murine lupus. Others have described a mouse model of lupus with T cells that are resistant to anergy induction and increased levels of IL-2 (350). T cells from newly diagnosed, untreated lupus express increased levels of IL-2 mRNA (166).

Since certain strains of mice with defects in T cell expression of Fas or Fas Ligand develop an SLE-like disease, there has been a considerable interest in Fas expression in SLE. Fas-mediated cytotoxicity represents a major pathway leading to cell death (351 ,352 ,353 ,354) and is an important mechanism in peripheral tolerance. Mice that are homozygous for either lpr, a mutation in the fas gene (354) or for gld, a point mutation resulting in defective or absent Fas ligand expression, develop generalized lymphadenopathy in association with lupus-like features (355 ,356 ,357 ,358 ,359 ,360 ,361). These mice have impaired deletion of self-reactive B cells and expansion of autoreactive T cells (288). Conversely Medical Research Laboratory (MRL)-lpr/lpr mice that are transgenic for the intact fas gene under the control of a T cell–specific CD2 promoter and enhancer do not develop the lymphadenopathy, glomerulonephritis, or clinical autoimmunity that their nontransgenic littermates do (362). Although Fas expression is not decreased in human SLE and actually may be increased (363 ,364), the potential ramifications of the observations in murine lpr and gld mice for human disease have been highlighted by an association in humans between fas mutations and clinical autoimmunity and/or lymphadenopathy (365 ,366 ,367). Moreover, soluble Fas protein, which can inhibit apoptosis of stimulated cells under appropriate conditions, has been reported to be elevated in the serum of many patients with SLE (368 ,369), although this finding has been challenged (370 ,371).

T Cell Signaling Pathways in SLE

The molecular basis for T cell hyperactivity and their resistance to apoptosis is beginning to be understood (Table 9-5). In response to T cell receptor (TCR) stimulation intracellular calcium increases and there is phosphorylation of signaling proteins in the immunologic synapse. In SLE both of these events are enhanced and prolonged. This ultimately results in “rewiring” of the TCR with replacement of the TCR chain signaling molecule with Fc receptor chain. The result is a lower activation threshold for SLE T cells (372).

The increased calcium fluxes also contribute to the prolonged expression of CD40L on lupus T cells. As stated above, CD40L is a very potent costimulatory molecule only transiently expressed by normal T cells following activation. Persistent expression of CD40L, in turn, contributes to the resistance of lupus T cells to anergy induction. Antagonism of CD40L by a pharmacologic agent restores the ability of lupus to cells to become anergic (295).

In an attempt to explain anergy resistance in SLE, one group looked for differences in the phosphorylation of signaling proteins in anergic T cells. T cells become anergic following ligation of TCR without costimulation. This study

revealed that there was a lack of phosphorylation of cbl-b, a critical T cell silencer in SLE T cells. This was an especially important observation since a defect in this silencer could explain the exaggerated phosphorylation of adapter proteins in SLE and enhanced expression of the ERK signaling pathway, which should be shut down in anergic cells (295).

Table 9-5: Defects of Signal Transduction in SLE T Lymphocytes

Exaggerated increase in intracellular free calcium and increased phosphorylation of signaling proteins following T-cell receptor stimulation.
Decreased expression of CD3/T cell receptor ζ chain
Decreased expression of protein kinase C
Decreased activity of protein tyrosine phosphatase activity of CD45
Decreased protein kinase A type I and type II isoenzyme activity
Decreased levels of the p65-RelA subunit of the NF-κB nuclear transcription factor
Increased binding of the transcriptional inhibitor pCREM (cyclic AMP response element modifier) to the IL-2 promotor
Defective phosphorylation of Cbl, an adaptor protein that negatively regulates transmembrane-signaling; correlates with increased expression of CD40 ligand and resistance to tolerance induction
Reduced levels of the intracellular signaling protein, LCK in lipid rafts

Cyclic AMP, 3, 5 8-cyclic adenosine monophosphate.

Although some T cells in patients with active SLE are IL-2 producing cells, SLE T cells generally produce decreased levels of this cytokine following TCR stimulation. Progress has been made in understanding the molecular basis of this cytokine defect. Decreased transcription of the IL-2 gene is a result of decreased enhancer and increased repressor activity (296). The activity of nuclear factor-κ B a gene that has a central role in immune responses and inflammation is decreased in SLE (373). This transcription factor not only controls IL-2 production, but also many other cytokines and other immune response genes. Normally, IL-2 activity is downregulated by a transcriptional adaptor protein called CREM (cyclic AMP response element modifier) (374). CREM binds to the IL-2 promotor and suppresses its activity and expression of this adaptor protein is increased in SLE T cells (375).

The reasons for increased CREM expression in SLE are complex. CREM is regulated by $Ca2^{+}$calmodulin-dependent kinase, a protein found to be increased in the nucleus of SLE T cells. Although the increased TCR-mediated free intracytoplasmic $Ca2^{+}$ complexes observed in SLE can account for the increase in $Ca2^{+}$calmodulin-dependent kinase, this kinase by itself cannot account for increased CREM. Overexpression of $Ca2^{+}$calmodulin-dependent kinase in normal mice could not reproduce the effect (268). Protein kinase A (PKA), protein kinase C (PKC) and ERK also regulate CREM expression and defects of each of these kinases have been reported in SLE (152 ,376 ,377 ,378 ,379 ,380 ,381 ,382 ,383 ,384 ,385 ,386). Abnormalities of both type I (PKA-I) and type II (PKA-II) isozyme activities have been described in SLE (386 ,387 ,388).

Although genetically inherited traits probably account for some of the signaling abnormalities, it has become clear that the host response to chronic antigenic stimulation strongly contributes to the defects described in SLE. For example, stress related oxidants contribute to the downregulation of TCR ζ chain expression in SLE. Resting SLE T cells in the presence of anti-oxidants reversed this defect (389). Moreover, anti-TCR/CD3 autoantibodies in lupus serum have been reported to increase expression of CREM and increase the binding of CREM to the IL-2 promotor. Thus, anti-CD3/TCR antibodies present in SLE sera activate a kinase that facilitates the binding of a transcriptional repressor or to the IL-2 promoter (268). This finding is in agreement with previous studies that revealed that the inhibitory effects of lupus serum on T cells from SLE patients and healthy controls was more striking than intrinsic defects of lupus T cells (169 ,254 ,255).

Defective expression of the lymphocyte-specific protein kinase (LCK) is also associated with disease activity of SLE (390). LCK has a critical role in maintaining the resting state of T cells and for initiating the activation of signaling cascades. Again resting lupus T cells in vitro reversed defects in LCK expression and other alterations in lipid rafts where TCR signaling is taking place (391). This defect, therefore, can be attributed to uncontrolled, persistent T cell activation in SLE.

The Role of T Cells in the Initiation of SLE

Table 9-7 outlines a proposed sequence of events leading to the various SLE syndromes.

Genetic Factors

SLE results from the interaction of several genetic loci, which by themselves cannot induce clinical disease (392). In mice one or more loci have been described on 15 different chromosomes that affect T cell activation, differentiation and cell death. SLE 1 on chromosome 1 is associated with loss of tolerance to nuclear antigens. A recent study of SLE 1 mice illustrates many of the points discussed above. T cells from these mice have a decreased activation threshold and $CD4^{+}$ cells from these mice can provide help to antichromatin B cells. Moreover, levels of $CD4^{+}CD25^{+}$ regulatory cells decrease just prior to appearance of autoantibodies. This finding provides strong evidence that $CD4^{+}CD25^{+}$ cells have an important role in controlling autoimmunity. Although there is a loss of tolerance to nuclear antigens, SLE 1 $CD4^{+}$ cells had a limited ability to provide help for other autoantigens, and these mice do not develop lupus (393). Thus, genes localized to a single locus can have major effects on both T helper and regulatory cells, but cannot induce full blown disease.

SLE3 is a lupus susceptibility locus expressed on murine chromosome 7 (347). Congenic recombination has resulted in mice with antinuclear autoantibodies. DC and macrophages appeared to be more mature, more resistant to apoptosis, more pro-inflammatory, and better at costimulating T cells in vitro (394). These mice also do not develop lupus. However, mice that express both SLE1 and SLE3 develop full blown lupus, a result indicating the genetic interaction of these two loci. In human SLE, a genome wide scan has also revealed linkage with SLE susceptibility in at least nine chromosomal loci (395).

As stated earlier, $CD8^{+}$ lymphocytes in SLE have an impaired ability to become killer cells (220 ,221 ,224 ,225). Indeed, the CTL defect in SLE is independent both of disease activity and of immunosuppressive medications (221). Moreover, as discussed, in monozygotic twins who are discordant for SLE, the defect often is detectable in the clinically healthy co-twins (225), raising the possibility that the CTL defect in SLE may be inherited. These individuals may have an increased risk of developing SLE if they are exposed to one or more inciting environmental factors.

Inciting Factors

The ultraviolet radiation from sunlight can generate the autoantigens that precipitate SLE. UVA and UVB in sunlight induce keratinocytes to undergo apoptosis and necrosis,

and reactive oxygen species alter nucleosomal DNA, Ro, La, and snRNPs. These altered structures appear as blebs on the cell surface of apoptotic cells where they can serve as autoantigens (280 ,324 ,396 ,397 ,398). The chemokines produced by keratinocytes in response to UVB irradiation can attract interferonγ producing plasmacytoid dendritic cells to the skin and increased numbers of these antigen-presenting cells have been reported in the skin of patients with lupus. These cells in turn can recruit other autoimmune T cells and IFN-γ producing DC to the skin to amplify and perpetuate cutaneous lupus (398).

Activated cells are more susceptible than resting cells to the induction of apoptosis (303 ,304). Thus, because of apoptosis of various cells and decreased clearance of the fragmented nuclei, plasma nucleosome levels may be increased in SLE patients (325) and various epitopes are recognized by self-reactive T cells.

Infectious agents can act as the trigger factors to induce polyclonal B cell activation and autoimmunity. A compelling argument for microbial superantigens has been offered in triggering polyclonal T cell helper activity, which may result in SLE (399). Microbial superantigens, like anti-CD3 monoclonal antibodies, activate T cells via surface CD3/TCR. Certain bacteria such as staphylococci, streptococci, and mycoplasmas bear structures called superantigens that simultaneously bind to specific structures on the variable (V) region of the TCR chain and to the class II MHC molecules of antigen-presenting B cells. Therefore, these structures bring $CD4^+$ cells into close contact with B cells. Such bridging of T and B cells by microbial superantigens can induce polyclonal IgM and IgG formation. Moreover, specific autoantibodies may be produced if there also is concurrent crosslinking of the B cell receptor by autoantigen. In addition, it has been reported that superantigens and TLR ligands interact with each other. Superantigens synergize with LPS in an IFN-γ–dependent pathway and prime the innate immune system to a subsequent challenge with endotoxin (400).

This finding is another example of interactions between the innate and adaptive immune system. EBV infection has been considered to be important in the pathogenesis of SLE. A peptide from the latent viral protein EBV nuclear antigen (EBNA) 1 cross-reacts with a 60 kDA Ro epitope, an initial autoantigenic epitope for some patients positive for these antibodies. It has been proposed that humoral autoimmunity in human lupus arises through molecular mimicry between EBNA 1 and lupus autoantigens (401). As discussed above, lupus $CD8^+$ cells have an impaired ability to clear this virus (226).

Failure of Feedback Control Mechanisms

Disruption of normal immunologic homeostasis provides the opportunity for the activation of autoreactive T cells. Decreased clearance of apoptotic cells, the persistence of infectious agents, certain drugs, and the loss of regulatory T cells are examples of conditions that can lead to breakdown of immune tolerance. Here also genetic factors are important since the same immunogenic peptides that can trigger lupus in susceptible mice only cause transient autoimmunity in resistant strains (348).

T cells have multiple molecular feedback regulators of activation to control activation of autoreactive T cells. These include the negative regulatory cell surface molecules CD30 and PD-1 (402 ,403). Deletion of PD-1, a transmembrane protein containing an ITIM (immunoreceptor tyrosine-based motif) in normal mice results in a lupus-like syndrome (404). A regulatory genetic polymorphism in PDCD-1 is associated with increased susceptibility to human SLE (405). PD-1 is expressed by $CD4^+CD25^+$ regulatory cells (406). A deletion of the cell cycle regulator p21also leads to a lupus syndrome in normal female mice (407). In mice deletion of the genes that encode TGF-ß, CTLA-4 and FoxP3 result in a similar T cell driven fatal lymphoproliferative disease with multiple organ pathology shortly after birth (242 ,243 ,244).

In SLE, decreased macrophage phagocytosis (308 ,311 ,408), decreased levels of the chromatin-binding C-reactive protein (CRP), and decreased levels of the early complement components (409 ,410) result in persistent high concentrations of potentially pathogenic autoantigens. Treatment of NZB/W F1 mice with CRP decreases autoantibody formation and increases survival (411). As a result of decreased clearance of apoptotic debris, increased enriched guanine plus cytosine (GC)-containing DNA fragments are found in the sera of SLE patients. Moreover, abnormal DNA methylation of GC-rich regions in apoptotic nucleosomes further increases the immunogenicity of these autoantigens (322).

Drugs that Inhibit DNA Methylation of $CD4^+$ Cells can cause lupus-like Syndromes Procainamide and hydralazine are capable of converting anergic, autoreactive T cells to immunocompetent ones through this mechanism. T cells from patients with active lupus have hypomethylated DNA. Mouse T cells have been treated in vitro with drugs that modify DNA methylation and then injected into syngeneic female mice. These mice develop a lupus-like disease (203 ,336).

Breakdown of Peripheral Tolerance

The continuous presence of chromatin and snRNPs resulting from decreased clearance of apoptotic cells, paired with hyperactive T cells and antigen-presenting cells set the stage for a breakdown of T cell tolerance and generalized autoimmunity. Because B cells that are reactive to nuclear antigens are not fully deleted, these cells also are prime candidates for being antigen-presenting cells that contribute to the breakdown of self-tolerance. Considerable evidence has been accumulated indicating that B cells have an important, if not critical role, as antigen-presenting cells in autoimmune diseases, including SLE (292 ,412 ,413 ,414 ,415 ,416 ,417 ,418). Since B cells generally lack the costimulatory molecules to trigger T cell activation, presentation of chromatin autoantigens by self-reactive B cells should result in T cell anergy. Like plasmacytoid dendritic cells, however, B cells express TLR9 (418), which enable them to bind

unmethylated deoxycytosine-deoxyguanosine (CpG) motifs (419). In this manner they can be activated by CpG stimulation (420). Moreover, the combination of CpG and IFN-α produced by plasmacytoid DC have been reported to trigger polyclonal B cell expansion and B cell differentiation toward Ig-producing plasma cells without T cell help (421). These hyperactive SLE B cells, therefore, may have the capacity to induce autoreactive T cells to become fully responsive to self-antigens. This effect of the innate immune system is analogous to experimental graft-versus-host disease where donor activated $CD4^+$ cells provide the costimulatory signal than permits B cell activation and an autoantibody–mediated lupus-like disease (422 ,423 ,424).

Initially, the T cell response to nuclear autoantigens is probably controlled by $CD4^+$ and $CD8^+$ suppressor cells. In lupus-prone mice $CD4^+CD25^+$ cells decrease shortly before the antibodies appear (393). The numbers of these cells is also decreased in patients with SLE (251 ,252). Moreover, $CD8^+$ cells in SLE provide B cell help instead of inhibiting antibody production (121). They also inhibit IL-2 production, a cytokine required for the development of $CD4^+$ $CD25^+$ regulatory T cells (425 ,426). Dysfunctional $CD8^+$ cells in SLE, therefore, probably contribute to the pathogenesis of this disease (201 ,221 ,224 ,241).

Thus, a combination of genetic and environmental factors ultimately results in a loss of tolerance to certain nuclear and cytoplasmic antigens. The factors include persistent exposure of immune cells to pathogenic autoantigens, hyperactive antigen-presenting cells, self-reactive T cells with a low threshold of activation and an inadequate number of functional suppressor/regulatory T cells to interrupt this response. The autoantigen–specific T cells that emerge have the ability to provide B cell help for a panoply of autoantibodies. Although a single cryptic peptide presented by B cells may be the antigen that is recognized by T cells, the phenomenon of epitope spreading will broaden the response to include many nucleosomal antigens. Recruitment of other nontolerant self-reactive T cells will broaden the response even further and lead to generalized autoimmunity. Thus, pathogenic Th1 cells provide help for IFN-related IgG complement fixing dsDNA autoantibodies associated with nephritis and other autoantibodies that contribute to other lupus syndromes. It is important to note that a breakdown of immune tolerance, by itself, is probably insufficient to trigger clinical disease (393). Appropriate alleles of other susceptibility genes that regulate the inflammatory response to autoantibodies are also required for disease onset. While high titers of anti-dsDNA autoantibodies are generally associated with lupus nephritis, studies reporting a lack of correlation of autoantibodies and disease have also been reported (427 ,428).

T Cells in the Perpetuation of SLE

Genetically inherited elements that control T cell function, T cell abnormalities secondary to persistent activation, the cytokine milieu, certain autoantibodies, regulatory T cell defects, and the consequence of disease activity all contribute to the perpetuation of SLE. Polymorphisms of the negative regulators PD1 and CTLA-4 contribute to the perpetuation, as well as the initiation of SLE (405 ,429). Since most of the various T cell signaling defects listed do not change with disease activity (Tables 9-5 and 9-6), these probably reflect inherited traits (Table 9-7). Table 9-8 shows the correlations of T cell abnormalities that correlate with disease activity and could, therefore, contribute to the perpetuation of disease. The defects that do not correlate with disease activity are also indicated.

As stated earlier, the costimulatory element, CD40L is upregulated in SLE and persistent ligation of CD40 with CD40L may result in chronic T cell help for antibody and autoantibody production (125 ,215 ,216 ,217). The interaction between CD40 expressed by B cells and CD40L expressed by activated T cells is a critical signal for B cell differentiation (207 ,208). A brief treatment of lupus mice with a monoclonal antibody against CD40 ligand greatly decreased the onset of lupus nephritis (430). As stated above, hyperexpression of CD40L is associated with a defect in anergy induction (295). Interestingly, $CD8^+$ T suppressor cells directly inhibit the CD40 signaling pathway (280), and the activity of these cells is decreased in SLE.

Sustained, high levels of IL-10, discussed in the following chapter, directly sustain B cell proliferation and differentiation. This cytokine also contributes to enhanced T cell apoptosis in SLE (144). Moreover, IL-10 downregulates IL-2, TNF-α, and TGF-ß (36 ,280 ,281 ,282), cytokines that are needed for feedback regulation of B cell activity. TNF-α has a protective effect in lupus mice (431), and the treatment of rheumatoid arthritis with a monoclonal antibody that antagonizes TNF-α leads to the appearance of anti-DNA antibodies in approximately 10% of the patients (432).

As reviewed above, suppressor/regulatory T cell function is decreased in SLE and both IL-2 and TGF-ß induce $CD4^+$ and $CD8^+$ cells to develop this function (28 ,29 ,35 ,36). Insufficient levels of these cytokines probably contribute to decreased $CD4^+CD25^+$ cells in SLE and why $CD8^+$ cells support rather than suppress B cell activity in SLE (121). The transfer of T cells from young lupus-prone mice to older mice that had developed SLE reverses the disease (433). This is probably because cytokine production is intact in young mice and they have functional regulatory T cells (393).

Several autoantibodies contribute to impaired T cell function in SLE and perpetuate the disease. IgG anti-CD3/TCR antibodies trigger signaling pathways to lead to impaired IL-2 production (268). Anti-Brn autoantibodies correlate with increased expression of heat shock protein 90 in SLE (434). Besides their housekeeping and cytoprotective functions, heat shock proteins are now believed to regulate the immune response (435).

Finally, the effects of active disease can have negative effects on T cell function. For example, increased levels of interferon alpha correlate with disease activity (52 ,53). As stated above, this cytokine has negative effects on the number and proliferative ability of $CD4^+$ cells, negative effects on suppressor

activity, but has positive effects on the on the number of HLA-DR+ T cells and the antigen-presenting ability of DC. A correlation with disease activity, T cell function and inhibitory IgG has been reported previously (254). Table 9-6 shows the correlations with disease activity and abnormalities of T cell function.

Table 9-6: Relationship of T Cell and Related Defects to Clinical Activity of SLE

Correlation	No correlation
Elevated levels of the anti-apoptotic Brn-3 family members with autoantibodies to these molecules	Decreased threshold for T-cell activation
Serum levels of IFN-γ	Resistance to activation-induced apoptosis
Levels of antilymphocyte antibodies	Decreased clearance of apoptotic cells
Lymphopenia	Impaired response to mitogenic stimulation (Most studies)
Decreased CD4+ cells	Decreased production of IL-2 and active TGF-ß
Increased HLA-DR+ T cells	IgG antibodies to CD3/TCR
Non-responsiveness to skin test antigens	**T-cell signaling abnormalities**
Levels of soluble costimulatory molecules	Enhanced and prolonged rise in intracellular calcium following CD3/TCR stimulation
Decreased T-cell cytotoxicity	Increased intracellular phosphorylation of signaling adaptor proteins
Decreased CD8+ cell suppressive activity	Decreased phosphorylation of the signal silencer Cbl-b (correlates with increased expression of CD40 ligand and resistance to tolerance induction)
Decreased NK cell activity (Some groups)	Decreased expression of TCR ζ chains
Decreased antibody-dependent cytotoxicity	Increased binding of the transcriptional inhibitor pCREM to the IL-2 promotor
Activated CD8+ granzyme B and perforin positive cells	Decreased Protein Kinase C activity
Serum inhibition of T-cell proliferation	Decreased Protein Kinase A type I and type II isoenzyme activity
Decreased production of total TGF-ß	Decreased amounts of MAPK signaling proteins
Decreased Varicella Zoster-specific CD4+ cells	Decreased levels of p65-RelA subunit of the NF-κB nuclear transcription factor factor
Decreased Fc receptor expression by monocytes	
T-cell signaling abnormality	
Decreased LCK in lipid rafts	

Table 9-7: Initiation of Systemic Lupus Erythematosus

1. Genetic predisposition	Genes that decrease the activation threshold of T and B cells
	Genes that result in increased generation and decreased clearance of apoptotic cells, the source of pathogenic nucleosomal autoantigens.
	Genes that increase the production of IL-10, and decrease the production of TGF-ß.
	Genes that regulate the production of inhibitory receptors and T-cell signaling molecules
	Major histocompatibility complex class II susceptibility alleles (for autoantibodies)
	Resistance genes such as C1, C4, or C2 complement components that clear immune complexes
2. Triggering events	Female sex hormones
	The adjuvant effect of infectious agents that stimulate cells of the innate immune system to produce large amounts of interferon α and γ
	Sunlight injury that results in cell death and the release of immunogenic nucleosomal autoantigens.
	Drugs that decrease T-cell methylation of DNA and thereby enhance their activation by autoantigens can induce a lupus-like syndrome.
3. Breakdown of peripheral tolerance (nonresponsiveness)	Presentation of nucleosomal autoantigens by hyperactive antigen-presenting cells to naive autoreactive T cells that havea low activation threshold.
	Stimulation of naive autoreactive naive T cells and their transition to the activated or memory phenotype
4. Failure of fail-safe, feedback control mechanisms	Failure of a regulatory cell network consisting of CD4+, CD8+ and probably NKT cells to block the response of self-reactive T cells to autoantigens, to inhibit the migration of activated Tregs to inflammatory sites, and to prevent their effector functions.
	Failure of expression and/or function of receptors that inhibit T-cell responsiveness
5. Onset of clinical disease in genetically susceptible hosts	T-cell help to selfreactive B cells
	Pathogenic autoantibodies and immune complexes
	Systemic inflammatory disease

Table 9-8: Perpetuation of SLE

Continuous presence of immunogenic nucleosomal autoantigens
Continuous stimulation of clonally expanded, autoreactive T and B memory cells
Production of cytokines such as IL-10 that sustain B-cell activity and also inhibit T-cell function
Decreased production of IL-2 and TGF-β, the cytokines required for the generation of CD4+ and CD8+ regulatory
T cells needed to control antibody production.
IgG autoantibodies that perpetuate T-cell hyperactivity in SLE
Depressed cell-mediated immunity that enhances susceptibility to infections

A principal difference between human and mouse lupus is the regularity of remissions and exacerbations of the human disease in contrast to the progressive disease in mice. This finding suggests that feedback regulatory mechanisms persist in humans, but are not strong enough to induce remission. Long term improvement in SLE patients has been observed in patients that have received autologous stem cell transplantation (436) or rituximab therapy (437). There is now evidence that stem cell transplantation restores the regulatory T cell network. Thus the immune system can be reset in a manner that allows normal homeostatic mechanisms to be reestablished and autoimmunity can be controlled.

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Chapter 10
Cytokines and Interferons in Lupus

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Introduction

The immunopathology of systemic lupus erythematosus (SLE) has traditionally been attributed to the deposition in tissues and organs of immune complexes or autoantibodies with specificity for or crossreactivity with locally expressed antigens. These mechanisms are likely to account for an important component of the inflammation that generates tissue damage in this disease, but accumulating data suggest that additional mechanisms should be considered. The complement of soluble mediators, particularly cytokines and chemokines, that are produced in the context of innate and adaptive immune system activation in patients with lupus is likely to be a product of whatever endogenous and exogenous triggers are inducing autoimmunity, as well as the efforts of immune system cells to gain control over its activated components. These molecules may shape the character of the immune system dysfunction and organ system involvement. In SLE, given the heterogeneity of the disease, distinct cytokine pathways may operate in different patients and those pathways may, in part, determine the different organ systems affected. In addition, different cytokine pathways may be important at different stages of the disease. Understanding the balance of cytokines that are expressed in a given patient may ultimately guide medical management as new approaches to modulating cytokine pathways therapeutically become available.

Properties of Cytokines and Their Receptors

Cytokines are small soluble proteins that are produced by immune system cells and mediate activation or functional regulation of nearby cells by binding to cell surface receptors (1). Cytokines act in an autocrine or paracrine manner, in close proximity to their source, and have been considered to serve a similar function to neurotransmitters in the nervous system. Basal production of most cytokines is negligible, and activation of the producer cells, through nonspecific or antigen-specific receptors, results in either new gene transcription or translation of pre-existing cytokine mRNAs and protein secretion. Cytokine protein is typically not detected in serum from healthy individuals, but in patients with SLE, elevated levels of some cytokines are measurable and some vary with disease activity (Table 10-1).

The level of cell surface expression of the receptors to which cytokines bind is also highly regulated and contributes to the impact of the cytokine on immune system activity. Once the receptors are engaged, complex multi-component molecular pathways transduce a signal from cell surface to nucleus, resulting in new gene transcription. The Janus kinase (Jak)-signal transducer and activator of transcription (STAT) pathways are common mediators of cytokine-cytokine receptor interactions (2). The strength of these signaling pathways can be affected by the state of activation of the target cell and the additional signals that it has received, with the mitogen-activated protein (MAP) kinase and other signaling systems modulating the function of the Jak-STAT pathway. In addition, negative regulators of cytokine signals, such as the suppressors of cytokine signaling (SOCS) proteins, further modulate the strength of target cell response to the cytokine (3).

The degree of expression of individual cytokines or activation of their pathways is also regulated based on genetic differences among individuals that translate into variable efficiency in cytokine production or response (4). The extent of genetic polymorphisms that contribute to SLE has not been fully characterized. However, variable sequences in the promoter region of tumor necrosis factor (TNF) may contribute to other autoimmune diseases, including juvenile diabetes mellitus, and IL-6 polymorphisms have been associated with SLE (5 ,6 ,7 ,8). Genetic variability can be localized to regulatory regions of genes, potentially modifying the level of expression, or can be in coding sequences, sometimes resulting in an altered amino acid sequence and modified conformational structure. More complete study of the genetic variants that are associated with disease activity and clinical disease subsets is likely to provide new understanding of the basis of the immune system alterations that contribute to autoimmune disease and inflammation.

Assessment of Cytokine Production

The expression of cytokines and the capacity to produce cytokines in an individual can be assessed using numerous distinct and complementary approaches (9). The identification of genetic polymorphisms that modify expression or function of cytokine gene products, along with understanding the role of those cytokines in immunopathogenesis, may permit assignment of disease susceptibility to an individual.

Measurement of mRNA encoding a cytokine can be used to provide a reasonable indication of the amount of cytokine protein produced. However, the variable stability of one or another mRNA must be considered, and the presence of mRNA may not necessarily indicate that the mRNA is translated and the corresponding protein generated.

Table 10-1: Role of Cytokines and Interferons in Lupus Pathogenesis

Mediator	Role in Pathogenesis
Products of the Innate Immune Response	
Type I Interferon	Increased in active SLE
-IFN-α, IFN-β, IFN-ω	Mediates multiple immune system alterations, including dendritic cell maturation, immunoglobulin class switching, and induction of IL-10, interferon-γ, and other immunoregulatory molecules. IFN-α being targeted by new therapeutics in clinical trials.
Tumor Necrosis Factor	Role in SLE not clear.
Interleukin-1	Increased levels associated with active SLE.
Interleukin-10	Complex role in SLE.
Promotes B cell expansion and immunoglobulin class switching. Pro-B cell effects may dominant its anti-inflammatory effects.	
BLyS/BAFF	Increased levels in SLE.
Promotes B cell survival and may contribute to immunoglobulin class switching.	
Interleukin-6	Increased levels in active SLE.
Promotes terminal B cell differentiation. Being targeted by new therapeutics in clinical trials.	
Interleukin-12 and -18	Support expansion of Th1 cells and NK cells.
Interleukin-8, IP-10, MIG, MCP-1, fractalkine	Chemokines that may be increased in active SLE and may recruit inflammatory cells to sites of organ inflammation, particularly in lupus nephritis
Products of the Adaptive Immune Response	
Interleukin-2	Decreased production in SLE in in vitro studies. Mediates T cell proliferation and activation induced cell death.
Interferon-γ	Produced by Th1 cells and NK cells.
Implicated in lupus nephritis in murine models and human SLE.	
Interleukin-6 and -10	Produced by T and B lymphocytes, as well as monocytes.
TGFβ	Produced by multiple cell types.
Role in SLE not clear, but may contribute to T regulatory cell function and renal scarring.	

Direct measurement of protein, as by enzyme-linked immunoabsorbent assay (ELISA), is a fairly reliable indicator of the presence of that protein, but issues of protein degradation and variable detection, based on the antibodies used and the availability of their corresponding epitopes, suggest that confirmation of protein concentration using alternative approaches can be valuable. While ELISA determines quantity of protein per volume of fluid, usually serum or plasma, intracellular staining for cytokine protein and the enzyme-linked immunospot assay (ELISPOT) determine the percentage of cytokine-producing cells in a cell preparation and permit identification of those cells (10 ,11 ,12 ,13 ,14). The latter approach provides important information, as some of the pathogenic cytokines are products of multiple cell types. Knowing the major cell source can assist in development of therapeutic strategies to inhibit (or augment) production of the cytokine.

Because some cytokines may be short-lived, in some cases measurement of the activation of signaling molecules induced by a cytokine or expression of the target genes regulated by a cytokine may be a more sensitive measure than assay of the cytokine itself. For example, phosphorylation of components of the Jak-STAT pathway of signaling molecules can indicate recent binding of the relevant cytokine to its receptor (15). A more “downstream” target, gene transcription, can also be measured as a readout of recent cytokine activity. For example, recent use of micro-array technology has indicated that a set of genes regulated by interferons (IFNs), the first-described cytokine family, is activated in patients with SLE, based on increased relative expression of mRNA corresponding to those target genes (16 ,17 ,18 ,19 ,20). As any given gene target can usually be induced by multiple triggers, inhibition of gene expression with an antibody that neutralizes activity of a specific cytokine can

be used to demonstrate the relevance of that cytokine to the induction of the target mRNA (or protein) being measured.

Use of Microarray to Study Cytokine Effects

Micro-array analysis, a system in which thousands of oligonucleotide sequences are spotted on a solid substrate, usually a glass slide, and RNA-derived material from a cell population is hybridized to the gene array, is an innovative technology that permits a global view of the profile of genes expressed in a cell population at a point in time, including genes in the cytokine pathways (19). Applying micro-array analysis to heterogeneous cell populations in peripheral blood of patients with autoimmune diseases raises technical challenges. The variable proportions of different cell populations in each individual, each making variable contributions to the mRNAs in the blood sample, adds complexity to the comparison of study groups. Additionally, the statistical analysis of thousands of gene sequences studied in multiple individuals is daunting. Recent investigations have demonstrated that in spite of these technical challenges, significant and useful micro-array data can be derived from complex cell samples, including peripheral blood mononuclear cells stimulated in vitro and peripheral blood preparations from patients with autoimmune disease. The view that IFN-α might play a central pathogenic role in SLE has only recently gained momentum with the completion of several large-scale studies of gene expression profiling using micro-array technology (16 ,17). At least four groups have used this powerful technology to demonstrate that mRNAs encoded by IFN-regulated genes are among the most prominent observed in peripheral blood cells of lupus patients (Fig. 10-1) (16 ,17 ,18 ,19 ,20).

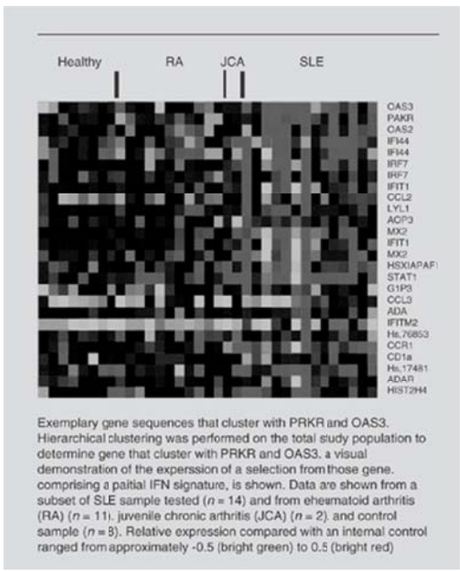

Figure 10-1. (See color plate.) Exemplary gene sequences that cluster with PRKR and OAS3. Hierarchical clustering was performed on the total study population to determine genes that cluster with PRKR and OAS3. A visual demonstration of the expression of a selection from those genes, comprising a partial IFN signature, is shown. Data are shown from a subset of SLE samples tested (n = 14) and from rheumatoid arthritis (RA) (n = 11), juvenile, chronic arthritis (JCA) (n = 2) and control samples (n = 8). Relative expression compared with an internal control ranged from approximately -0.5 (bright green) to 0.5 (bright red). (From Crow MK, Wohlgemuth J. Microarray analysis of gene expression in lupus. *Arthritis Res* Ther 2003:5: 279-287. Used with permission.)

It should be emphasized that microarray is a screen to identify genes potentially altered in expression in a cell preparation or disease state. Micro-array data should be confirmed using more quantitative techniques, such as real-time polymerase chain reaction (PCR), and data derived from patient samples should be confirmed in additional patient cohorts.

Activation of the Immune Response in SLE

The mechanisms that account for aberrant production of autoantibodies, cytokines, and other soluble mediators in SLE can be modeled in parallel to the production of antibodies and mediators in a productive immune response to microbial pathogens in a healthy individual. The initial encounter with the microbe is mediated by cells of the innate immune response. While those cells have traditionally been considered to initiate an immune response through nonspecific cell surface receptors, the recent elucidation of the Toll-like receptor (TLR) family has altered that picture (21 ,22). Although the innate immune response does not have the fine level of specificity that characterizes the adaptive immune response generated by T and B lymphocytes, the members of the TLR family do recognize classes of stimuli with characteristic structural features. Among the TLR ligands are nucleic acids, including hypomethylated CpG DNA and single-stranded or double-stranded RNA. These nuclei acids are typical components of viruses and bacteria but might also be a product of apoptotic or necrotic host cells. Whether microbe-derived in the setting of infection or self-derived, these oligonucleotides provide an adjuvant-like stimulus that can initiate a heightened level of immune system activity, including the production of cytokines.

It is the production of cytokines in the context of innate immune response activation that permits the activation and maturation of antigen-presenting cells (APC), such that T cells of the adaptive, highly specific, component of the immune response can become engaged. Whether the antigenic target is a viral or bacterial protein or a self-antigen

concentrated in the cell surface blebs of apoptotic cells, antigen-specific T cell receptors and T cell surface costimulatory molecules, such as CD28, interact with the antigenic peptide-major histocompatibility complex and the costimulatory ligand, CD80 or CD86, on the surface of an APC and stimulate biochemical signals, new gene transcription, and cell activation. The activated T cell is then able, through expression of new cell surface molecules that mediate cell-cell interactions with B cells and other target cells, along with production of cytokines, to drive the humoral immune response and activate effector cells. It is the nature of the cytokines produced by the APC, T cells and B cells that shape the quality of the adaptive immune response to a microbe. It is likely that parallel mechanisms account for the induction of immune responses to self-antigens, although genetic factors must be important in setting a threshold for lymphocyte activation that favors an immune response to stimulation by self-antigens in a lupus-susceptible individual. In both innate and adaptive immune responses to foreign and self-antigens, the antigens determine the specificity of the response but cytokines determine the quality of the response. The isotype of antibodies produced and the extent of amplification of an inflammatory response by chemokines and cells will be determined by the particular cytokines generated.

Cytokines of the Innate Immune Response

Cells of the innate immune system—macrophages, neutrophils, and dendritic cells (DC)—are among the first cells to encounter pathogens in the setting of infection and are likely to be early players in the lupus autoimmune response. In either case, the cells respond to their stimuli with production of soluble mediators, including cytokines. Recent studies support a contribution of signals through TLRs to the activation of the innate immune response in lupus (23 ,24 ,25 ,26). Among the documented triggers relevant to SLE are immune complexes containing DNA or RNA, along with specific antibodies (25 ,26 ,27). A consequence of TLR ligation is production of type I IFN, predominantly IFN-α, that then mediates numerous functional effects on immune system cells. Plasmacytoid dendritic cells, a rare cell type that is enriched in skin lesions of lupus patients, are presumed to be active producers of IFN-α (28 ,29 ,30 ,31 ,32 ,33 ,34 ,35). Interaction of IFN with widely expressed cell surface receptors activates intracellular signaling pathways and induction of transcription of a large number of IFN-responsive genes, including those associated with maturation of myeloid dendritic cells (36 ,37). The result is predicted to be increased antigen presenting cell function and augmented capacity to trigger self-reactive T cells (38).

In addition to plasmacytoid and myeloid dendritic cells, mononuclear phagocytes are essential for the inactivation of pathogenic infectious organisms and for the clearance of potentially pathogenic immune complexes and senescent or apoptotic cells. These cells are also important in SLE. Impaired clearance of apoptotic cells has been supported in some studies, and IFN-mediated maturation of monocytes into effective APCs has been shown in another study (38 ,39). Macrophages bind, process and present antigenic peptides to T cells; they physically interact with T cells, delivering secondary activation signals through cell surface adhesion and costimulatory molecules; and they secrete a panoply of soluble products, including TNF, IL-1, IL-6, IL-10, IL-12, and B-lymphocyte stimulator (BLyS), that provide important accessory and regulatory signals to both T and B cells. The role of these products of innate immune system cells will be highlighted, with a particular emphasis on the type I IFNs.

Type I Interferons (IFN)

Productive infection of host cells by a virus, leading to synthesis of RNA or DNA molecules of viral origin, induces production of host proteins, including the IFNs (40). The function of these proteins is to inhibit viral replication and to modulate the immune response to the virus, with the aim of controlling infection. The type I IFN locus on chromosome 9p21 comprises genes encoding 13 IFNα isoforms, as well as IFN-ß, IFN-ω, IFN-κ, and IFN-ε, the latter mostly restricted to trophoblast cells and produced early in pregnancy (41 ,42). The IFN-α gene complex is likely to have been generated by repeated gene duplications and recombinations. While the need for and function of each of the IFN-α genes is not clear, specific virus infections are associated with induction of one or another IFN-α isoform (43 ,44). Recent data from two groups have identified additional IFNs that are encoded by a gene family related to the classic type I IFNs (45 ,46). IFN-λs (IL-28 and IL-29) have only moderate sequence similarity to IFN-α, bind to a distinct receptor, yet induce genes similar to those induced by IFN-α. The relative functional roles of IFN-λ and the chromosome 9p-encoded IFNs are under study (47).

IFN-α can probably be produced by all leukocytes, but plasmacytoid dendritic cells (PDC) are the most active producers. Rapid progress in study of type I IFN regulation indicates that cell type (PDC vs. fibroblast), stimulus (dsRNA, single-stranded RNA, DNA), and signaling pathway used (TLR3 vs. TLR7/8 vs. TLR9) all contribute to determining the specific IFN isoforms that are produced (47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59). The TLR family of innate immune system receptors and their downstream signaling components play a central role in mediating activation of type I IFN gene transcription (Fig. 10-2). The details of these pathways are now being elucidated; TLR3 is triggered by dsRNA, TLR7 and 8 are triggered by ssRNA, and TLR9 is triggered by CpG DNA (21). TLRs 7, 8, and 9 signal through the MyD88 adaptor. IFN regulatory factors and additional transcription factors, including nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) and ATF-2, bind to and activate an IFN stimulated response element (ISRE) present in the IFN-α and IFN-ß gene promoters (49 ,50 ,51). Although the details of these complex pathways are being modified on a weekly basis, with new publications providing new insights into the complex regulation of the IFN

system, what is clear is that tracking the specific intracellular factors that mediate transcription of specific IFN isoforms can provide clues to the innate immune system receptors and the relevant triggers that drive production of those IFNs.

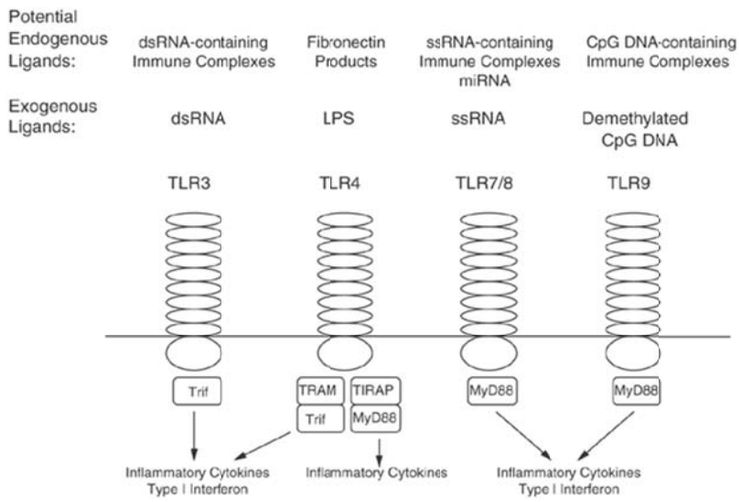

Figure 10-2. Induction of the type I interferon pathway through toll-like receptors. Both exogenous and endogenous stimuli can induce TLR activation, resulting in new gene transcription. Among potential endogenous ligands are immune complexes containing DNA or RNA or matrix-derived components. TLR ligands trigger activation of intracellular adaptors (including Trif, TIR domain-containing adapter inducing IFN-ß; TRAM, Trif-related adaptor molecule; TIRAP, TIR-domain-containing adapter protein; or MyD88, myeloid differentiation primary response protein 88) and induce transcription of type I interferons or inflammatory cytokines.

Type I IFN production represents the first line of defense in response to viral infection. Following invasion of the host by a virus, IFN-α is secreted by PDC, along with other immune system cells, and binds its receptor on many target cells, resulting in engagement of intracellular signaling molecules and induction of a gene transcription program (60). The IFNs were used as model cytokines when Darnell et al. defined the requirements for cytokine-mediated signal transduction (61 ,62 ,63). Binding of IFN-α to its cell surface receptor was shown to activate Jak1 and then STAT1. Subsequently, it was shown that Tyk-2, also a Jak kinase, is constitutively associated with the α subunit of the type I IFN receptor (IFNAR), while Jak-1 is associated with the ß subunit of the receptor. Cytokine binding leads to activation of Tyk-2 and Jak-1 and phosphorylation of the α receptor subunit and part of the ß subunit. Subsequent events include activation of STAT1, 2, and 3, the insulin receptor substrate proteins 1 and 2 (IRS-1 and IRS-2) and vav (64). STAT1-to-STAT1 and STAT1-to-STAT2 dimers bind to the pIRE element and ISGF3, including STAT1, STAT2, and a third protein, p48, binds the ISRE element (49 ,62). The Jak-STAT pathway seems to be sufficient to mediate the antiviral effect of IFN-α, while the IRS proteins, as well as other undefined factors, are also required for the antiproliferative effect of IFN-α (64).

Activation of the type I IFN pathway has diverse and numerous functional effects on immune system cells. IFN-α matures DC by inducing ICAM-1, CD86, MHC class I, and IL-12p70 expression, and promotes expression of some T cell activation molecules (65 ,66 ,67). However, IFNα has anti-proliferative effects on T cells, and it is generally described as a suppressor of T cell immune activity. IFN-α inhibits expression of some pro-inflammatory cytokines, including IL-8, IL-1, and GM-CSF, and it preferentially promotes Th1 responses, by decreasing IL-4 and increasing IFN-γ secretion (68 ,69). In the setting of coculture of monocytes with lipopolysaccharide (LPS) or CD4+ T cells with anti-CD3 and anti-CD28 monoclonal antibodies, IFNα augments IL-10 production (68 ,70). IFN-γ does not have these effects and in fact inhibits IL-10 production. Although IL-10 has important suppressive effects on T cell proliferative responses, its capacity to promote B cell proliferation and immunoglobulin class switching suggests that IFNα may favor antibody production (71 ,72 ,73 ,74 ,75 ,76 ,77). Finally, IFN-α leads to increased NK- and T cell–mediated cytotoxicity (78 ,79 ,80). This effect on CTL function has been exploited in the treatment of several malignancies with IFN-α in order to augment tumor lysis, although the mechanism that accounts for the increased killing has not been elucidated fully. At least one such mechanism is the induction of FasL expression on NK cells and increased Fas-mediated apoptosis (80). IFN-α can also promote an inflammatory response. Among IFN-α–inducible gene targets are several chemokines, soluble mediators that attract lymphocytes and inflammatory cells to

tissues. In brief summary, IFN-α helps to initiate an adaptive immune response that results in increased cytotoxic T- and NK-cell activity, increased Fas-mediated apoptosis, increased antibody production and inflammation but decreased T cell proliferation. Many of these immune system effects are reminiscent of those observed in patients with SLE (Fig. 10-3).

Several sets of compelling data suggest an important pathogenic role for IFNs in SLE. Papers published as early as 1979 described increased serum levels of IFN in patients with SLE, particularly those with active disease (81 ,82 ,83 ,84 ,85). At that time, the distinct type I and type II IFNs had not yet been documented, but within several years, IFN-α was cloned and it became clear that IFNα was present in particularly high levels in SLE blood. This IFN was said to be “acid-labile,” a characteristic that is still not fully understood but may relate to its glycosylation state (85 ,86 ,87). Soon after, it was observed that tubuloreticular-like structures in the renal endothelial cells of SLE patients and in murine lupus models were associated with IFN-α and that in vitro culture of cell line cells with IFN-α–induced similar intracellular structures (88). These observations suggested that IFN-α was not only increased in concentration in SLE blood but also that it might have a functional impact on cells and perhaps contribute to disease. Another key observation was first reported in 1990 and has been noted many times subsequently. Therapeutic administration of IFN-α to patients with viral infection or malignancy occasionally results in induction of typical lupus autoantibodies and, in some cases, clinical lupus (89 ,90 ,91 ,92 ,93). This demonstration of induction by IFN-α of SLE in some individuals indicated that given the appropriate genetic background and perhaps in the setting of concurrent stimuli, SLE could be induced by IFN-α. Twenty percent to 80% of patients treated with IFN-α have been noted to develop autoantibodies specific for thyroid or nuclear antigens, including anti-DNA autoantibodies (94). Clinically apparent disorders include autoimmune thyroiditis, inflammatory arthritis, and SLE. Hints regarding possible mechanisms of these IFN-α toxicities come from an animal model of autoimmune diabetes (95). Expression of IFN-α by pancreatic islets correlates with development of type I diabetes, and transgenic mice overexpressing IFN-α acquire diabetes. These mice develop autoreactive CD4 T cells that are Th1 and can kill islet cells. Blanco et al. recently showed that IFN-α is one component in lupus serum that can promote maturation of blood monocytes to have increased antigen-presenting activity (38). These data are consistent with the demonstration that IFN-α is one of several maturation factors for immature DC, permitting efficient antigen presenting function to T cells (96 ,97). Generation by IFN-α of an antigen-presenting cell functional phenotype competent for activation of autoantigen–specific T cells could be an important immune mechanism that incorporates many of these findings (98). Murine studies have supported a role for type I IFN in SLE. Both NZB lupus mice and B6/lpr mice deficient in the IFN-α/β receptor show significant improvement in some manifestations of autoimmunity as well as improvement in clinical disease (99 ,100).

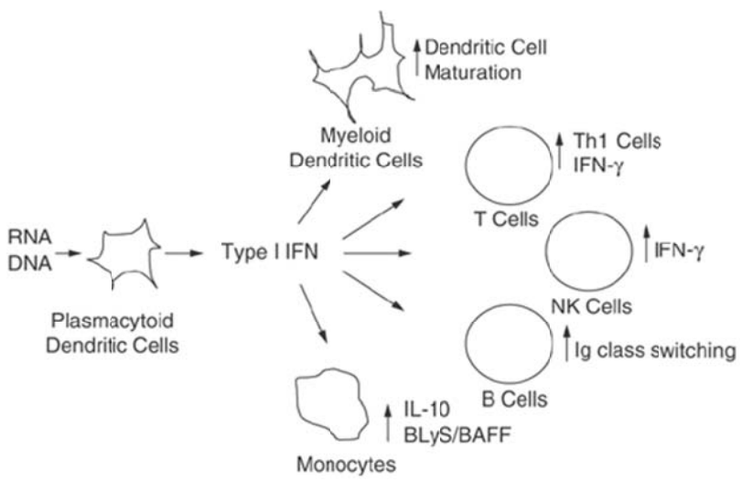

Figure 10-3. Regulation of the immune response by type I interferons. Activation of plasmacytoid dendritic cells through Toll-like receptors, perhaps triggered by endogenous DNA or RNA, results in production of type I interferon. Actions of type I interferon on the immune system include dendritic cell maturation; increased Th1 cytokine production, particularly IFN-γ; activation and IFN-γ production by NK cells; augmented immunoglobulin class switching by B cells; and increased IL-10 and BLyS/BAFF expression by monocytes. Many of these functions are among the features of the altered immune system that have been described in SLE.

In the early 1990s, the major cellular source of IFN-α had not yet been identified, but Ronnblom et al. were able to demonstrate that immune complexes containing lupus autoantibodies and cellular material could induce production of IFN-α by peripheral blood mononuclear cells in vitro (23 ,28 ,29 ,101 ,102). With the assignment of PDCs as the major source of IFN-α, lupus immune complexes were shown to be active inducers of IFN-α by those cells, while additional recent data implicate TLR9 and FcγRIIa in the induction of IFN-α by some of those complexes (27 ,30 ,31 ,32). A similar scenario has been demonstrated in a collaboration between the Marshak-Rothstein and Shlomchik laboratories in another system relevant to rheumatic diseases, the activation of rheumatoid factor producing B cells by DNA enriched in CpG immunostimulatory sequences opsonized with anti-DNA antibody (24 ,25). Means et al. recently reported similar data using lupus immune complexes (26). PDC appear to be somewhat reduced in the blood, but they have been demonstrated in the skin lesions of lupus patients (33 ,34 ,35). It is possible, or likely, that the IFN-α–producing cells have also been recruited to other sites of active disease, including lymph nodes and kidney. Monoclonal antibody to BDCA-2, a cell surface C-type lectin that may contribute to internalization of immune complexes, has been used to identify PDC (103).

Several previous reports documented increased expression of IFN-α–induced genes in SLE, including dsRNA-dependent protein kinase (PRKR) and oligoadenylate synthase (OAS), as well as Mx1, present in lupus-involved skin (104 ,105 ,106). Recently, microarray studies have reproducibly demonstrated that in SLE, IFN-induced genes are the most significantly overexpressed of all those assayed on the microarray (16 ,17 ,18 ,19 ,20). While these data could have initially been interpreted as

attributable to either type I IFN (IFN-α) or type II IFN (IFN-γ), recent experiments have used quantitative real-time PCR analysis of SLE PBMC to show that those genes that are increased in expression in SLE are those that are preferentially induced by IFN-α, not those induced by IFN-γ (107 ,108). With the description of the new type III IFN gene family (IFN-λ), its gene products can also be considered candidate inducers of the genes overexpressed in SLE. High expression of IFN-inducible genes is seen in approximately 40% of adult SLE patients. These patients are characterized by autoantibodies to RNA-binding proteins (Ro, La, Sm, and RNP) and frequent renal involvement (108).

Genetic contributions to variability among individuals in production and signaling of IFN have been suggested by a recent study. Data document an association of a single nucleotide polymorphism in one of the TLR pathway transcription factors, IRF5, with SLE in Swedish and Finnish populations (109). This observation draws attention to the pathway that utilizes IRF5, the TLR7 and 8 pathway, but also suggests that genomic variations in additional innate immune system pathways could potentially impact efficiency of induction of IFN production.

Given all of the described observations, there is strong support for the hypothesis that inhibition of the type I IFN pathway may benefit lupus patients, particularly those with increased expression of IFN-inducible genes. However, IFN pathway blockade might weaken the innate and adaptive immune responses to viral infection. Potential approaches to inhibit the type I IFN pathway could include antibodies specific for the IFN-α receptor or for one or more of the various IFN subtypes noted above. Other approaches might include inhibition of upstream (e.g. TLR pathways) or downstream (e.g., Jaks or STATs) signaling molecules (110). Humanized monoclonal antibodies to IFN-α are currently available and clinical studies are eagerly awaited (111).

Tumor Necrosis Factor

Tumor necrosis factor (TNF), the prototype member of the TNF family, is expressed as a trimer on the cell surface and in soluble form after activation of innate immune system cells, including macrophages and dendritic cells, through TLRs, Fc receptors, and receptors for other cytokines. Like type I IFN, TNF is produced early during immune responses to microbes and is particularly effective in promoting influx of inflammatory cells into sites of microbial invasion and in stimulating granuloma formation. The important role of TNF in controlling microbial infections is demonstrated by the reactivation of *Mycobacterium tuberculosis* that can occur in the setting of TNF blockade.

The role of TNF as a central upstream inducer of inflammation has been clearly shown in rheumatoid arthritis based on in vitro studies and on the impressive clinical response experienced by some patients treated with TNF inhibitors. The importance of TNF in lupus is still being debated. In murine lupus models it has been described as both protective and as harmful, depending on the mouse strain and stage in disease development (112 ,113 ,114 ,115). In SLE patients, data are variable, but at least some studies show high levels of TNF in sera and kidneys (116 ,117 ,118 ,119 ,120). The observation that anti-TNF agents can sometimes induce anti-dsDNA antibodies and occasionally clinical lupus raises interesting questions regarding the mechanisms by which reducing TNF might promote autoimmunity, as well as concern about treatment of SLE patients with TNF inhibitors (121 ,122). Nevertheless, an open-label safety study with infliximab has been carried out in SLE and documented efficacy with regard to arthritis and proteinuria in 6 patients (123). Urinary tract infections occurred in three patients of whom one had *Escherichia coli* bacteremia. Interestingly, anti-dsDNA and anticardiolipin titers increased transiently but without concomitant complement level depression or clinical flare.

A potential mechanistic relationship between TNF and type I IFN has been suggested with some experimental support (124). In some in vitro and in vivo settings, TNF can inhibit synthesis of type I IFN and vice versa (125). It is possible that when availability of TNF is reduced by anti-TNF agents, negative regulation of IFN production is abrogated, allowing increased activation of the type I IFN pathway and augmented immune system capacity to develop autoimmunity. Addition investigation will be required to address this hypothesis.

IL-1

IL-1 and its physiologic inhibitor IL-1 receptor antagonist (IL-1ra) are produced by monocytes and macrophages in the early stages of an immune response and are also demonstrated at local sites of inflammation. High serum IL-1 levels have been associated with active SLE and correlate with serum C-reactive protein levels (126). Interestingly, low serum IL-1ra levels correlated with renal flares of the lupus (126). Only limited clinical experience is available for therapy with recombinant IL-1ra in SLE. In one study of 3 patients, arthritic symptoms but not myositis improved (127). Moreover in another study of 4 SLE patients with arthritis, IL-1ra therapy resulted in improvement in all (128). However, two relapsed despite continued therapy. At this time, there is neither strong rational nor experimental support for a central role for IL-1 in SLE.

IL-10

IL-10, a pleiotropic cytokine produced by monocytes and lymphocytes, is considered to have anti-inflammatory effects in that it inhibits activation of APC, reduces expression of costimulatory molecules on their cell surface, thereby blunting T cell activation, and inhibits TNF production. However its functional effects are complex, since when it binds to activated monocytes, as may occur in autoimmune disease, IL-10 may not effectively generate intracellular signals. For example, in the presence of IFN-α, it can mediate pro-inflammatory effects on target monocytes (129). Additionally,

IL-10 augments B cell proliferation and immunoglobulin class switching, resulting in increased secretion of antibodies with the capacity to enter extravascular compartments and promote inflammation and disease in SLE (130).

Immune complexes, present at increased levels in many SLE patients, can stimulate production of IL-10 after binding to FcγRII (CD32) (131). Indeed, IL-10 levels are increased in the serum of active lupus patients (132 ,133). Disease activity in lupus has been shown to correlate with an elevated ratio of IL-10-secreting to IFN-γ–secreting cells (134). Increased IL-10 has also been associated with increased activation-induced apoptosis of SLE T cells, an effect reduced by anti-IL-10 antibodies (135). Increased burden of apoptotic cells could potentially contribute to increased load of self-antigens that are ultimately targeted by autoantibodies.

When considering the diverse activities of IL-10 in a host with an otherwise activated immune system, its overall effects may contribute to disease, based on its less efficacious inhibition of activated, compared with unstimulated, monocytes and its positive actions on B cells (130 ,131).

In animal models of lupus there is evidence that therapy with anti-IL-10 mAb (136 ,137), or IL-10 itself, might be beneficial (138). In humans, treatment of six SLE patients with a murine anti-IL-10 monoclonal antibody resulted in significant improvement in cutaneous lesions, joint symptoms, and SLEDAI, even 6 months after the 21-day therapy (139). Although this study showed benefit, additional studies with humanized reagents will be needed to assess the value of this therapy in SLE.

BLyS

B lymphocyte stimulator (BLyS; also called B cell-activating factor, BAFF) and a related molecule, a proliferation inducing ligand (APRIL), belong to the TNF ligand superfamily, and like TNF they can exist in a soluble trimeric form (140). These molecules are produced by myeloid lineage cells and act exclusively on B cells through several receptors, transmembrane activator and CAML interactor (TACI), BAFF receptor and less so through B cell maturation factor (BCMA), to induce B cell maturation and survival (140). BLyS supports survival of transitional and mature B cells and also supports immunoglobulin class switching to mature immunoglobulin isotypes, although with less activity than that provided by CD40 ligation (141). In mice, BLyS is overexpressed in NZB × NZW F1 and MRL/lpr lupus mice, and BlyS inhibition ameliorates disease (142). SLE patients express high levels of BLyS as well (143 ,144). In a recent phase II study, LymphoStat-B (or belimumab, a humanized monoclonal antibody to BLyS) was apparently well tolerated and showed clinical effect, although it did not meet the predetermined efficacy endpoints of the study (reported at: http://www.hgsi.com/news/press/05–10–05_LSB_Phase_2_Results.htm). The publication of this study is awaited to better assess the role of this agent in SLE management.

IL-6

IL-6 is a pleiotropic cytokine that is secreted mainly by monocytes, fibroblasts, endothelial cells and also by B cells and T cells. It is induced by inflammatory signals (such as LPS) and cytokines (such as TNF and IL-1), as well as by anti-dsDNA antibodies (145). Among the many properties of IL-6 is its ability to activate and mediate terminal differentiation of B cells to secrete immunoglobulin, as well as the induction of acute phase protein synthesis, including C-reactive protein (146). Interestingly, although IL-6 is primarily thought of as a pro-inflammatory cytokine, it can inhibit TNF and IL-1 synthesis. With regard to kidney function, IL-6 can induce mesangial cell proliferation.

IL-6 has been implicated in lupus, both in animal models and in human disease (146). Blockade of IL-6 ameliorates murine lupus and inhibits anti-dsDNA production (147 ,148). Moreover, IL-6 has been noted to be present at increased levels in SLE sera and has been associated with active disease in some but not all studies (149 ,150 ,151). Indeed in one large recent cross-sectional study IL-6 levels were associated only with hematologic disease activity (mainly reflected in an inverse correlation with hemoglobin levels) but not with any other organ disease activity as measured by the BILAG index (152). High levels of IL-6 have also been noted in the urine of active nephritis patients.

Inhibition of IL-6 by a humanized monoclonal antibody to IL-6 receptor (IL-6R) has been attempted in other autoimmune diseases, including rheumatoid arthritis and juvenile idiopathic arthritis, with good responses (153 ,154). The therapy was tolerated well, but significant hypercholesterolemia was noted in many patients, while one patient died of reactivation of chronic EBV infection. The antibody, called tocilizumab or MRA, binds soluble and membrane bound IL-6R, blocking its binding to IL-6 and thereby inhibiting IL-6–mediated signaling. Signaling by other IL-6-like cytokines, such as IL-11, is spared (155). In summary, there is some evidence that anti-IL-6 therapy could decrease anti-dsDNA levels and ameliorate disease activity, including renal disease, in SLE patients. An ongoing randomized controlled trial with MRA may provide additional data on these points.

Other Cytokines

In addition to the cytokine products of the innate immune response discussed above, IL-12, IL-18, and IL-8 have also been found to be high in sera of active SLE patients (156 ,157 ,158 ,159). Both IL-12 and IL-18 are produced by activated macrophages and can promote the differentiation of IFN-γ–secreting T cells and NK cells. Inhibition of IL-18 in MRL/lpr lupus mice reduced renal damage and mortality, suggesting that the cytokine plays a pathogenic role in that model (160). IL-8 is a chemokine with potent chemoattractant activity. IL-8, along with the chemokines IP-10, MIG, MCP-1, and fractalkine have been observed at high levels in SLE sera and are candidate markers of increased disease

activity (156 ,161). Although some of these may be attractive candidates to therapeutically target in patients with active end-organ disease, such as nephritis, there is as yet no significant clinical experience with inhibitors of those mediators in lupus patients.

Cytokines of the Adaptive Immune Response

SLE is characterized by production of autoantibodies, and abundant data indicate that those autoantibodies are both antigen-driven and depend on T cell help. The T cell–derived signals that drive B cell expansion and immunoglobulin class switching to produce the potentially pathogenic isotypes IgG and IgA comprise those delivered by cell contact, such as those mediated by the CD154 (CD40 ligand)/CD40 pathway, as well as signals delivered by T cell–derived cytokines (162). The degree of activation of T cells as well as the effector pathways to which T cell differentiation is directed depend on many factors, including the avidity of the interaction between antigenic peptide-major histocompatibility complex antigen and the T cell antigen receptor, the level of expression of costimulatory ligands and receptors on APC and T cells, and the cytokines produced by those APC. Inherent features of T cells, including structure and expression of cell surface molecules, intracytoplasmic T cell signaling pathways and transcription factors, show variability among individuals based on genetic polymorphisms. These differences can contribute to variable T cell function, including cytokine production. The nature of the cytokines produced by T cells has an important impact on the character of the B cell immune response, particularly with regard to selection of immunoglobulin isotypes, and on induction or control of inflammation, through effects on mononuclear phagocyte Fc receptor expression, phagocytic activity, and production of effector cytokines.

Cytokines Generated in the Adaptive Immune Response: T Cell–Derived Cytokines

The Th1/Th2 Paradigm

The concept that T lymphocytes differentiate along one of two possible vectors, termed T-helper-1 (Th1) and T-helper-2 (Th2), was presented by Mossman et al. (163). Each of these T cell types was characterized by production of distinct cytokines (IL-2 and IFN-γ for Th1 and IL-4,-5,-6,-9,-10, and -13 for Th2). Subsequent studies elucidated some of the determinants of differentiation along one or the other pathway, including cytokines to which T cells were exposed (IL-12 supporting Th1 and IL-4 supporting Th2 development) and transcription factors expressed in the T cell (T-box expressed in T cells, T-bet, in Th1 cells and GATA3 in Th2 cells) (164 ,165 ,166). The two T cell types have been generally associated with distinct functions, with Th1 cells viewed as promoting cell-mediated immunity and inflammation by supporting T cell expansion and monocyte activation, and Th2 cells considered to support humoral immunity, including immunoglobulin class switching to produce some IgG subclasses as well as IgE.

Although the classical Th1/Th2 paradigm might suggest that Th2 cytokines would predominate in SLE, as Th2 cytokines are thought to drive B cell differentiation and production of pathologically significant autoantibodies is a central feature of lupus, in fact the cytokine picture in SLE is complex (167). In murine lupus models, the IgG subclasses that make up a substantial proportion of the autoantibodies that are found in serum are IgG2a, a subclass supported by the Th1 cytokine IFN-γ (168). Moreover, IFN-γ deficient lupus mice are protected from nephritis, suggesting an important role for that cytokine in end-organ inflammation and tissue damage (169 ,170 ,171). On the other hand, IL-10, a product of Th2 cells, is elevated in SLE as discussed. Careful measurement of T cell, monocyte, and DC–derived cytokines, as well as definition of the cells that produce those cytokines, will be important for more complete characterization of the pathogenic mechanisms that contribute to disease in SLE and other autoimmune syndromes.

Interleukin-2

IL-2, a classic Th1 cytokine, is produced by T cells after activation through the T cell antigen receptor and the costimulatory molecule CD28. The regulation of IL-2 occurs through activation of signaling pathways and transcription factors that act on the IL-2 promoter to generate new gene transcription, but also involves modulation of the stability of IL-2 mRNA. IL-2 binds to a multi-chain receptor, including a highly regulated α chain and ß and γ chains that mediate signaling through the Jak-STAT pathway. IL-2 delivers activation, growth and differentiation signals to T cells, B cells, and NK cells. IL-2 is also important in mediating activation-induced cell death of T cells, a function that provides an essential mechanism for terminating immune responses. Perhaps because IL-2 was among the first cytokines to be studied in detail by immunologists investigating basic mechanisms of T cell and general immune function, the level of expression and functional role of IL-2 in the cellular alterations that characterize SLE were the focus of numerous studies over the past 25 years.

In general, the consistent observations were that IL-2 production by T cells stimulated in vitro was low in SLE (172 ,173 ,174). However, when studied in vivo, there are some reports of increased serum IL-2 protein, and IL-2 mRNA transcripts have been documented in unstimulated SLE peripheral blood cells (175). Regarding IL-2 receptors, in vitro studies have indicated impaired induction under conditions of cell activation, but serum levels of soluble IL-2 receptor are increased in patients with active disease (176). Taken together, the data are most consistent with in vivo activation of CD4 T cells in SLE, with the additional possible interpretation that chronic or repeated exposure of those cells to self-antigens

presented by mature APC in vivo alters their capacity to demonstrate normal activation responses when studied in vitro. At this time there is not strong support for therapeutically manipulating the IL-2 pathway in SLE.

Interferon-γ

IFN-γ is the sole type II IFN. Early in an immune response, IFN-γ is mainly generated by NK cells, and once the adaptive immune response is engaged, it is a major product of Th1 cells activated by APC that produce IL-12 or IL-18. IFN-γ implements a broad spectrum of effects on immune responses, including activation of monocytes, and when produced in excess can promote tissue injury (177). Among its activities are the induction of other pro-inflammatory cytokines such as TNF and induction of apoptosis in renal parenchymal cells. The relationship between IFN-α and IFN-γ is complex (178). IFNα inhibits the induction of IFN-γ by NK cells in the presence of STAT1. In contrast, in the absence of STAT1 IFN-α can stimulate production of IFN-γ by T cells. Like IFN-α, IFN-γ signals cell activation through STAT1 but can also utilize a poorly defined STAT1-independent pathway (179).

The role of IFN-γ in the pathogenesis of SLE has been best illustrated in studies of murine lupus. Experiments using IFN-γ–deficient mice have demonstrated a requirement for IFN-γ in development of significant nephritis and for expression of IgG2a anti-dsDNA antibodies in MRL/lpr and NZB × NZW F1 mice (168 ,169 ,170 ,180 ,181 ,182 ,183). However, in pristane-induced lupus, the pristane treatment was sufficient to induce some IgG2a anti-Sm/RNP autoantibody, even in the absence of IFNγ (184). Additional approaches supporting a requirement for IFN-γ for most manifestations of lupus include administration of anti-IFN-γ antibody, soluble IFN-γ receptor, and study of IFN-γ receptor deficient mice. Nephritis appears to be particularly dependent on IFN-γ (168 ,185). It is likely that the different murine models will show variable dependence on either type I or II IFN for development of autoimmunity and disease, perhaps based on their baseline relative expression of those cytokines. While murine studies support important roles for both type I and II IFN in lupus, support for IFN-γ in human lupus is less well documented. Gene expression studies of peripheral blood cells do not show increased levels of CXCL9 mRNA, a gene product that is highly induced by IFN-γ (107). However, IFN-γ may be more highly expressed in kidneys of patients with lupus nephritis and could play an important role in augmenting expression of chemokines that contribute to recruitment of inflammatory cells and tissue damage.

Th2 Cytokines in SLE

As described above, IL-6 and IL-10, typical Th2 cytokines, are increased in the serum of active lupus patients, but the production of those cytokines is more likely to be attributable to monocytes and B cells than to T cells (186 ,187). IL-4 and IL-5 are additional Th2 cytokines, but the role for these mediators in SLE is less well supported than for others discussed. Increased production of IL-4 has not been consistently demonstrated in SLE. A recent report suggests that Th2 cytokines may actually be protective from lupus nephritis (188).

TGF-ß

TGF-ß is a pleiotropic and multifunctional cytokine. While produced by many cell types, it is included among the Th2 cytokine family. TGF-ß is produced as a latent molecule that is then activated by plasmin-mediated cleavage and release of the biologically active fragment. When TGF-ß binds to its receptor, SMAD proteins translocate from cytoplasm to nucleus and promote generation of new mRNAs. TGF-ß plays an important role at each stage of an immune response and in the context of wound healing (189). Early in an immune response, TGF-ß promotes activation of innate immune system cells. Once an adaptive immune response is well underway, it inhibits activation and proliferation of T cells to provide regulation of cellular immunity. Finally, TGF-ß is a central mediator of tissue repair (190).

A role for TGF-ß in the inhibitory activity of Tregulatory cells (Tregs) has been recently investigated (189). Regulatory T cell function is now felt to reside in the CD4+CD25+ T cell population and is associated with production of TGF-ß and IL-10, as well as expression of a transcription factor, FOXP3 (190 ,191 ,192). TGF-ß also appears to contribute to induction of Tregs from precursor T cells.

As is the case for other T cell-derived cytokines, interpretation of data addressing the expression and function of TGF-ß in SLE is challenging, particularly in view of the fact that most of the cytokine present in serum is present in the latent form. Most data indicate that production of TGF-ß in peripheral blood cells is decreased in SLE which would be consistent with impaired regulation of T cell activation (193). However, TGF-ß may be expressed at sites of inflammation, such as the lupus kidney, and potentially contribute to renal scarring (194). Intracellular pathways activated by TGF-ß are well known to target genes, such as those encoding collagen and fibronectin, that are implicated in tissue fibrosis.

Additional T Cell–Derived Cytokines

IL-16 is produced by CD8 T cells and mediates chemotaxis of CD4 T cells and monocytes. IL-16 serum levels are increased in patients with severe disease and the serum levels showed correlation with measures of disease activity (195 ,196).

Cytokines Generated in the Adaptive Immune Response: B Cell–Derived Cytokines

B lymphocyte function in SLE is most simply characterized as hyperactive. A high proportion of peripheral blood B cells are activated by morphologic criteria. SLE B cells in vitro proliferate and differentiate to antibody-secreting cells spontaneously, without the addition of traditional mitogens (197).

The spectrum of B cells that secrete antibody in patients with SLE represents a polyclonal assortment, but characteristic of SLE is the selective and high-level secretion of a restricted population of autoantibody specificities including those reactive with nucleic acids and nucleic acid-associated proteins.

Studies of B lymphocytes have focused on their exclusive role in generating antibody-producing plasma cells, with some additional emphasis on the capacity of activated B cells to effectively present antigen to T cells. Current thinking has expanded the function of B cells to include production of soluble mediators, including cytokines. Most of the products of B cells are not exclusive to those cells, but are also expressed by monocytes and T cells. Among those, IL-6 and IL-10 have been discussed. As noted, these cytokines have been demonstrated to be expressed at high levels in patients with active SLE, and both contribute to B cell expansion and differentiation. While it is likely that multiple cell types produce these cytokines in lupus, activated B cells may be particularly active in this function.

Summary

The scope of immune system alterations in SLE is so extensive that it has been difficult for investigators to determine which of those altered functions is a primary contributor to lupus pathogenesis. The recent resurgence of interest in the type I IFN system and documentation of a prominent and broad activation of the IFN pathway in cells of lupus patients, along with rapid progress in the elucidation of the TLR system, has helped to reformulate the view of lupus pathogenesis to include an important role for innate immune system activation, by either exogenous or endogenous adjuvant-like triggers, in generating type I IFN and many of its downstream effects on immune function (110). As in immune responses to microbes, the adaptive immune system is engaged subsequent to activation of the innate immune system, but in SLE it is focused on self-antigens. Activation of T and B lymphocytes results in production of a diverse complement of cytokines, along with autoantibodies, that contribute to the character of the disease. IFN-γ produced by T cells and NK cells is a potent inducer of chemokines that attract inflammatory cells to involved tissues and organs. BLyS/BAFF, IL-6 and IL-10 are products of the innate immune response but promote survival and differentiation of B cells, amplifying their production of pathogenic autoantibodies. Each of these cytokines represents a rational therapeutic target. With some good fortune, we can look forward to the opportunity to evaluate data from clinical studies of potentially therapeutic cytokine inhibitors, allowing new insights into lupus pathogenesis and new hope for patients.

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Chapter 11

B Cells in Systemic Lupus Erythematosus

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The hallmark of immune cell dysregulation in systemic lupus erythematosus (SLE) is B cell overactivity. B lymphocytes in SLE produce a spectrum of autoantibodies (autoAb) against soluble and cellular constituents. Although all cellular constituents represent potential targets of the lupus autoAb response, the most characteristic, as well as the most common autoAb in SLE, are those targeting the macromolecular complexes of the cell nucleus, the antinuclear antibodies (ANA). While the spectrum of autoAb specificity in SLE may seem unrestricted, it is only a handful of those that have been shown to contribute convincingly to disease-related tissue injury. The latter are best represented by the anti-blood cell antibodies that activate complement and cause cytopenias and the cationic anti-dsDNA autoAb that are thought to contribute to the expression of nephritis (1 ,2). The presence of some autoAb correlates with clinical subsets of the disease and is used in disease diagnosis. Besides patients with SLE and patients with a variety of autoimmune and nonautoimmune diseases, normal individuals have autoAb in their sera albeit transiently and in low concentrations (3). In this chapter we will review pertinent human data and we will refer to and integrate useful information produced from the study of B cells in different murine lupus models.

Autoreactive B Cells

Older theories portrayed the view that normally autoreactive B cells do not exist because their presence would provoke overt autoimmunity and they are, therefore, eliminated during the maturation process. Nevertheless, it is currently well established that such B cells do exist in the normal person. Moreover, while the naturally autoreactive B cell pool was initially thought to be rather small, novel studies have presented evidence that this is an underestimation. B cells often respond to autoantigens for which they have such small affinities that cannot be detected with the use of older, conventional assays (4).

Under normal conditions or in disease states ranging from viral infections to malignancy, normal, autoreactive B cells produce a variety of natural autoAb that do not cause autoimmune disease or tissue damage. Natural autoAb usually belong to the IgM isotype, and do not undergo isotype switching and affinity maturation. They are poly- and cross-reactive with auto- and alloantigens, their appearance may be helpful for the host in order to efficiently eliminate an invader, and finally, natural autoAb usually appear in the circulation for a short time because of tight regulatory mechanisms imposed on their existence. Natural antibodies are also expected to “dispose” dying or dead cells, but when tissues are exposed to stressors such as ischemia, then they may bind to newly revealed antigens, activate complement, and confer extensive tissue damage. The nature of the antigen(s) natural antibodies bind may represent proteins or phospholipids (5 ,6 ,7 ,8). The above characteristics contrast with those of the autoAb response in SLE where isotype switching and affinity maturation occur, their presence is not helpful for the host since they can cause tissue damage, their production is continuous and their presence is long-lived, indicating that regulatory mechanisms governing their appearance and/or elimination are profoundly defective.

Autoreactive antibodies arise from autoreactive B cells, but the mechanisms involved in the preservation of autoreactive B cells are unclear. One view favors that immature B cells having autoreactive potential are eliminated by negative selection so the mature B cell population contains a few, or, no autoreactive cells. Escaping the negative selection process may thus represent a mechanism of nonelimination of immature autoreactive B cells. The generation of the hen-egg lysozyme (HEL) transgenic mouse model improved our understanding on the preservation of autoreactive B cells and the appearance of autoimmunity. Data derived from the study of this model indicate that all B cells inherently exhibit a certain level of autoreactivity. Thus, it may be dangerous for the host to eliminate too many developing immature B cells that bear autoimmune potential because it may restrict severely the spectrum of the normal immune response repertoire. It may be equally dangerous to eliminate too few, because this may cause overt autoimmunity. Such a dynamic equilibrium between immunity and autoimmunity is maintained at the stage of the pre-immune B cell and is driven by specific selection processes. These selection functions include the strength of B cell antigen receptor (BCR)-generated intracellular signals (the stronger the BCR-initiated signal, the more likely the rapid elimination of the preimmune B cell) and the interclonal competition. In the presence of a normal B cell repertoire, the autoantigen weakly-binding B cells cannot compete for entry into the follicles of the lymph nodes and spleen in

order to receive cell-survival signals. The nonautoimmune B cells are arithmetically superior and they enter and occupy the follicles rather easily. The follicle-excluded B cells deprived of important survival signals die. When the B cell repertoire becomes compromised, the autoantigen–binding B cells face less competition, so they eventually enter the follicles, receive survival signals and become long-lived cells (9 ,10 ,11 ,12 ,13 ,14). The increased occurrence of autoimmunity in conditions of immunodeficiency is in agreement with this theory (15 ,16). Further supporting the above are data produced in high- and low-affinity anti-Sm autoAb producing transgenic mice. High-affinity anti-Sm producing B cells enter lymphoid follicles and differentiate appropriately into B1 cells, while low-affinity anti-Sm producing B cells still enter the follicles but become partly anergic long-lived B2 cells (17).

Circulating B cells in patients with SLE are not present in increased numbers, have increased rates of proliferation, and spontaneously secrete increased amounts of immunoglobulin consisting of natural antibodies, natural autoantibodies and autoantibodies reactive with nuclear, cytoplasmic, and cell-membrane self antigens (18 ,19). The number of B cells spontaneously releasing immunoglobulin correlates rather accurately with disease activity (20 ,21). Significantly increased telomerase activity, an indication of increased activation and proliferation, is also found in B, but not in T cells, from patients with SLE (22).

If we consider that detection of autoAb indicates the presence of autoreactive B cells, it is currently understood that such cells exist in patients with SLE several years before the development of clinically evident disease. A recent study conducted using serum samples obtained from previously healthy individuals that subsequently developed SLE (*n* = 130) disclosed that autoAb were present in 115 (88%) of them as early as 9.4 years (mean: 3.3 years) before diagnosis was made. AutoAb were present in 3.8% of sera obtained from healthy control individuals. It is of interest to note that autoAb were present in the first available serum samples obtained from many patients suggesting that autoreactive B cells were present and functional even earlier (23).

Autoreactive B cells in SLE arise early in B cell ontogeny, and a significant number of antigen-inexperienced naïve B cells are capable of producing autoAb. As shown from the exhaustive analysis of different early B cell subpopulations in three patients with juvenile-onset SLE the two discrete tolerance checkpoints imposed during B cell ontogeny are violated although to a different degree in different patients. The first checkpoint is at the immature B cell stage in the bone marrow where the majority of polyreactive and ANAs are lost. The second checkpoint is in the periphery before maturation of new emigrant B cells into naive immunocompetent lymphocytes. Single B cell analysis in the B cell compartment newly emigrated from the bone marrow and in the mature naïve B cell compartment disclosed that the two tolerance checkpoints in patients with SLE are compromised. Twenty five percent to 50% of the mature naïve B cell compartment is autoreactive in this limited number of patients with SLE before they encounter autoantigen(s) while in healthy individuals 5% to 20% of such B cells are capable of producing self-reactive autoAb (24). The differential development and fate of specific autoreactive B cells in patients with SLE and healthy individuals was addressed in another study. B cells producing natural autoAb bearing the VH4.34 heavy chain were followed from immaturity to terminal development into plasma cells. In healthy donors the naïve VH4.34 B cells are numerous and follow a sequence of specific steps of positive and negative selection into secondary lymphoid organs. Negative selection forces in the normal host predominate in such a manner that progression into terminal stages of differentiation is efficiently blocked resulting in underrepresentation of VH4.34 B cells in the memory B cell pool. This block can be violated ex vivo following culture with IL-2, IL-10 and CD70 in the normal host; in patients with lupus this ex vivo step was not necessary since VH4.34 B cells readily progress in vivo to the final plasma-cell stage. It is therefore suggested that tolerance checkpoints in SLE are malfunctioning (25). Despite the striking B cell overactivity challenging SLE patients with standard immunizations or stimulating lupus peripheral B cells with polyclonal activators in vitro can result paradoxically in substantially decreased amounts of specific Ab production compared to the responses of B cells obtained from normal individuals (26 ,27).

B Cell Subpopulations

B-1 cells are phenotypically distinguished from the conventional B cells (B-2 cells) because they bear the T cell surface marker CD5 in humans (Ly-1 in mice). B-1 cells, the majority of B cells during fetal life, predominate in cavities such as the peritoneum; nevertheless, their numbers in the circulation progressively decrease following birth until they become no more than 20% of the circulating B cell pool in the adult. B-1 cells have interesting and distinct properties because they are self-reconstituting and long-lived and because they produce highly cross-reactive and low-affinity antibodies that usually belong to the IgM class (28 ,29).

B-1 cells may be involved in the pathophysiology of some human and murine autoimmune diseases such as Sjögren syndrome and rheumatoid arthritis because they produce IgM rheumatoid factor (30 ,31). Moreover, B-1 cells are expanded in certain murine lupus-prone models where they contribute significantly to the production of important autoAb. While this may be the case for autoimmunity models such as the NZB, the (NZB × NZW) F1 and the motheaten mouse where B-1 cells produce anti-erythrocyte and anti-DNA antibodies, it is not the case for other experimental animal lupus models (29 ,32 ,33). In other murine lupus models B-1 cells are not expanded in the circulation, and their induced expansion in nonautoimmune backgrounds does not correlate with autoimmunity (32 ,34). The potential pathogenic role of B-1 cells in human lupus is questionable principally because in patients with SLE both B-1 and

B-2 cells contribute to the production of pathogenic autoAb such as anti-dsDNA (35).

A detailed analysis of circulating B cells in patients with SLE disclosed that there are major disturbances in the different peripheral B cell compartments. A recently described B cell subpopulation representing immature transitional B cells (generated in the bone marrow but not having undergone final maturation steps in peripheral lymphoid organs) named type I transitional (T1) B cells were reportedly increased in the circulation in patients with SLE (36). In patients with active but not in those with inactive SLE there was a marked reduction in the numbers of naïve ($CD19^+CD27^-$) B cells and an enhanced representation of the $CD27^{high}CD38^+CD19^{dim}sIg^{low}CD20^-CD138^+$ plasma cells in the periphery (37). A naïve B cell–specific glycoform of the surface molecule CD45 was recently shown to be the target of IgG VH4.34 autoAb produced by the VH4.34 B cell subpopulation, which is greatly expanded in SLE patients. This CD45 glycoprotein in memory B cells has a different glycosylation pattern from that of naïve B cells and does not bind VH4.34 autoAb (38). Immunosuppressive therapy, commonly used in lupus patients, induces changes in the different B cell subsets: it reduces significantly the numbers of $CD27^{high}$ plasma cells and of $CD27^-$ naïve B cells but does not affect the population of $CD27^+$ B cells (37). In one study, increased numbers of CD27 high plasma cells correlated with increased disease activity more accurately than a combination of clinical and serologic parameters, suggesting that this may be of value in monitoring disease activity (39). In another study of pediatric patients with SLE it was found that both naïve and memory peripheral B cells were reduced by 90% and that plasma-cell precursors were expanded threefold. The predominant B cell subpopulation in such young patients with SLE was a novel B cell subset with pregerminal center phenotype. These B cell subset alterations were independent of disease activity (40).

The Abnormal Immunoregulatory Environment

The features of the autoAb (isotype switching and affinity maturation) indicate that the lupus autoAb response is a T cell dependent, (auto)antigen-driven immune process. But is the T cell compartment responsible for B cell hyperreactivity? If this is correct then B cells should be under either decreased T cell–mediated suppression or under excessive, unopposed T cell–derived help or both. In the past it was considered that autoreactive B cells in SLE are not properly controlled by suppressor T cells (41 ,42 ,43). There is evidence supporting the opinion that increased T cell help is responsible for the increased production of antibody and autoAb in SLE. Apart from $CD4^+$ helper T cells, other T cell subpopulations can provide help to SLE B cells to produce pathogenic autoAb such as $CD8^+$, or $CD3^+CD4^-CD8^-$-TCRαß + or $CD3^+CD4^-CD8^-$-TCRγδ+ cells (44 ,45 ,46). Increased, nonspecific help to autoreactive B cells can also lead to autoimmunity. When anti-dsDNA heavy chain transgenic B cells were generated in a nonautoimmune background and alloreactive T cell help was provided in the setting of chronic graft-versus-host disease, such transgenic B cells were specifically expanded (70%) and secreted high titers of anti-dsDNA autoAb. Analysis of these autoAb disclosed that they had undergone receptor editing evidenced by the detection of light-chain rearrangements (47).

The recent interest in the role of T regulatory cells in the development of autoimmune diseases (48) has rekindled interest in the role of these cells in the pathogenesis of human SLE. Indeed, a study showed decreased numbers of T regulatory cells in the peripheral blood on SLE patients (49) but it has not been shown yet whether they control B cell function also.

The above studies conclude that B cell overactivity and the production of autoAb are a result of factors exogenous to the B cell and lie within the T cell compartment. While the previously mentioned data clearly document the contribution of the T cell compartment to the production of autoAb, there are functional as well as genetic studies challenging the view of an entirely T cell–dependent process. Studies of murine and human lupus have produced direct or indirect evidence that B cells in SLE are not innocent bystanders but that their contribution to disease initiation and perpetuation may be central.

The Intrinsically Abnormal B Cells in SLE: Functional Studies

A number of studies have concluded that the B cell in lupus may contribute in a T cell independent manner to the production of autoAb. These studies cumulatively have shown that:

- The immunologic tolerance of B cells can be violated rather easily in vitro under conditions that could be readily reproduced in vivo (50). B cell tolerance can be broken during viral infections, during immunizations, and upon stimulation with polyclonal activators (51 ,52 ,53 ,54 ,55 ,56). Polyclonally triggered B cells are found in the circulation of patients with SLE and Sjögren syndrome (57 ,58). Additionally, chronic polyclonal B cell stimulation may lead to autoimmunity in mice (59). Nevertheless, polyclonal activation is not the principal cause for the production of autoAb (60). Bretscher and Cohn have proposed the two-signal hypothesis suggesting that contact with (self) antigen in the absence of T cell–derived help tolerizes B cells (61). Initial B cell contact with a self antigen and T cell–derived help at the same time results in B cell tolerance breakdown (62 ,63). Nevertheless, experimental data support that this rule can be violated, and B cell tolerance can be broken without the support of T cell–mediated help (64).
- Lupus-prone murine strains genetically manipulated to have no T cell antigen receptor (TCR)$αß^+$ (and no $CD4^+$) T cells were still able to produce significant amounts of pathogenic IgG autoantibodies (65).

MRL/lpr mice congenitally deficient in TCRαß T cells develop autoimmunity characterized by hypergammaglobulinemia, autoAb to native DNA and to small nuclear ribonucleoproteins and also manifest immune-complex mediated nephritis (66).

- Finally when pre-B cells obtained from the embryonic liver of (NZB × NZW) F1 mice were transferred to mice with severe combined immunodeficiency (SCID), they were able to produce anti-dsDNA autoAb and to sustain a lupus-like disease in the SCID recipients (67). These data are in contrast with previous studies reporting that the transfer of autoreactive B cells into nonautoimmune animals caused the production of relatively small amounts of autoAb (68). In contrast, transfer of normal B cells into autoimmunity-prone recipients was associated with autoantibody production within a month from the transfer (69 ,70).

The former studies underscore that B cells of lupus-prone experimental animals are able to produce autoAb and to cause lupus-like disease even in host environments where they receive no $CD4^+$ - mediated T cell help. This ability is preserved in the absence of αß T cells and even in the total absence of T cells, and hence in the total absence of either T cell–mediated increased help or decreased suppression. But is it the presence of B cells that is causally related to the development of disease, or the presence of autoAb that results from B cell overactivity? A study elegantly approached this question. Lupus-prone MRL/lpr mice genetically manipulated to express B cells bearing sIg BCR but unable to secrete any antibody were employed. A proportion of these mice still developed autoimmunity characterized by nephritis with cellular infiltrates, indicating autoimmune B cells themselves without the production of autoAb and immune complexes, contribute directly to disease pathogenesis (71). The absolute dependence of the expression of SLE on either direct or indirect interactions of B cells with either hyperactive or autoreactive T cells is therefore questionable under certain experimental conditions.

The expression of B cell antigen receptor (BCR) on bone marrow cells limits subsequent rearrangements of Ig by downregulation of the expression of recombination activating genes (RAG) 1 and RAG2. If those B cells re-express RAG activity then autoreactive receptors may be eliminated or, nonautoreactive ones may acquire significant self-reactivity (72). Data from one lupus patient failed to show aberrant Ig editing (73) but the jury may be still out on this issue.

The lupus B cell is an efficient autoantigen-presenting cell.

Other investigators taking into account the particularly efficient antigen presenting capacity of the B cell have produced data indicating that the B cell in lupus may represent the central pathogenic cell that triggers other immune cells toward hyperactivity. Studies from the MRL/lpr lupus murine model have shown that when these mice undergo genetic manipulations in order to have no B cells, they do not display autoimmune manifestations, lack circulating autoAb and deposits of immune-complexes in tissues. These animals do not have circulating activated T cell that appear in the MRL/*lpr* animals and inflamed tissues with infiltrating T cells. While the vast majority of the circulating T cells in the unmanipulated animal have a particular activated/memory phenotype, the vast majority of the T cells in the manipulated animals are naïve T cells. It is thus proposed that the abnormal B cells of the $MRL/^{lpr}$ model are not only responsible for the production of pathogenic autoAb, but also mediate the activation of the T cell compartment (74 ,75 ,76). More specifically, this T cell activating power of B cells has been shown for $CD8^+$ T cells in the MRL/*lpr* mouse (77). In the above models the presence or the absence of B cells was the one and only determinant of the appearance or not of the autoimmune murine syndrome. In the absence of B cells these autoimmune mice had neither activated T cells nor lupus. It is thus possible, that under certain circumstances, lupus B cells are not restricted to the production of autoAb only, but also mediate abnormal T cell activation.

Patients with SLE treated with anti-CD20 antibody the clinical effect was associated with decreased numbers of circulating T cells expressing CD40L (78), suggesting that, as in mice, in human lupus B cells are involved in the generation of activated T cells.

Genetic Studies

The description of functional aberrations in lupus B cells suggest a potentially intrinsic defect and that such a defect(s) may reflect the product of genetic alteration(s). Studies of the genetic composition of lupus-prone mice began several years ago. Three independent research groups analyzing the whole genome of the (NZB × NZW) F1 mouse mapped genetic loci that contribute to development of murine lupus (79 ,80 ,81). It is currently thought that these disease-predisposing genetic loci work in an additive manner; the more disease-predisposing loci an animal inherits, the higher the likelihood it will develop the full-blown lupus-like illness. Three independent groups reached similar conclusions during mapping of such disease-susceptibility loci (82 ,83).

One lupus-prone strain analyzed was the NZM2410 mouse. This is a substrain of the (NZB × NZW) F1 strain characterized by highly penetrant and early onset lupus-like illness. In the NZM2410 model disease-susceptibility maps to a few genetic intervals named *Sle1, Sle2, Sle3*, and so on (84). The other two groups also mapped disease susceptibility loci; one lupus-related genetic locus called nba-1 by one group and the one called by others *Lbw-2/Sbw-2* was identified as the locus with a particularly strong pathogenetic contribution (79 ,81). *Nba-1, Lbw-2/Sbw-2* as well as *Sle2* map in a 9cM-spanning region of murine chromosome 4, possibly within the context of a single gene. Crossing experiments produced a new strain expressing *Sle2* only, that developed a clinical picture characterized by hypergammaglobulinemia and IgM class autoAb, which could be attributed to dysfunction of the B cell compartment only. Nevertheless, *Sle2*-only expressing

mice did not develop full-blown lupus, nor did the other strains that expressed other isolated disease-susceptibility genetic intervals. Such experiments propose that *Sle2* may represent a “lupus B cell overactivity genetic locus” (85).

Equally interesting are the data stemming from the novel *Sle1* expressing murine models. *Sle1* maps on murine chromosome 1 and is associated with the production of anti-histone autoAb in lupus mice. Histones are T cell dependent autoantigens and the production of antihistone autoAb is a well-characterized T cell dependent autoimmune-response. *Sle1* expressed in a lupus-resistant background led to the production of IgG anti-H2A/H2B/DNA autoAb. It is now understood that *Sle*1 is expressed in both B and T cells. When expressed in B cells in the absence of T cells it mediates a break of tolerance and the production of autoAb and when expressed in T cells in the absence of B cells it mediates the abnormal production of cytokines and activation markers. Expression of glomerulonephritis requires the expression of *Sle1* in both B and T cells (86). Isolated expression of the genetic interval *Sle3* that maps on chromosome 7 was associated with low-grade polyclonal T- and B cell activation and mildly penetrant glomerulonephritis (87). However, the simultaneous presence of *Sle1* and *Sle3* was associated with an aggressive autoimmune phenotype, particularly in female mice. *Sle1* and *Sle3* bi-congenic mice developed splenomegaly, significantly expanded B- and $CD4^+$ T cells with activated phenotypes, widespread humoral autoimmunity including pathogenic autoAb, and a highly penetrant glomerulonephritis (88).

Based on their elegant studies the Wakeland group propose an epistatic model for the development of autoimmunity, where a combination of multiple defects in the genome allow for the expression of autoimmune aberrations culminating in the expression of lupus-like disease. To complicate things further, the same group identified autoimmunity–suppressing modifiers. One of these, designated *Sles1* was shown to specifically suppress the autoimmune features that should ensue because of the presence of *Sle1*, but not of *Sle2* or *Sle3* loci (89). It is thus the presence of autoimmunity–promoting and/or the absence or loss of autoimmunity–suppressing genetic elements that integrate into the development of overt autoimmunity in experimental models.

The *Sle1* interval has been further divided into *Sle1a, b,* and c. Chapter 7 lists further details about the major genes contributing to murine SLE.

The Deranged Cytokine Environment

The production of and the response to cytokines such as IL-6 and IL-10 is abnormally increased in SLE B cells. Both IL-6 and IL-10 are B cell stimulatory cytokines, while IL-10 also inhibits type-1 cytokine responses (90 ,91). Patients with active SLE have elevated serum levels of IL-6 (92 ,93), increased numbers of IL-6 secreting cells (94) and increased IL-6 mRNA content in their peripheral blood mononuclear cells (95). B cells from patients with SLE express constitutively cell-surface IL-6 receptors, but normal B cells do not (96). IL-6 generates an in vitro increase in the lupus B cell output of IgG and an increase of IgG anti-DNA autoAb, and the addition of anti-IL-6 mAb inhibits the IL-6 induced effects (92 ,96). The autocrine action of IL-6 has been shown clearly in B cells from (NZB × NZW) F1 and from MRL/*lpr* mice (97 ,98 ,99).

Similarly to IL-6, patients with SLE have elevated serum levels of IL-10 (100), increased numbers of IL-10 secreting cells (94) and increased IL-10mRNA content in their peripheral blood mononuclear cells (95). Interleukin-10 overproduction was shown to have functional significance since an anti-IL-10 mAb used in that study suppressed the in vitro production of autoantibodies from B cells obtained from patients with SLE but recombinant human IL-10 promoted it (101). In support of the above is that continuous administration of anti-IL-10 mAb delays the onset of autoimmunity in (NZB × NZW) F1 mice (102). It could be proposed that increased production of IL-10 may represent a product (rather than a cause) of a hyperproliferating B cell population. Because the degree of B cell proliferation parallels closely the activity of the disease and the overproduction of IL-10 was disease activity-independent, these data point toward a putative intrinsic B cell defect in SLE. In agreement with this hypothesis are reports that increased production of IL-10 is found in B cells from relatives of SLE patients. The mRNA of IL-10 was found in lupus B cells but was undetectable in control B cells. Using flow cytometry it was reported that circulating, unmanipulated lupus $CD5^+$ B cells spontaneously produce IL-10, an effect not seen in healthy individual B cells (103). Additional in vitro studies have shown that IL-10 producing B cells from patients with SLE express the surface marker CD40L (104).

Phenotypic Changes of the Lupus B Cell

The “ectopic” expression of CD40 ligand (CD40L, CD154) was first described on the surface membrane of lupus B cells (105). In the peripheral blood of patients with active SLE $CD40L^+$ B cells were 20.5-fold more when compared to healthy donors. In patients with inactive SLE the numbers of CD40L+ B cells were comparable to those of the controls. Nevertheless, activation-induced CD40L expression on the surface of lupus B cells was 17-fold the baseline levels, compared to a 7.6-fold increase recorded in control subjects. CD40L expressed on the surface of lupus B cells is functional because the addition of a neutralizing anti-CD40L mAb in in vitro cell-culture settings decreased the production of cationic anti-dsDNA. Because not only T cells, but also B cells from SLE patients express functional CD40L on their cell surface, and because both kinds of lymphocytes express CD40, a bidirectional cognate stimulatory loop may function between lupus T and B cells (106). Moreover, it was reported that CD40L expressed on the surface of B cells costimulates other B cells (107). CD40L-expressing circulating

B cells from patients with active SLE were also shown to express the recombination activating genes 1 and 2. These two genes are not expressed by circulating B cells of healthy volunteers (108). In lupus-prone mice continuous administration of anti-CD40L ab resulted in decreased IgG anti-dsDNA autoAb production and delayed the onset of autoimmunity. Nevertheless, autoreactive IgM-producing B cells were still detected and following cessation of treatment such cells underwent a series of activation events and became again fully competent ab-secreting cells (109). The role of CD40L-expressing B cells in the production of autoAb in patients with SLE was supported by a study analyzing the fate of such cells and the production of autoAb in a few patients with active SLE and nephritis treated briefly with anti-CD40L mAb. The frequency of IgG- and IgG anti-DNA autoAb-producing lupus B cells was markedly reduced for months. This effect was also seen in EBV-transformed B cell lines established from the patients with SLE (110). Finally, the transgenic expression of CD40L on the surface of B cells in a nonautoimmune murine background correlated with the development of autoimmune manifestations in some of the ageing animals. Several different autoAb were detected in their sera and half of the animals developed immune-complex mediated nephritis, underscoring a potentially crucial role for CD40L-expressing B cells in the appearance of autoimmunity (111). Another costimulatory pair of molecules, the inducible costimulator (ICOS) and its ligand (ICOS-L), is also aberrantly regulated in lupus lymphocytes. While the expression of ICOS on the surface of circulating lupus T cells was increased, the expression of ICOS-L on the surface of circulating memory B cells was significantly decreased. The authors inferred that this effect was a result of recent contact between B and ICOS+ T cells in patients with SLE (112).

In addition to CD40L, CD80 and CD86 are also aberrantly expressed on the surface of lupus B cells. Fresh, small, resting, peripheral B cells but also large, activated B cells from patients with SLE were studied for the expression of CD80 and CD86. While CD80 expression was only slightly increased on the surface of large, activated lupus B cells, the expression of CD86 was 2.5- and 7-fold increased on the surface of activated and resting lupus B cells respectively, when compared to B cells obtained from patients with allergic disorders (113). Although all patients studied were in remission, and the functional integrity of the costimulatory molecules was not assessed, this study provided evidence that B cells from patients with SLE overexpress molecules that belong to the B7 family of costimulatory molecules. Expression of B7-family molecules has been shown to be a prerequisite for the disruption of immune tolerance toward self antigen (114). A recent study disclosed that both CD80 and CD86 were significantly upregulated on the surface of circulating B cells from patients with SLE. CD86 was not found on the surface of normal B cells but was induced following coculture with CD40L-expressing T cells. In the presence of anti-CD40L mAb the expression of CD86 on B cells from patients with lupus was inhibited. In the presence of anti-CD86 but not of anti-CD80 mAb the production of polyclonal Ig and of antisingle-stranded DNA was inhibited, suggesting a functional role for the abnormally expressed CD86 molecule on B cells from patients with lupus (115). Another group of investigators reported aberrant expression of the B cell surface molecule RP105. In patients with SLE there is an over-representation of RP105-negative B cells; such cells are reportedly responsible for the production of autoAb. When cultured in vitro with IL-6 RP105-, but not RP105+ B cells from patients with SLE were capable of secreting anti-dsDNA autoAb (116) (Table 11.1).

Table 11-1: Molecules Abnormally Expressed on the Surface of B Cells in SLE

Molecule abnormality	Normal Function
CD40L (CD154) ↑	Costimulation
ICOS-L ↓	Costimulation
CD80 Slightly ↑	Costimulation
CD86 ↑	Costimulation
RP105 ↓	Unknown
CR1 ↓	Complement receptor
CR2 ↓	Complement receptor EBV receptor

Aberrant B Cell Antigen Receptor Signal Transduction

The studies discussed above present evidence supporting the view that B cells from patients with SLE have functional as well as phenotypic abnormalities that are at least in part independent of the activity of the underlying disease. It is thus possible that such aberrations represent intrinsic lupus B cell defects. It is also possible that the heterogeneous defects described above may have a common underlying central biochemical abnormality. Crucial aspects of lymphocyte function, such as activation, proliferation, cytokine production, effector functions, and apoptosis are determined by the signaling biochemical pathway initiated following ligation of the surface antigen receptor (117 ,118 ,119 ,120 ,121 ,122 ,123). Physiologically, the ligand for BCR is the relevant antigen, and for the autoreactive B cell it is the autoantigen.

We have addressed the question of possible antigen-receptor-mediated signaling aberrations in B cells of lupus patients using anti-Ig antibodies (124). Stimulation of circulating B cells from patients with SLE through their sIgM or sIgD BCR produced significantly higher fluxes of free intracytoplasmic $Ca2^{+}$ when compared to similarly induced responses of B cells from patients with other systemic rheumatic diseases, or to the responses obtained from normal B cells. The elevated $Ca2^{+}$ responses come primarily from the intracellular calcium stores. Nevertheless, the production of inositol 1,4,5-trisphosphate (the principal mediator of free

calcium release from the intracellular compartment) was only slightly elevated, raising the possibility of either a hypersensitive $Ca2^+$ release machinery or of dominant, and as yet incompletely understood, inositol triphosphate-independent pathway(s) of $Ca2^+$ release.

The earliest known BCR-mediated signaling event is the activation of protein tyrosine kinases, which results in tyrosyl phosphorylation of cellular proteins (125 ,126 ,127). The tyrosyl phosphorylation reaction has numerous substrates; only a few of these substrates have been identified. In lupus B cells, the overall level of IgM-initiated protein tyrosyl phosphorylation was significantly enhanced and correlated with the augmented BCR-mediated free calcium responses. More specifically, at least four cellular proteins with molecular sizes between 36 and 64 kD were significantly hyperphosphorylated in anti-IgM-treated lupus B cells compared to the response of B cells from normal controls (124). The aberrant BCR-mediated signaling process was detected in approximately three quarters of the studied patients and did associate with disease activity, treatment status or specific clinical manifestations. Moreover, enhanced tyrosyl protein phosphorylation was disease-specific, implying a possible intrinsic lupus B cell defect, which may have pathogenic impact. Furthermore, the increased $Ca2^+$ responses could represent a biochemical and molecular basis for the enhanced expression of CD40L on the surface of lupus B cells upon stimulation. It has been previously reported that CD40L upregulation on the cell-surface is predominantly NFAT-, and hence $Ca2^+$-dependent (128). Moreover, strikingly similar abnormalities of antigen-receptor signaling have previously been reported from the study of fresh T cells, T cell lines and autoantigen–specific T cells from patients with SLE (129 ,130), pointing towards a potentially unifying Ag-receptor-mediated signaling defect(s) in lupus lymphocytes. It has been proposed that the signaling abnormalities encountered in SLE lymphocytes may provide a biochemical and molecular background for such diverse functions as lymphocyte activation, anergy, and cell death (131).

It is interesting that BCR-initiated signaling abnormalities similar to those in human lupus were encountered in a study of experimental murine lupus. Feuerstein et al. (132) induced systemic lupus-like autoimmunity by inducing graft-versus-host disease in a background of double-transgenic sIg/sHEL murine tolerance model. Induction of autoimmunity correlated with phenotypical as well as with signal-transduction changes of B cells similar to the human disease. B cell surface expression of CD21 (part of the complement receptor 2, CR2, complex) was significantly decreased in the autoimmune state. Additionally, changes in the BCR-mediated protein tyrosyl phosphorylation pattern in B cells developed following the induction of autoimmunity. In the autoimmune but not in the tolerant state, two substrates with apparent molecular masses of 78 and 60 kD were hyperphosphorylated following BCR crosslinking. The similarity of BCR-induced signaling in patients with SLE and in this particular autoimmunity model underscore the potentially central pathogenic role of aberrantly functioning B cells in both conditions.

Estrogens and the Lupus B Lymphocyte

Estrogens are indisputably involved in the pathogenesis of SLE in humans as well as in murine models. Hormonal manipulations dramatically alter the expression of SLE in lupus-prone mice and women of child-bearing age are afflicted more commonly than men. The mechanisms involved in the contribution of estrogens to the pathogenesis of SLE are unknown.

A recent study elegantly showed that estrogens, estradiol in particular, can break B cell tolerance and cause a lupus-like disease in a previously nonautoimmune mouse that transgenically expresses the heavy chain of a pathogenic anti-DNA autoAb (133). Estradiol induced high titers of anti-DNA autoAb, and glomerulonephritis. Estradiol-induced autoimmunity in the above murine model was associated with increased expression of the antiapoptotic molecule Bcl-2 by such autoreactive B cells. The same group of investigators reported that sustained elevation of estradiol levels produced changes in B cell subpopulations because a subset of autoreactive marginal zone B cells were significantly expanded and activated (134).

Abnormalities of BCR-Signaling Regulatory Molecules

When B cells encounter antigen other B cell surface molecules are also engaged and some of them provide regulatory control over the intensity, the duration, and the fate of the biochemical signal generated by the interaction of BCR with the antigen. Engagement of signaling regulatory molecules triggers separate intracytoplasmic biochemical cascades; the net sum of these signals may result in an increased, or attenuated, or a qualitatively different BCR-initiated signal outcome (135) (Fig. 11-1).

Complement Receptors on Lupus B Cells

The most important signal-augmenting B cell surface molecule is the complement receptor type 2 (CR2). This heterooligomeric complex (the CR2 complex) is contributed by CD21 (CR2), CD19 (the signaling molecule of the complex), and CD81. Physiologically, CR2 binds iC3b, C3dg, or C3d. The cytoplasmic signal produced when antigens decorated with the aforementioned complement fractions bind to B cells, represents the sum of co-crosslinking BCR and CR2 and is several orders of magnitude higher when compared to the signal produced by the same antigens via the BCR alone (reviewed in (136)). The effects of CR2-BCR co-crosslinking in B cells from patients with SLE were directly addressed recently. A construct made of anti-IgD ab conjugated to the Epstein-Barr virus (EBV) protein gp350 was employed to stimulate fresh, unmanipulated B cells from patients with SLE and it was shown that despite a significant decrease in the expression of cell surface CR2 the free

cytoplasmic $Ca2^{+}$ responses recorded were significantly enhanced compared to normal B cells (137).

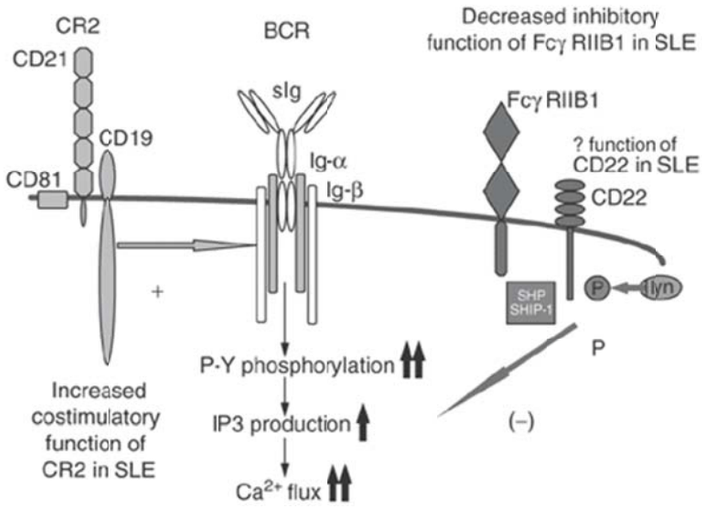

Figure 11-1. An outline of the AgR-mediated signal transduction aberrations encountered in lupus B cells, and possible contributions from B cell surface coreceptors.

BCR (sIgM or sIgD)-triggered early signal transduction events in lupus B cells leads to significantly enhanced production of tyrosine-phosphorylated cellular proteins and to increased formation of inositol triphosphate, when compared to B cell responses from either normal or disease-control individuals. These events are followed by a significantly increased free Ca2+ flux in the cytoplasm, which is contributed primarily by the intracellular calcium stores. Free Ca2+ is an important second messenger for numerous cellular functions.

B cell surface coreceptors regulate the magnitude of BCR-generated signals. Complement receptor type 2 (a complex of CD21, CD19, CD81) is a signaling enhancer. CR2 and BCR co-crosslinking significantly lowers the threshold for B cell activation. In lupus sera, ligands of CR2 (C3d, C3dg and iC3d) are commonly found in abundance.

Of the negative regulatory coreceptors, FcgRIIB1 is functionally characterized best. BCR and FcgRIIB1 co-crosslinking (i.e., when Ag is presented to B cells in the form of immune complexes) causes an early termination of BCR-initiated signals. Lyn, a tyrosine kinase phosphorylates inhibitory motifs on the intracytoplasmic domains of FcRgIIB1 and CD22 to which the tyrosine phosphatases SHP and SHIP-1 bind. This results in suppression of the signaling process initiated by BCR.

In SLE, the function of Fc receptors is defective, and abnormalities in other types of Fcg receptors have been characterized at the molecular level. The functional status of other regulatory B cell-surface molecules (CD22, CD5, CD72) in human lupus is unknown.

Previous studies have shown that expression of both CR2 and of complement receptor type 1 (CR1, an alternatively spliced product of the CR2 gene) is decreased on the surface of B cells from patients with SLE. Because this defect was also present in healthy relatives of patients with SLE it was proposed that it may be a genetically determined alteration (138). Decreased expression of B cell surface complement receptors is also seen on MRL/lpr B cells during the development of autoimmunity (139). When the expression of CR1/CR2 was partially or totally disrupted in the MRL/lpr background, survival and development of nephritis were not altered. Decreased expression of CR1/CR2 correlated with decreased levels of total IgG3 and IgG3 rheumatoid factors and significantly higher levels of IgA deposition in the glomeruli, suggesting that CR1/CR2 expression plays a role in autoAb production and/or clearance (140). According to a more recent study decreased CR2 expression levels correlate well with increased disease activity (141).

CR2 also represents the B cell surface receptor for EBV. A recent epidemiologic study disclosed that young patients with recent onset SLE had evidence of EBV infection more commonly than their healthy counterparts (99% vs. 70%) (142). A previous in vitro study reported that in circulating lymphocytes, EBV infection of lupus B cells cannot be contained by lupus T cells (143). This potentially defective T cell–mediated EBV control in patients with SLE was also reported in a recent study (144). Blood cells infected with EBV were detected in abnormally high frequencies in patients with SLE along with aberrant EBV-gene expression and correlated with disease activity (145). Additionally, EBV causes hyperactivation of B cells in SLE and decreases their apoptotic death because it induces overexpression of the anti-apoptotic molecule bcl-2 in the cytoplasm (146).

CR2 deficiency on the surface of lupus B cells does not help to explain the pathophysiology of the overactive lupus B cell. Nevertheless, a novel concept on CR2 (and CR1) function supported by recently produced experimental data proposes that complement receptor-mediated signaling maintains B cell immune tolerance to self antigens (147). This view may provide a molecular background to understand how lupus B cells, expressing low amounts of membrane complement receptors (a result of genetic or acquired defects), display deranged immune self-recognition. It has been proposed that complement participates in the early stages of B cell negative selection. According to this proposal, circulating natural autoantibodies identify highly conserved self-antigens and activate the classical pathway of complement. Such complexes bind to the BCR and complement receptors of autoreactive immature B cells arising daily in the bone marrow; this process represents an efficient means to delete these highly autoreactive B cells. In the case of early complement factor (or complement receptor) deficiencies, autoreactive immature B cells are not efficiently removed, and lupus-like autoimmunity develops (148). In support of the above it was recently reported that B cells from mice that partially or totally lack C4 produce anti-DNA autoAb. It is of interest that production of autoAb can be triggered in vitro months earlier, before their detection in the sera of the experimental animals (149). Finally, complement activation products can trigger B cell signaling events. The membrane-attack complex (C5b-9) found in the serum at sub-lytic concentrations may induce activation of the B cell kinases Ras, Raf-1, and extracellular regulated kinase (ERK) 1 in a B cell line via a mechanism involving G proteins (150). Circulating sub-lytic C5b-9 complex concentrations are reportedly increased in the serum of patients with SLE (151).

Stimulatory B Cell Surface Molecules

B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) represent two novel members of the TNF-superfamily (152 ,153). They are distinct but closely related and they can stimulate B cells both in vitro and in vivo (152 ,154), APRIL being a weaker B cell stimulator compared to BLyS. Apart from similarities in their structure and function APRIL and BLyS also share common cell-surface receptors, i.e., the B cell surface receptors TACI and BCMA. TACI is an important inhibitory receptor found on the surface of B cells; TACI(-/-) murine models develop autoimmune glomerulonephritis and autoantibodies and fatal lymphoproliferation (155). So far, while the function of BCMA has been characterized as redundant (156), the receptor BAFFR is thought to be the principal B cell stimulating receptor that mediates the action of BLyS (157 ,158 ,159). Therefore, BlyS can bind to BCMA, TACI, and BAFF, whereas APRIL can bind only BCMA and TACI. Much attention has been focused on BLyS because it has clearly been shown that BLyS protein is vital and indispensable for normal B cell development. Use of soluble fusion proteins composed of BLyS receptors (BCMA and TACI) and the Fc fragment of IgG resulted in inhibition of both T cell dependent and T cell independent antibody responses (154 ,160 ,161). BLyS-knockout mice are B cell lymphopenic and have reduced antibody responses to T cell dependent and independent immune responses (162 ,163). Genetically manipulated mice expressing the TACI-Ig construct display a similar phenotype (162 ,164).

Administration of recombinant BLyS to mice induces polyclonal hypergammaglobulinemia (152). Lupus-prone mice like the NZB × NZW (F1) and the MRL-*lpr/lpr* mice display increased serum levels of BLyS during the onset and progression of their lupus-like disease, and genetically manipulated mice overexpressing BLyS develop lupus-like autoimmunity (165). Some patients with SLE have increased levels of BLyS protein in their sera, and the titers of BLyS correlated well with the titers of anti-dsDNA autoAb but not with disease activity (166 ,167). The biologic activity of circulating BLyS in patients with SLE was higher compared to normal controls (167). Nevertheless, there is a heterogeneity in BLyS levels among different lupus patients, and there are patients with SLE that have normal or even lower than normal levels of circulating serum BLyS (166).

Based on the central role of BLyS in the B cell immune response, different groups evaluated the effects of BLyS blockade in experimental lupus. Treatment of NZB × NZW (F1) and MRL-*lpr/lpr* lupus-prone mice with the soluble construct TACI-Ig delayed disease progression and improved survival (165). Treatment with adenovirally encoded TACI-Ig diminished polyclonal hypergammaglobulinemia and autoAb production in lupus-prone mice (168). Anti-BlyS is currently in phase 2 clinical trials in patients with SLE.

B Cell Surface Receptors That Provide Negative Regulation

There are several B cell surface signaling inhibitory receptors (169). Among them, the functions of CD5, CD22, and FcγRIIB1 are best understood. The ligand for CD5 was recently shown to be CD72 (170). The role of $CD5^+$ B cells in SLE and in experimental animal lupus is discussed above. The role of coreceptor CD22 as a signaling inhibitory molecule was clarified in CD22-knockout mice. Young CD22-knockout mice display autoimmunity, increased BCR-initiated cytoplasmic calcium responses, hypergammaglobulinemia, and circulating IgM autoAb (171). Adult CD22-knockout mice display glomerulonephritis, circulating IgG anti-dsDNA and anticardiolipin autoAb (172). This is an interesting model of systemic lupus-like autoimmunity, because it is monogenic, and because one B cell molecular defect created an autoAb response that included isotype switching and affinity maturation of the autoantibody, features reminiscent of a T cell–dependent immune response. Nevertheless, CD22 expression on the surface of B cells from patients with either active or inactive SLE is similar to that of normal controls (173 ,174). Expression of CD22 ligand on the surface of B cells has not been addressed in humans but has been studied in lupus-prone mice. Different lupus-prone strains were analyzed and it was reported that B–cell expression of CD22 ligand increases in parallel with the progression and severity of the autoimmune disease, but it could not be concluded if this was a primary or secondary effect (175).

Another B cell surface inhibitory receptor is the one for the Fc fraction of IgG type IIB1 (FcγRIIB1, CD32). When antigen bound to IgG is presented to the BCR, then BCR and FcγRIIB1 are co-crosslinked resulting in a net signal of smaller magnitude than the signal generated by antigen alone (176 ,177). The cytoplasmic free $Ca2^+$ response is of shorter duration and the resulting B cell response is incomplete activation and proliferation. Previous studies have reported that the system of receptors for the Fc fraction of IgG is overall malfunctioning in SLE resulting perhaps in the production of excess antibodies and the accumulation of immune-complexes (178). Genetically manipulated mice having B cell surface FcγRIIB1 deficiency manifested hypergammaglobulinemia (179). In NZB as well as in (NZB × NZW) F1 mice, the B cell surface expression of FcγRIIB1 was reported to be decreased in follicular germinal center B cells of adult (autoimmune) mice, but not in the circulating B cell pool. The NZB allele for FcγRIIB1 was found to have two deletion sites that included transcription factor-binding sites, increasing thus the likelihood that such deletions have a functional impact (180). This defect was correlated with IgG hypergammaglobulinemia. Additionally, a single-nucleotide polymorphism (a G-C substitution at position -343) in the human *FCGR2B* promoter was recently identified; homozygosity for this polymorphism was overrepresented in European-American patients with SLE. The -343

G/C polymorphism results in decreased binding of the AP-1 transcription factor to the FCGR2B promoter and hence to decreased transcription, providing thus a molecular explanation for the dysregulation of FcγRIIB1 in some patients with SLE (181).

The role of FcγRIIB1 as a suppressor of autoimmunity was demonstrated when the Fas(lpr/lpr) phenotype was crossed with FcγRIIB1-sufficient and FcγRIIB1-deficient B6 mice. Fas mutations were not sufficient to cause autoimmunity in wild-type B6 mice but in FcγRIIB1-/- animals an aggressive autoimmune lupus-like systemic disease developed characterized by multiple autoAb, glomerulonephritis, arthritis, and a markedly shortened life span (182). More recently data stemming from the study of FcγRIIB1 deficient IgG anti-DNA autoAb producing mice suggested a novel role for FcγRIIB1 as a potential distal peripheral checkpoint limiting the accumulation of autoAb-producing plasma cells (183). In B cells from patients with SLE the surface expression of FcγRIIB1 was similar to controls but the inhibitory function of this molecule was impaired. Co-crosslinking the BCR and FcγRIIB1 resulted in a significantly defective suppression of the free cytoplasmic $Ca2^{+}$ response in circulating B cells from patients with SLE compared to B cells from disease-control or healthy individuals (184).

The coreceptors CD22 and FcγRIIB1 bear a signaling inhibitory domain in their cytoplasmic tail called immunoreceptor tyrosine-based inhibitory motif (ITIM) (185 ,186 ,187). ITIM become functional when their tyrosyl residues become phosphorylated under the influence of protein tyrosine kinases (188). Tyrosyl phosphorylated ITIMs become the docking and activating sites for SH2-domain containing protein tyrosine phosphatases, particularly SHP-1 and SHIP (189 ,190 ,191). It is interesting that SHP-1 is absent or dysfunctional in another autoimmunity model, the motheaten and the viable motheaten mouse respectively (192).

The phosphorylation of ITIM tyrosyl residues is accomplished by protein tyrosine kinases. The ITIM lying in the cytoplasmic tail of FcγRIIB1 and CD22 coreceptor undergoes tyrosyl phosphorylation by the src-family kinase lyn (193). In the absence of lyn the CD22-initiated signaling inhibitory pathway is not triggered, despite adequate amounts of B cell surface CD22 expression (194). Until recently, lyn was considered as an integral part of the BCR-initiated B cell activation machinery (195 ,196). Yet, knockout experimental models disclosed that lyn is redundant in its stimulatory mediator role. In lyn-/- animals immature B cells developed normally but mature B cells were decreased. Rather unexpectedly it was shown that BCR-initiated signaling events were not only propagated but in fact they were enhanced. The lyn-/- mice develop autoimmunity with features quite reminiscent of lupus; they have hypergammaglobulinemia, increased sensitivity to IL-4, and develop autoAb and glomerulonephritis (197 ,198 ,199 ,200).

Lyn is crucial for the phosphorylation of CD22 and FcγRIIB1, and phosphorylation of CD22 and FcγRIIB1 are crucial for the activation of SHP-1 and SHIP respectively. Based on recent experimental animal studies, it appears that lyn, CD22 and SHP-1 are present in limiting quantities in B cells. A reduction in the quantity of each of the above mediators promotes a state of B cell hyperreactivity that eventually correlates with the development of autoimmunity (201).

Furthermore, it also appears that the signaling inhibitory coreceptor CD22 and the signaling enhancer CD19 molecule (part of the CR2-complex) are interrelated and form a regulatory feedback loop that tunes BCR-initiated signaling (202). CD19-deficient B cells are hyporesponsive but B cells expressing even slightly increased CD19 levels are hyperresponsive and develop autoimmunity (203 ,204). CD19 amplifies src-family protein tyrosine kinase activity and has been shown to allow for optimal CD22 function (205). Similarly, CD22 expression negatively regulates CD19-mediated functions. There are several pieces of experimental data suggesting that CD19 amplifies the activity of lyn that in turn phosphorylates CD22. CD22 ITIM phosphorylation recruits SHP-1 that negatively regulates the activity of CD19 by tyrosyl dephosphorylating it (203). A study recently investigated the expression of CD22 and lyn in B cells from patients with SLE (206). Preliminary data disclose that while CD22 expression is comparable to control B cells, the content of lyn in lupus B cells is significantly decreased in two thirds of patients with SLE examined (207). Although the function of the CD22/lyn signaling inhibitor was not addressed, it is likely that decreased lyn in SLE B cells contributes to B cell overactivity by deficiently regulating the BCR-initiating response.

Summary

The single most characteristic abnormality of immune cells encountered in both patients with SLE and in animal lupus models is B cell overactivity (Fig. 11-2). The pathogenic contribution of factors exogenous to the B cell itself, be it an aberrantly functioning T cell compartment or the local cytokine environment are well established. Recent data support a more central pathogenic role for the lupus B cell itself. It is revealed that lupus-like autoimmunity can ensue with either minimal or no contribution from T cells. Genetic as well as functional studies support a role for lupus B cell as an independent contributor to the appearance of the disease, apart from the well-known contribution of potentially harmful autoAb production. Studies unraveling the biochemistry of lupus B cell function reveal that there are disease-specific signal transduction aberrations that may represent a common background for other disturbed effector functions. The contribution of signal transduction regulators is appreciated using well-characterized genetically manipulated experimental models. Improving our understanding of the lupus B cell physiology and pathophysiology will ultimately improve our understanding of the disease and may provide us with useful tools to deal with SLE more rationally.

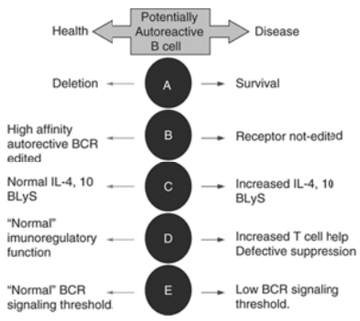

Figure 11-2. Processes that may lead to B cell-mediated autoimmune response. Any of the indicated processes, A through E, alone or in any possible combination can lead to B cell-mediated autoimmune response and autoimmune disease.

A. B cells with "proper" affinity for autoantigen are expected to be deleted in the bone marrow. Inability to delete autoreactive cells may lead to expansion of certain B cell subsets as recorded in the peripheral blood of lupus patients. Genetic defects of molecules involved in B cell apoptosis can limit cell deletion.

B. Once a BCR has been selected it can be edited to adjust for autoreactivity. Escaping autoreactive BCR can be edited to lower affinity or autoantigen. Although this process has been shown to be important in the regulation of the BCR repertoire, it has not been shown operative in human SLE.

C. Genetic, hormonal or environmental factors can cause increased production of cytokines that promote B cell function and in a proper background the expansion of autoreactive cells.

D. Increased T cell help and possibly diminished T regulatory function can promote excessive autoimmune B cell function.

E. Genetic, hormonal and possibly environmental factors can lower the excitation threshold for B cells through several distinct processes. First, gain of function or loss of function changes in the expression of enhancing and repressive signaling molecules can lead to increased B cell signaling responses as recorded in SLE patients. Defects (numerical or functional) in the expression of coreceptors (see Fig. 11-1) can alter the BCR excitation threshold.

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Chapter 12

Abnormalities in Immune Complex Clearance and Fcγ Receptor Function

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Systemic lupus erythematosus (SLE), the prototype human disease mediated by immune complexes, is characterized by circulating antigen/antibody complexes that may be removed by the mononuclear phagocyte system or deposited in tissues. The fate of circulating immune complexes depends on the lattice of the immune complexes (i.e., number of antigens and antibody molecules in a given complex), the nature of the antigen and antibodies composing the immune complexes, and the status of the mononuclear phagocyte system. The efficiency of mononuclear phagocyte system immune complex clearance depends on the function of Fcγ receptors, receptors recognizing the Fc region of immunoglobulin, and the complement receptors. In SLE, inadequate clearance results in tissue immune complex deposition, detected by immunofluorescence and electron microscopy, that initiates release of inflammatory mediators and influx of inflammatory cells. If sustained, this leads to tissue damage with resultant, clinically apparent disease, such as glomerulonephritis. Through in vivo and in vitro studies of patients with SLE, there clearly are both FcγR-dependent and complement-dependent mononuclear phagocyte dysfunction in SLE that have inherited and acquired components. This chapter reviews the role of the mononuclear phagocyte system in immune complex clearance, describes abnormalities in the mononuclear phagocyte function in SLE, and discusses mononuclear phagocyte system Fcγ receptor dysfunction as a mechanism for abnormal immune complex clearance in SLE.

The Role of the Mononuclear Phagocyte System in the Clearance of Immune Complexes

Early studies of the blood clearance of bacteria in mice, rabbits, and guinea pigs demonstrated that the mononuclear phagocyte system (previously known as the reticuloendothelial system) performed this function for opsonized particles. Infused bacteria were internalized by hepatic and splenic phagocytes (1). The rate of clearance of bacteria from the blood and the site of their clearance depended on the level of antibodies to the bacteria in the serum of the animal. Rapidly cleared, well-opsonized bacteria were principally phagocytosed in the liver, while the more slowly cleared, less efficiently internalized bacteria (and presumably less opsonized) were removed by splenic phagocytes. These observations are remarkable for their similarity to the models of immune complex clearance in animals and humans that are described later.

The role of the mononuclear phagocyte system in the clearance of soluble immune complexes infused into the circulation has been defined in several experimental animal models. In mice and rabbits a major proportion of infused immune complexes formed with rabbit antibodies is taken up by the liver, indicating that the mononuclear phagocyte system serves an important role as a site for complex removal (2 ,3). This system may be saturated with increasing amounts of infused immune complexes, resulting in glomerular deposition of immune complexes (4). These early studies suggested that modulation of mononuclear phagocyte system function regulates the localization of immune complexes.

Several lines of evidence support this model and demonstrate that defective mononuclear phagocyte system clearance of immune complexes may be important in the development of immune complex diseases, especially glomerulonephritis. Increased glomerular deposition of immune complexes is found when the clearance rates of infused immune complexes are decreased by blockade of the mononuclear phagocyte system with colloidal carbon (5), by cortisone treatment (6), or by reduction and alkylation of antibodies (7 ,8). In mouse strains with intrinsically lower clearance rates, there is a high degree of immune complex glomerular deposition (9); in contrast, decreased deposition of immune complexes in the kidney is found when mononuclear phagocyte system clearance is enhanced by pretreatment with *Corynebacterium parvum* (10) or zymosan (11).

While impaired immune complex clearance leads to increased tissue deposition, the absence of activating FcγR on phagocytes prevents an inflammatory response at the sites of immune complex deposition. Mice with targeted deletions of stimulatory FcγR are protected from fatal antigen-antibody Arthus reactions and immune complex-mediated glomerulonephritis. In contrast, mice lacking inhibitory FcγR have exaggerated responses to immune complexes (12 ,13 ,14).

Animal models of endogenous immune complex deposition also support the relationship between depressed mononuclear phagocyte system clearance and the genesis of glomerulonephritis. In chronic serum sickness, there is decreased clearance of aggregated albumin (15) and aggregated human immunoglobulin G (IgG) (16). Decreased clearance of heat-aggregated IgG in murine nephritis (associated with lymphocytic choriomeningitis virus infection) (17) and of polyvinyl pyrrolidine in New Zealand black/white (NZB/W) mice (18) has been observed, although some studies of endogenous immune complex-mediated disease have not found dysfunction of the mononuclear phagocyte system. Studies of Heyman nephritis (19) and NZB/W nephritis (20) have concluded that mononuclear phagocyte system function is either normal or supranormal. These results are not in conflict, however; rather, they highlight the importance of the mononuclear phagocyte system probe, the site of clearance, and the timing of the study in relation to the genesis of disease (discussed later).

The principle to be derived from these animal models of immune complex disease, whether from infused immune complexes or endogenous disease, is that immune complex deposition is influenced by the efficiency of mononuclear phagocyte system clearance. Specifically, impairment of mononuclear phagocyte system clearance is associated with tissue deposition of immune complexes and the potential for local organ damage.

Mechanisms of Immune Complex Clearance

A number of factors govern the physical characteristics of immune complexes and, hence their biologic properties (Table 12-1). These include the nature of the antibody in the complex, the nature of the antigen, and the antigen-antibody interaction. Antigen and antibodies in the circulation may rapidly form immune complexes, but the immunochemical properties of these circulating immune complexes determine their ultimate fate, either removal by the mononuclear phagocyte system or deposition in tissues. The potential of immune complexes to interact with FcγRs, to fix complement, and to react with complement receptors influences their rate of clearance. Immune complexes without complement will be cleared primarily by FcγRs on fixed tissue macrophages. Complexes that are opsonized with sufficient complement may bind to the receptor for C3b on circulating erythrocytes and subsequently be removed by FcγRs and complement receptors. Thus, two classes of receptors, the FcγRs on phagocytes and the complement receptors on both erythrocytes and phagocytes, participate in the clearance of immune complexes (Fig. 12-1).

Table 12-1: Factors Influencing the Characteristics of Immune Complexes

ANTIGEN
Availability
Valence, size
Epitope density and distribution
Tissue tropism/charge
ANTIBODY
Quantity
Class, subclass
Capacity to fix complement
Binding avidity
Charge and distribution
ANTIGEN-ANTIBODY INTERACTION
Molar ratio

Complement Mechanisms: Immune Adherence and Erythrocyte CR1 System

Complement component 3 and the receptor for C3b on erythrocytes are important in processing and transporting large immune complexes (21) (see Chapter 13). Incorporation of complement components, C3b in particular, modifies the solubility of large immune complexes (22 ,23) and mediates the binding of immune complexes to human and other primate erythrocytes. Although both the liver and spleen are the major sites of immune complex uptake, erythrocytes in primates (21 ,24), and platelets in rodents (25 ,26) are important in clearing/processing immune complexes from the circulation. It has long been known that large complement-opsonized immune complexes bind to human erythrocytes (27). Termed immune adherence, this reaction has recently been shown to participate in the handling of nascent circulating immune complexes in primates (28).

Human erythrocytes express complement receptor type 1 (CR1), which permits binding of complement-fixing immune complexes. CR1 on erythrocytes can be conceptualized as having three main functions, which are not mutually exclusive: buffering, transporting, and processing (Fig. 12-1). The role of immune complex buffer has been suggested because erythrocyte-bound immune complexes are unavailable for tissue deposition, whereas nonbound complexes can deposit in the tissues. Bound immune complexes are transported to the liver or spleen where fixed tissue phagocyte FcγRs and complement receptors strip the immune complexes from the erythrocytes, which then return to the circulation to continue this process, thus performing the transporting function. Finally, CR1 promotes degradation of captured C3b on immune complexes, thereby modifying their subsequent handling.

The human CR1 (the complement receptor for C3b/C4b and, to a lesser degree, iC3b) is a single-chain, intrinsic membrane glycoprotein expressed on several different cells, including erythrocytes, granulocytes, monocytes, and macrophages (see Chapter 13). There are four codominantly expressed alleles of CR1, with molecular weights of 220,000, 250,000, 190,000, and 280,000 d (29 ,30 ,31 ,32). This structural polymorphism reflects differences in the number of long homologous repeat units comprising the receptors (33). Inherited and acquired differences in the numeric expression of CR1 on erythrocytes also have been described and associated with SLE (34 ,35 ,36 ,37 ,38).

Two alleles with codominant expression determine erythrocyte CR1 number in healthy individuals (37 ,39). Although the CR1 number expressed on erythrocytes is low compared with that on leukocytes, approximately 90% of total circulating CR1 is on erythrocytes, because there are far more erythrocytes than leukocytes in the circulation (40 ,41).

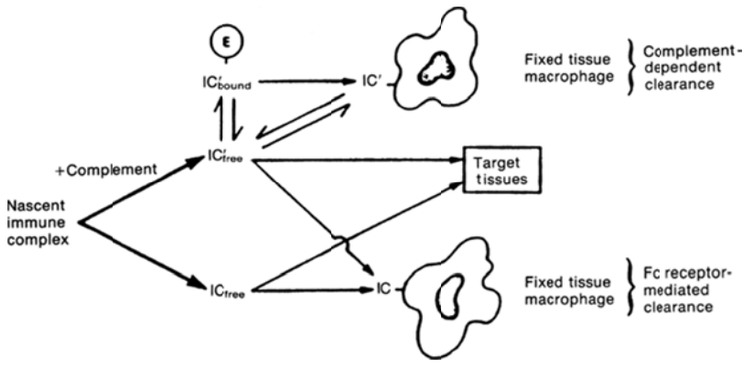

Figure 12-1. Framework for immune complex handling. Nascent immune complexes (ICs) that fix complement efficiently are rapidly bound by erythrocytes (E). ICs containing complement may cycle between E-bound and unbound; they usually are rapidly taken up in the liver. Unbound complexes also may deposit in the tissues, and with impaired complement-dependent uptake, they may be taken up by Fc receptor-dependent mechanisms. ICs that do not bind complement are either taken up by Fc receptor-dependent mechanisms or deposited in tissues. (Reprinted with permission from Elsevier from Kimberly RP. Immune complexes in rheumatic diseases. In: Pisetsky DS, Snyderman R, eds. Immunology of the Rheumatic Diseases, vol. 13. *Rheum Dis Clin North Am* 1987:583–596.)

The binding of immune complexes to CR1 occurs rapidly in vivo, and it represents multivalent binding between multiple C3b molecules on the complex and clusters of CR1 on erythrocytes (28 ,42 ,43 ,44 ,45). In vivo studies have demonstrated that immune complexes preferentially bind to circulating erythrocytes that express multiple CR1 clusters, and that the capacity of each erythrocyte for binding correlates with the density of cell surface CR1. Because CR1 on erythrocytes tends to cluster more than that on resting neutrophils, most immune complexes that are bound to circulating cells are bound to erythrocytes (22 ,24 ,40 ,41 ,46 ,47 ,48 ,49 ,50). A reduction in the number of functional CR1 limits the capacity of erythrocytes to transport and buffer immune complexes, and in vivo studies have demonstrated that repeated administration of antigens in immunized humans and primates with immune complex formation results in a decrease in erythrocyte CR1 levels (46 ,51). Studies with primates have suggested that circulating immune complexes that are not bound to erythrocytes are more easily trapped in the microvasculature and can be recovered in the lungs and kidneys (50 ,52). Taken together, these findings have obvious implications for immune complex-mediated diseases.

The efficiency of immune complex binding to erythrocytes via CR1 relates to the nature of the immune complex, particularly the ability to activate complement and capture C3b, the spatial organization of the captured C3b, and the final size of the complex (53). Several models have been used to analyze the characteristics of immune complexes interacting with the erythrocyte CR1 system, including DNA/human anti-DNA, bovine serum albumin (BSA)/anti-BSA, tetanus toxoid/human antitetanus toxoid, and hepatitis B surface antigen/human antihepatitis B surface antigen (24 ,28 ,48 ,50 ,54). In each system, large immune complexes bind to erythrocytes better than do small immune complexes. The antigen and antibody also influence this reaction, because the capacity to fix complement varies with antibody class and certain antigens alone may capture C3b. Erythrocyte CR1 immune complex binding is avid but reversible, and the rate of dissociation also varies according the particular immune complex, which dictates the nature of C3b capture (43 ,55).

The erythrocyte CR1 system also may have a second physiologic function: providing a processing mechanism for immune complexes (56). In addition to being a carrier for opsonized immune complexes, CR1 has a potent inhibitory function in the complement cascade, which may enhance clearance. It participates in the inactivation of C3b and may alter the size of complexes, thus affecting their subsequent handling. Specifically, CR1 is a cofactor for factor I in the cleavage of C3b to iC3b and then to C3dg (57 ,58). Therefore, the binding of immune complexes containing C3b to erythrocyte CR1 facilitates proteolytic cleavage of the C3b to iC3b and C3dg, which do not bind to CR1. This reaction is the basis for the degradation of complement on immune complexes with their subsequent release from the receptor (59), and its rate varies with the physicochemical properties of the individual complexes (55). If the immune complex can again activate complement and bind C3b, it can rebind to CR1 (60). Although

repeated cycles of binding and release are likely, these immune complexes are not constantly bound to erythrocytes and thus are available for either deposition or (enhanced) removal by the mononuclear phagocyte system. The fraction of immune complexes in whole blood that is erythrocyte bound depends on several dynamic processes: complement fixation and C3b capture, erythrocyte binding, and C3b degradation and immune complex release.

Fcγ Receptor Mechanisms

Immune complexes are removed from the circulation by the mononuclear phagocyte system of the liver and spleen through engagement of FcγRs and complement receptors. The interaction of immune complexes with the phagocyte involves a qualitatively different process than that with erythrocytes (47). The relative contribution of each receptor system depends on the immunochemical properties of the complex. The liver, which is much larger than the spleen, is the principal site for the uptake of immune complexes (52 ,61 ,62); however, immune complexes that escape clearance by hepatic macrophages, which may be smaller and of lower valence, are taken up by the spleen (61). The role of FcγRs in immune complex clearance of both soluble and particulate immune complexes is shown by studies wherein blockade of FcγRs by an infusion of aggregated IgG into the portal venous system (40) or of antibodies against FcγRs (47) suppresses uptake of these immune complexes (Fig. 12-2). Supporting the pivotal role of FcγRs in handling certain immune complexes, studies of complement depletion show no effect on the efficiency of uptake of immune complexes by the liver or spleen and actually show an acceleration in the rate of removal of complexes from the circulation, presumably resulting from trapping in the microvasculature (50).

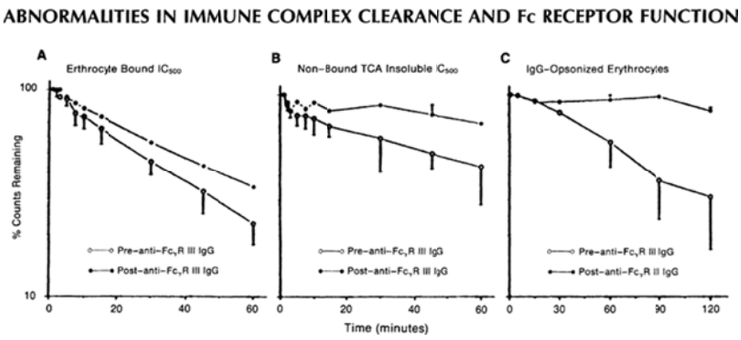

Figure 12-2. Effect of anti-FcγRIII monoclonal antibody (MAb) on the handling of soluble immune complex (IC). The effects of anti-FcγRIII MAb infusions on the handling of several different radiolabeled model IC probes in chimpanzees are presented with data expressed as the percentage counts remaining relative to the counts infused. A: Following intravenous infusion of soluble radiolabeled IC, clearance of erythrocyte (E)-bound IC was measured and found to be slowed by treatment with anti-FcγRIII MAb IgG. B: After intravenous infusion of soluble IC, clearance of non-E-bound IC was slowed more by anti-FcγRIII MAb IgG. C: Clearance of IgG-opsonized E was most markedly slowed by anti-FcγRIII infusions. (Reprinted with permission from Kimberly RP, Edberg JC, Merriam LT, et al. In vivo handling of soluble complement fixing Ab/dsDNA immune complexes in chimpanzees. *J Clin Invest* 1989;84: 962–970.)

FcγRs appear to play a key role in the transfer and retention of immune complexes by mononuclear phagocytes. Studies of DNA/anti-DNA complexes that are bound to radiolabeled erythrocytes and injected into chimpanzees show that while immune complexes are removed by the mononuclear phagocyte system, the erythrocytes are not sequestered; rather, they are stripped of immune complexes and promptly recirculated (24). Although the mechanism of this stripping is not well defined, the involvement of complement proteases has been implicated (63). In this model of immune complex clearance, infusion of erythrocyte-bound DNA/anti-DNA complexes after treatment with anti-FcγR monoclonal antibody results in a significant amount of nonerythrocyte-bound circulating immune complexes, documenting the participation of FcγRs in the retention of immune complexes by phagocytes (Fig. 12-2B) (47).

In addition to stripping erythrocyte-bound complexes, FcγRs as well as CR3/CR4 are responsible for the clearance of those complexes that are unable to bind to erythrocyte CR1 because of inadequate C3b capture or degradation of C3b. This interpretation is supported by experiments in primates that were treated with anti-FcγR monoclonal antibodies and showed impaired clearance of infused immune complexes, which was most pronounced in the fraction of complexes that did not bind to erythrocytes (47). It has been shown that immune adherence is not a prerequisite for the efficient handling of immune complexes by the mononuclear phagocyte

system (51), but immune complexes that do not fix complement or that fix complement poorly cannot be cleared if FcγR function is impaired (Fig. 12-1).

Abnormal Immune Complex Clearance in SLE

Human Models of Immune Complex Clearance

Probes that have been used to assess the efficiency of immune complex clearance in humans are: (a) autologous erythrocytes sensitized with IgG antibodies that are directed against the D antigen of the Rh system, (b) preformed immune complexes or aggregated IgG, and (c) antigen infused into passively immunized subjects. Because each of these probes has distinct immunochemical properties, they interact differently with the complement and FcγR systems, as expected. Thus, the results of in vivo studies comparing immune clearance in patients with SLE and in healthy individuals vary depending on the probe used.

Analysis of the Clearance of IgG-Sensitized Autologous Erythrocytes

The technique introduced by Frank et al. (64) to measure mononuclear phagocyte system function employs autologous 51Cr-radiolabeled erythrocytes that are sensitized with IgG anti-(Rh)D antibodies and injected into study subjects, and clearance or removal of these cells from the circulation is determined by serial bleeding. External surface counting of sensitized radiolabeled erythrocytes shows initial rapid sequestration in the liver, followed by splenic accumulation of most of the injected cells. The semilogarithmic plot of mean data for the clearance of sensitized cells in normal control subjects is curvilinear, with a rapid initial loss of radiolabeled cells followed by a slower, sustained loss of radioactivity (Fig. 12-3) (65 ,66 ,67).

Although originally conceptualized as a measure of FcγR capacity, kinetic analysis of in vivo clearance studies and in vitro studies with IgG anti-(Rh)D-coated erythrocytes suggests that complement also plays a role in clearance of this probe. Further support comes from studies of C4-deficient patients, who show delayed clearance relative to healthy individuals (68). A proposed model to describe the series of steps in handling of IgG anti-(Rh)D-sensitized erythrocytes is as follows: Circulating cells initially sequestered by a complement-dependent process are deactivated and released back into the circulation or are phagocytosed. Released cells are sequestered and phagocytosed by an FcγR-mediated process. Circulating cells also may be directly sequestered and phagocytosed by FcγRs (66 ,67 ,68).

The role of complement in the clearance of anti-(Rh)D-sensitized erythrocytes is a function of the level of antibody sensitization (69). Erythrocytes that are prepared with a low density of surface anti-(Rh)D are cleared primarily by splenic FcγRs, while at higher-density sensitizations, hepatic complement receptor-mediated clearance becomes increasingly important. This observation is highlighted in studies of splenectomized patients, which show that the clearance of erythrocytes sensitized with low levels of anti-(Rh)D antibody is more markedly prolonged than that of more densely opsonized erythrocytes, which fix complement and may be cleared by hepatic C3b receptors. Because the density of opsonization determines the relative contribution of FcγRs and complement mechanisms in clearance, the results of in vivo clearance studies in patients with SLE must be interpreted in the context of the sensitization of the probe.

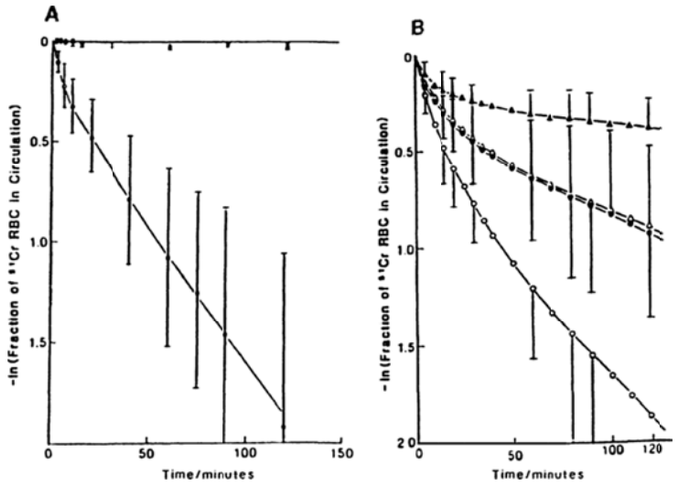

Figure 12-3. A, Survival of 51Cr-labeled autologous erythrocytes in normal controls. Data are shown for unsensitized erythrocytes in six normal controls (upper curve) and anti-Rh(D)-sensitized erythrocytes in 49 normal controls (lower curve). **B,** Survival of 51Cr-labeled autologous anti-Rh(D)-sensitized erythrocytes in 32 patients with SLE: comparison between disease active subgroups. o-o inactive/nonrenal (*n* = 5); ●-● active/nonrenal (n = 7); •-• inactive/renal (*n* = 12);▲-▲ active/renal (*n* = 8). (Reprinted with permission from Kimberly RP, Meryhew NL, Runquist OA. Mononuclear phagocyte function in SLE. I. Bipartite Fc- and complement-dependent dysfunction. *J Immunol* 1986;137:91–96.)

In Vivo Studies of IgG-Sensitized Autologous Erythrocytes in SLE

Abnormal mononuclear phagocyte system function in patients with SLE has been demonstrated in several studies performed with IgG anti-(Rh)D-sensitized erythrocytes (64 ,70 ,71 ,72 ,73). Clearance half-times for radiolabeled autologous IgG-sensitized erythrocytes were prolonged in these patients compared with normal individuals and were longer in patients with renal disease than in those without renal disease (Figs. 12-3 and 12-4). In these studies, the prolongation of clearance half-time of erythrocytes (low-density sensitization) was attributed to impaired splenic FcγR function. At this low level of sensitization, hepatic complement-mediated clearance is negligible, and the rate of clearance indeed reflects the efficiency and capacity of FcγRs. The abnormality in clearance is

receptor specific, because clearance of heat-damaged erythrocytes and aggregated albumin was not prolonged in these patients with SLE (64 ,74).

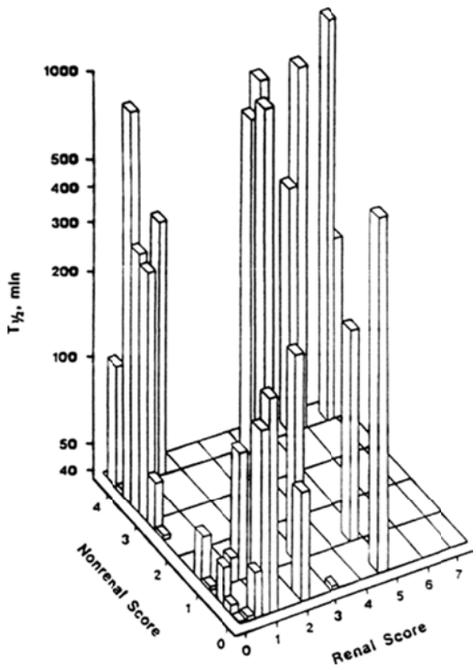

Figure 12-4. Relationship of clinical activity and FcγR-mediated mononuclear phagocyte system dysfunction. Clinical activity was assessed in terms of both renal and nonrenal manifestations. Longer (taller) clearance half-time values represent greater degrees of dysfunction. Patients with active renal and nonrenal disease showed the greatest degree of FcγR-mediated clearance impairment. (Reprinted with permission from Kimberly RP, Salmon JE, Edberg JC, et al. The role of Fc receptors in mononuclear phagocyte system function. *Clin Exp Rheum* 1989;7(Suppl):S130–S138.)

In a contrasting study, clearance of more heavily sensitized erythrocytes in patients with SLE was similar to that of normal controls (75). At this higher level of sensitization, clearance half-times are primarily a measure of hepatic complement receptor function rather than splenic FcγR function (69). Because complement-dependent clearance often is normal in patients with SLE but without renal involvement (discussed later), the overall clearance of heavy opsonized erythrocytes may be normal despite marked FcγR dysfunction.

When clinical activity in patients with SLE was assessed, there was a significant but independent association between impaired FcγR clearance and the level of both renal and nonrenal disease activity (72). Increased activity along either parameter was associated with more impaired clearance. As shown in Fig. 12-4 , patients with active renal and nonrenal disease are most likely to have the highest degree of clearance dysfunction. Longitudinal studies in patients with SLE showed that mononuclear phagocyte system function changed concordantly with changes in clinical status, indicating that clearance dysfunction is dynamic and closely related to disease activity (70 ,71).

Semilogarithmic plots of the mean data for clearance in patients with SLE, grouped according to disease activity and the presence or absence of renal involvement, show differences in slope and duration of the initial, rapid loss of radiolabeled cells from the circulation (predominantly complement-dependent clearance) and in the slope of the slow, sustained clearance reaction (predominantly FcγR-mediated clearance) (Fig. 12-3B). Rate constants governing the FcγR- and complement-dependent steps of the clearance process were derived from kinetic analysis of these curvilinear clearance data. Rate constants were evaluated for four steps: (a) complement-dependent sequestration, (b) C3b deactivation and release, (c) complement-mediated phagocytosis, and (d) FcγR-mediated sequestration and phagocytosis. Such analysis of the studies of patients with SLE grouped by disease activity revealed both FcγR-and complement-dependent dysfunction (67). Impaired FcγR function was evident in all patients except those with neither renal involvement nor any other manifestation of activity. Complement-mediated phagocytic dysfunction was seen only in patients with renal disease. These data suggest that altered complement-mediated phagocytosis in combination with abnormal FcγR-mediated phagocytosis by fixed tissue macrophages contributes to the pathogenesis of lupus nephritis.

In hypocomplementemic patients with SLE, examination of clearance rate constants revealed a good correlation between disease activity and FcγR-mediated clearance function (68). With decreased complement levels, there may be deficient complement opsonization of complexes, impaired binding and processing by erythrocytes, and reduced clearance by hepatic complement receptors. Such circulating immune complexes with little or no complement must be cleared by FcγRs. While rapid hepatic complement-dependent clearance appears to be the first line of defense against immune complex deposition, FcγR-mediated clearance becomes pivotal when this mechanism fails. In disease processes that are associated with hypocomplementemia, tissue immune complex deposition and increased disease activity occur when there is a concomitant defect in FcγR clearance. Similar to human SLE, studies in four murine models of lupus demonstrated an early progressive defect in FcγR-mediated clearance of IgG-sensitized erythrocytes, whereas the efficiency of complement-mediated clearance varied among the murine strains (76). The consistent finding of impaired FcγR-specific clearance in patients with active SLE emphasizes the potential importance of the mechanism for immune complex disease.

Given the role of immune complex deposition in the pathogenesis of SLE, circulating immune complex levels in patients were measured by a series of different assay systems (i.e., C1q binding, staphylococcal protein A binding assay,

Raji cell assay) at the time of in vivo erythrocyte clearance in many studies. There was a relationship between the levels of immune complexes and FcγR dysfunction in some, but not all, groups of patients with SLE. The lack of direct correlation between mononuclear phagocyte system function and immune complex level in all studies is not surprising, however, given the complexity of immune complex handling and the range of variables determining net complex levels (70 ,72).

Although partly acquired and related to disease activity, the FcγR mononuclear phagocyte dysfunction also may have a genetic component. Normal individuals with a human leukocyte antigen (HLA) haplotype containing either DR2 or DR3, which are some gene products found with increased frequency in SLE populations (see Chapter 6), are more likely to have an abnormally prolonged FcγR-mediated clearance of IgG-sensitized erythrocytes than their normal counterparts without these haplotypes (77 ,78). While the magnitude of the FcγR dysfunction is substantially larger in patients with SLE and has a large dynamic component associated with disease activity (64 ,70 ,72), individuals with an immunogenetically associated decrease in FcγR function might be more susceptible to the secondary FcγR abnormalities associated with SLE. Allelic polymorphisms of FcγR also are potential inherited factors influencing immune complex clearance (discussed later). Thus, basal genetically determined mononuclear phagocyte clearance in normal individuals may contribute to the predisposition and pathogenesis of SLE.

Analysis of Clearance of Infused Soluble Immune Complexes

As another measure of mononuclear phagocyte system function, the clearance of preformed, large, soluble, complement-fixing immune complexes has been studied in humans. Radiolabeled tetanus toxoid/anti-tetanus toxoid, hepatitis B surface antigen/antibody, or aggregated human IgG are infused and then sequential blood samples obtained and analyzed for whole blood and erythrocyte-bound radioactivity to monitor clearance (48 ,54 ,79). Clearance of these preformed immune complexes (free or erythrocyte bound) from the circulation of humans has been shown to involve the activation of complement with capture of C3b, binding to erythrocyte CR1 receptors, uptake by complement, and FcγR tissue mononuclear phagocytes as described earlier. Factors that cause the erythrocyte transport system to fail, such as hypocomplementemia or CR1 deficiency, are associated with an initially more rapid disappearance of immune complexes, presumably caused by trapping in capillary beds outside the mononuclear phagocyte system. For example, clearance of injected hepatitis B surface antigen/antibody complexes in patients with essential mixed cryoglobulinemia is accelerated compared with that in normal subjects, presumably because of immune complex deposition in tissues outside the liver and spleen as a result of impaired immune adherence. The basis for defective erythrocyte transport and buffering of complexes in these patients appears to be a result of complement depletion and monoclonal rheumatoid factor inhibition of immune complex opsonization and binding (54).

Because the relevance of these large, preformed complexes (40S) to human SLE is not entirely clear, studies of the clearance of smaller (19S) complexes formed in vivo have been performed. Patients with ovarian cancer were infused with murine antitumor antibodies followed by human anti-murine IgG (46). In this model, complexes were cleared rapidly by the liver, although the role of erythrocyte CR1 was unclear. In vivo immune complex formation was associated with systemic complement activation, reduction in erythrocyte CR1, and increased erythrocyte C3 and C4 (similar to the findings in SLE described later), which could predispose to less efficient handling of further complexes.

Given the different kinds of information obtained from each of these in vivo probes, examination of multiple models of immune complex clearance is necessary to define the mechanisms of immune complex deposition in SLE.

In Vivo Studies of Infused Soluble Immune Complexes in SLE

In vivo studies of infused soluble immune complexes complement the sensitized erythrocyte model of clearance and demonstrate multifactorial mononuclear phagocyte dysfunction. Abnormalities in erythrocyte CR1 system, the early buffer for circulating immune complexes, are described in patients with SLE, and for these models it is important to recognize that such patients tend to have an acquired, decreased numeric expression of CR1 on erythrocytes that correlates with disease activity (80 ,81) and may result from repeated immune complex/erythrocyte CR1 interactions (48 ,51). There also is evidence of an inherited deficiency of CR1 in some patients (36 ,38).

Diminished CR1 resulting in impaired immune adherence is one of the mechanisms for abnormal clearance of infused soluble complexes in SLE. For example, patients with SLE who were infused with radiolabeled aggregated human IgG showed decreased binding of the probe to erythrocytes and a more rapid initial distribution phase compared with that in normal controls (62 ,78). Similarly, complement-mediated binding of tetanus toxoid/antitetanus toxoid immune complexes to erythrocytes was decreased in SLE, and this correlated with erythrocyte CR1 number (48). With these model immune complexes, a rapid first phase of elimination was noted in patients with low complement, low CR1, and low immune adherence, which was ascribed to inappropriate tissue deposition of complexes (49).

The second, slower elimination phase of infused aggregated IgG also is abnormal in SLE, presumably because of impaired splenic uptake as well as generalized mononuclear phagocyte dysfunction. Whereas studies in normal individuals show preferential hepatic uptake of aggregated IgG and those who are splenectomized show hepatic compensation for splenic loss with normal elimination half-times, patients with SLE have both minimal splenic uptake and prolonged clearance half-times (62 ,78 ,82). Studies of the infusion of large, soluble

immune complexes (hepatitis B surface antigen/antibody) in patients with SLE reveal both impairment of hepatic clearance and retention of complexes as well as impairment of splenic clearance and retention of complexes (83). With this probe, the dysfunction is related to hypocomplementemia and decreased number of erythrocyte CR1 receptors. Similarly, in a patient with hereditary homozygous C2 deficiency, treatment with fresh frozen plasma to normalize classical pathway complement activity normalized the binding of hepatitis B surface antigen/antibody complexes to erythrocytes and corrected defects in splenic clearance and retention (84). Regardless of the mechanism, however, abnormalities in both splenic and hepatic clearance function allow for a spillover of complexes beyond the mononuclear phagocyte system in SLE.

Blockade of FcγRs by elevated levels of IgG interferes with this key mechanism for the elimination of soluble circulating immune complexes (85 ,86). That serum concentrations of IgG are an important factor predicting the rate of aggregated IgG clearance in SLE (78) emphasizes the importance of FcγR mechanisms in this model and supports the conclusions derived from the sensitized erythrocyte model of immune complex clearance. Specifically, FcγR-mediated clearance efficiency is crucial in SLE because of the defects in complement-dependent function.

Biology of Human Fcγ Receptors

With evidence for both the genetic and acquired components of FcγR-mediated clearance defect, further information about FcγR structure and function should enhance our understanding of immune complex handling and provide novel therapeutic options. Human FcγR structure is much more varied than simply being one type of receptor for IgG, as assumed in many of the early studies cited in this chapter. Recent, dramatic growth in our knowledge of FcγRs has revealed extreme diversity accompanied by great complexity. The nomenclature for FcγR follows that proposed by Ravetch and Kinet (87): uppercase letters refer to genes, and lower case letters refer to proteins (i.e., gene products).

Structure and Distribution

FcγRs are an essential receptor system that is engaged by immune complexes as they trigger internalization, release of inflammatory mediators, cytokines and degranulation. In contrast to complement receptors, FcγR recognize ligand in its native form. In humans, there are three distinct but closely related classes of FcγR—FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16)—that are identified by immunochemical and physicochemical properties, cellular distribution, and complementary DNA (cDNA) sequences (88 ,89 ,90 ,91 ,92). There are eight FcγR genes, each of which may lead to unique protein products. Extensive structural diversity among FcγR family members leads to differences in binding capacity, distinct signal transduction pathways, and cell specific expression patterns (Fig. 12-5). This chapter considers structure-function relationships in the context of each receptor family and provides a framework for understanding how FcγR may contribute to susceptibility, pathogenesis, and therapeutic intervention in SLE.

FcγR on surface of hematopoetic cells are often expressed as stimulatory and inhibitory pairs. Given the protean and potent reactivity initiated by stimulatory FcγR, cell activation must be modulated to respond appropriately to variations in environmental stimuli. Studies have suggested that inhibitory FcγRs, which modulate thresholds for activation and terminate stimulating signals, are a key element in the regulation of effector function (13 ,14). When co-aggregated with stimulatory receptors on the cell surface, inhibitory FcγRs can abolish cellular signaling, whereas when self-aggregated, they do not trigger effector functions. Inhibitory FcγRs play a central role in afferent and efferent immune responses as negative regulators of both antibody production and immune complex-triggered activation.

FcγRs belong to the immunoglobulin supergene family and are encoded for by multiple genes, which have been mapped to the long arm of chromosome 1q21-23 (93 ,94 ,95). Within the three FcγR families, the presence of multiple distinct genes (arising from gene reduplication) and alternative splicing variants leads to a variety of receptor isoforms that are most strikingly different in transmembrane and intracellular regions, whereas they share similar but not identical extracellular domains (Fig. 12-5).

FcγRs capable of triggering cellular activation possess intracellular activation motifs, termed immunoreceptor tyrosine-based activation motifs (ITAMs), similar to those of B cell receptors, and T cell receptors (96 ,97). Inhibitory FcγRs have extracellular domains that are homologous to their activating counterparts, but their cytoplasmic domains contain an immunoreceptor tyrosine-based inhibitory motif (ITIM). Stimulatory FcγRs are typically multichain receptors composed of a ligand-binding α subunit, which confers ligand specificity and affinity, and associated signaling subunits with ITAMs in the cytoplasmic domains (Fig. 12-5). FcγR α chains are transmembrane molecules that share the structural motif of two or three extracellular immunoglobulin-like domains, but vary in their affinity for IgG and in their preferences for binding different IgG subclasses (IgG1, IgG2, IgG3, and IgG4). There are also allelic variations in the ligand-binding region of specific FcγR that influence the ability to bind certain IgG subclasses and alter the responses of phagocytes to IgG-opsonized antigens (98 ,99 ,100). The transmembrane domains of the α subunits contain a basic residue to mediate the physical interaction with associated signaling chains required for efficient expression and signal transduction. The two multichain FcγR isoforms are termed FcγRI, a high affinity receptor for IgG that binds monomeric IgG, and FcγRIIIa, an intermediate affinity receptor, that binds only multivalent IgG. Homodimeric γ-chains are transducing modules for FcγRI and FcγRIIIa (Fig. 12-5). Heterodimers of γ-ζ chains or homodimers of ζ chains can also transduce signals through FcγRIIIa in human natural killer (NK) cells. The other isoform, FcγRIIIb, has neither an ITAM nor

a transmembrane domain, but is maintained in the plasma membrane outer leaflet by a glycosyl phosphatidylinositol anchor (Fig. 12-5). In addition to multichain receptors, there are two other types of activating FcγR and one inhibitory receptor with two different splice variants. FcγRIIa and FcγRIIc are single-chain receptors that include an extracellular ligand-binding domain and ITAM in the cytoplasmic domain. Inhibitory FcγRs, FcγRIIb1 and FcγRIIb2, are single-chain receptors with extracellular domains highly homologous to their activating counterparts and cytoplasmic domains with ITIMs (Fig. 12-5) (101).

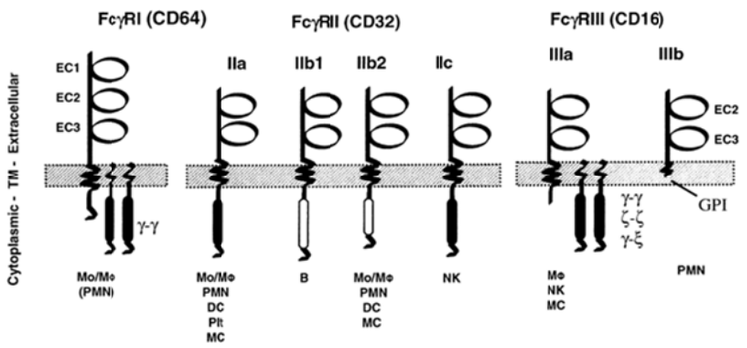

Figure 12-5. Schematic representation of the human Fcγreceptor family members. FcγR α chains contain two or three disulphide-linked immunoglobulin-like extracellular domains (ellipses) that mediate binding to IgG. All FcγRs, except the glycosyl phosphatidylinositol-anchored FcγRIIIb, have a transmembrane region (TM), some of which can interact with accessory chains to yield a multichain signaling complex. The cytoplasmic domains of FcγR or their associated subunits are responsible for signal transduction. FcγRIIIb is the only FcγR that lacks a cytoplasmic tail. FcγRI and FcγRIIIa are multichain receptors that associate with immunoreceptor tyrosine activation motif (ITAM)-containing γ- or ζ~- chain dimers (black cylinders) to mediate positive signaling. FcγRIIa and FcγRIIc are single chain stimulatory receptors containing ITAM motifs in their cytoplasmic tails. FcγRIIb (isoforms FcγRIIb1 and FcγRIIb2) are single chain inhibitory receptors containing immunoreceptor tyrosine inhibitory motif (ITIM) in their cytoplasmic tails (white cylinders). The cellular distribution of FcγRs is listed below each receptor: monocytes (Mo), macrophages (MΦ), dendritic cells (DC), mast cells (MC), B lymphocytes (B), platelets (Plt), natural killer cells (NK) and polymorphonuclear leukocytes (PMN). (Adapted from Salmon JE, Pricop L. Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. *Arthritis Rheum* 2001;44:739–750, with permission.)

FcγRI (CD64) is distinguished by its relatively high affinity for IgG (102). It is the only FcγR that is capable of binding monomeric IgG; other FcγRs require multivalent ligands. In contrast to the low-affinity FcγRII and FcγRIII, which have only two immunoglobulin-like domains homologous to the first two extracellular domains of FcγRI, FcγRI has three extracellular immunoglobulin-like domains, the last of which is postulated to confer high affinity (103 ,104 ,105) (Fig. 12-5). FcγRI is encoded for by at least three different genes, thus suggesting the possibility of the expression of different isoforms. Only FcγRIa has been detected on the cell surface. FcγRIa, a heavily glycosylated 72-kd protein, associates with homodimers of the γ-subunit of the high-affinity receptor for IgE (106) (Fig. 12-5). FcγRIa is present on monocytes, macrophages, and dendritic cells (107). Monocyte expression of FcγRI is markedly enhanced by interferon-γ (IFN-γ) (108 ,109), and neutrophils that do not constitutively express FcγRI can be induced to express this receptor by IFN-γ and granulocyte colony-stimulating factor (G-CSF) (110 ,111).

The FcγRII (CD32) family contains 40-kd receptors with low affinity for IgG, interacting only with IgG in complexes. They are the most widely expressed FcγR, and this family has the greatest range of structural heterogeneity. FcγRII family members are present on nearly all cells that bear FcγRs, including most leukocytes and platelets (112 ,113 ,114). Density of expression varies with cell type but generally is higher than that for FcγRI (115). The structural heterogeneity of FcγRII has a complex genetic basis with at least three genes (FcγRIIA, FcγRIIB, and FcγRIIC) and alternative splicing, resulting in the expression of six different isoforms (89 ,90 ,91 ,92 ,116) (Fig. 12-5). The three genes are nearly identical in their extracellular and transmembrane domains but differ in their cytoplasmic domains. The divergence in cytoplasmic tails determines the effector functions that are mediated by each isoform. FcγRIIA, FcγRIIB, and FcγRIIC represent single-protein gene products (α-chains) that are competent for ligand binding (extracellular domain) and signal transduction

(cytoplasmic domain incorporating structural motifs essential for signaling). This contrasts with the other FcγRs that assemble in multimolecular signaling complexes (Fig. 12-5). Among the FcγRII family members, there are activating and inhibiting receptors which differ mainly in the signaling motif in the cytoplasmic domain. FcγRIIA and FcγRIIC contain ITAMs and they are preferentially expressed on cells of myeloid lineage, monocytes, neutrophils, platelets and dendritic cells. FcγRIIC has recently been identified on NK cells (117).

FcγRIIB is the only FcγR gene encoding for an inhibitory receptor. It encodes for single-chain low-affinity receptors with extracellular domains highly homologous to FcγRIIA and FcγRIIC, but with cytoplasmic domains containing an ITIM (101) (Fig. 12-5). Alternative splicing generates two isoforms, FcγRIIb1 and FcγRIIb2, which differ only in their intracytoplasmic regions (116). FcγRIIb1 contains an insertion of 19 amino acids that significantly alters receptor function. FcγRIIb is widely expressed on hematopoetic cells: FcγRIIb1 on B lymphocytes and FcγRIIb2 on myeloid cells. Neither isoform can trigger cell activation. Instead, both isoforms of FcγRIIb, when coaggregated with ITAM-bearing receptors, are negative regulators of activation. In addition, FcγRIIb2 participates in endocytosis of multivalent ligands by phagocytes and antigen presenting cells while the intracytoplasmic insertion in FcγRIIb1 inhibits internalization (118). FcγRIIb can modulate cell activation by stimulatory FcγR, as well as responses triggered by B cell receptor (BCR), the T cell receptor (TCR), and Fc receptors for IgE (119). However, to inhibit cell activation, FcγRIIb must be co-aggregated with ITAM-expressing receptors by a multivalent ligand, and cell activation must be triggered by the receptors that are co-aggregated with FcγRIIb (120). For example, FcγRIIb co-aggregation with FcγRIIa by IgG-opsonized particles blocks phagocytosis, and FcγRIIb coligation to BCR by antibody-antigen complexes inhibits B cell proliferation and antibody production (121 ,122). Thus, FcγRIIb-mediated negative regulation of ITAM-dependent cell activation endows IgG-containing immune complexes with the capacity to regulate B cells and inflammatory cells. Because FcγRs are often expressed as activation/inhibitory pairs, the balance between stimulatory and inhibitory input determines cellular response.

In addition to different isoforms, there are two allelic forms of FcγRIIA (R131 and H131), resulting in further polymorphism in this receptor class. The FcγRIIA alleles differ functionally because of a single base difference that encodes for the amino acid position 131 in the second extracellular domain (arginine and histidine, respectively). These alleles are expressed codominantly on neutrophils, monocytes, and platelets and have differing IgG subclass-binding specificities (discussed later) (90 ,98 ,99). For FcγRIIb, allelic polymorphisms have been described in the promoter region which alter receptor expression (123).

The FcγRIII (CD16) family of low-affinity receptors for IgG contains two members, each of which is encoded for by different but highly homologous genes (FcγRIIIA and FcγRIIIB), and each is selectively expressed in specific cell types (90 ,91 ,124). FcγRIIIa is the most abundant class of FcγR on macrophages and thus is a key receptor of the mononuclear phagocyte system. It is present at high density on Kupffer cells in the liver and on macrophages in the spleen, both important areas for immune complex clearance binding and internalization. Additionally, it has been described on a subpopulation of monocytes (125) and on mesangial cells (126). One source of diversity between isoforms of FcγRIIIa is differences in glycosylation, which may account for the variations in receptor affinity noted for NK versus macrophage forms of FcγRIIIa and for FcγRIIIa versus FcγRIIIb (127). FcγRIIIa associates with members of a family of signal transduction molecules that bear ITAMs within their cytoplasmic domains (Fig. 12-5) (128). These molecules also are used by FcγRI and the high-affinity receptor for IgE and the T cell receptor/CD3 complex. These accessory molecules form disulfide-linked dimeric complexes (homo- or heterodimers) that noncovalently associate with the transmembrane region of FcγRIIIa to enable cell surface expression and signal transduction. FcγRIIIa also has the capacity to associate with the γ-subunit of the high affinity IgE receptor (129).

FcγRIIIb is the most densely expressed FcγR on neutrophils and therefore is the most abundant FcγR in the circulation. The major difference between the two subclasses of FcγRIII is that FcγRIIIa, which is the form expressed on macrophages and NK cells, is a conventional transmembrane protein, whereas FcγRIIIb, which is the form expressed exclusively on neutrophils, is anchored to the outer membrane leaflet by a glycosyl phosphatidylinositol moiety (130 ,131) (Fig. 12-5). Further diversity in FcγRIII structure is provided by an allotypic variation in FcγRIIIb. The two recognized allelic forms of the glycosyl phosphatidylinositol-anchored neutrophil isoform of FcγRIIIb, termed NA1 and NA2, differ by several amino acids and N-linked glycosylation sites (132 ,133). The alleles are inherited in a classic mendelian manner and are expressed in a codominant fashion. In addition to different isoforms, there are two allelic forms of FcγRIIIA (F176 and V176), which differ in one amino acid at position 176 in the extracellular domain (phenylalanine or valine, respectively) (100 ,134 ,135). These alleles differ in binding capacity for IgG1 and IgG3 (discussed later).

Another potentially important form of FcγR in the context of immune complex handling is circulating soluble receptor. Normal sera contain soluble FcγRII and FcγRIII (136 ,137). These soluble FcγRs lack transmembrane domains and presumably derive from alternative splicing or release from circulating leukocytes (136 ,137 ,138). The physiologic significance of these circulating IgG-binding proteins is not yet clear, but a proposed biologic function of plasma FcγRs is to suppress IgG production by B cells (139). The role of plasma FcγRs in immune complex clearance is open to speculation. Certainly, they may influence the clearance of immune complexes by blocking the ligand-binding site for FcγRs and thereby inhibiting both FcγR-mediated clearance and FcγR-triggered inflammation at sites of tissue deposition. Immune complex binding of plasma FcγR also has the potential to affect clearance by changing the size of the complexes, altering their solubility, modifying their capacity

to activate complement and capture C3b, and shifting binding from macrophage FcγRs to complement receptors.

Ligands

Ligand specificity for FcγRs is relative rather than absolute, and it depends on the valence or degree of opsonization of the study probe. Table 12-2 shows the binding specificity of human FcγRs for human IgG subclasses. The IgG subclass of the antibody in immune complexes and the valence of the complex influence the efficiency of the interaction with FcγRs (140). Further, a single immune complex may bind simultaneously to different classes of FcγR. FcγRI, the high-affinity receptor and the only FcγR capable of univalent binding of IgG (89 ,90), and FcγRII and FcγRIIIb, which are lower-affinity FcγRs, preferentially bind IgG1 and IgG3. There is differential binding affinity for allelic variants of FcγRIIIa (100). While the affinity of FcγRIIIa-expressed on macrophages and NK cells is higher than that of FcγRIIIb on neutrophils, the pattern of specificity for subclasses is similar for all FcγR (127). For all three classes of FcγR, IgG2 is the ligand with lowest affinity (Table 12-2), although studies have shown efficient binding to IgG2 by the H131 allele of FcγRIIa (discussed later) (98 ,99 ,141).

In addition to classic IgG-FcγR interactions, FcγR may bind ligands through lectin-carbohydrate interactions. The internalization of nonopsonized *Escherichia coli* bearing mannose-binding lectin requires neutrophil FcγRIIIb, which has high mannose oligosaccharides (142). Monocyte FcγRs also participate in lectin-carbohydrate interactions (143). These observations suggest that nonclassic engagement of FcγRs through lectin-carbohydrate interaction, perhaps by antigens in complexes, can affect clearance by phagocytes. Additionally, recent studies have shown that FcγRI and FcγRIIa are critical receptors for C-reactive protein (CRP) and that FcγRIIa is the main receptor on human phagocytes for CRP, which raises the possibility that FcγRs are important for the clearance of nucleosomes bound to CRP, which may also be influenced by allelic polymorphisms (144 ,145 ,146).

FcγR Signal Transduction

Effector cell activation is initiated when FcγRs are clustered at the cell surface by multivalent antigen-antibody complexes. Monovalent ligand binding is insufficient to generate a signal. Signal transduction by FcγR following aggregation involves a number of early cellular biochemical changes that lead to cellular activation. Like many other immune system receptors, such as the TCR and BCR, FcγR initiates tyrosine phosphorylation as a critical early signaling event (96 ,97). Stimulatory FcγRs have no intrinsic enzymatic activity, but are associated with membrane anchored src family kinases. The presence of two YxxL motifs separated by seven variable residues in the signaling subunit (γ-chain) of activating FcγRI and FcγRIIIa, or 12 residues in the case of FcγRIIa and FcγRIIc, is necessary for docking the protein tyrosine kinase syk and for initiation of positive signaling. Tyrosine kinases phosphorylate many intracellular substrates, including phospholipid kinases, phospholipases, adapter molecules, and cytoskeletal proteins. Activation of phospholipase C and phosphatidylinositol-3 (PI3) kinase by syk leads to the production of phosphoinositol messengers and a sustained increase in cytoplasmic Ca^{2+} (147). Recruitment of the adaptor protein shc allows signals triggered by FcγR to reach the nucleus via the ras pathway, leading to phosphorylation of mitogen-activated protein (MAP) kinase, activation of transcription factors, and induction of gene expression (148).

Table 12-2: FcγR Affinity and IgG Subclass Specificity

	MW(kD)	Genes	Affinity for IgG (Ka)	IgG Specificity
FcγRI	72	FcγRI	10^{8}–10^{9} M^{-1}	1, 3 > 4 ≫ 2
FcγRII	40–50	FcγRIIA-R131	<10^{7} M^{-1}	1, 3 ≫ 2, 4
		FcγRIIA-H131	<10^{7} M^{-1}	1, 3, 2 > 4
		FcγRIIB, C,	<10^{7} M^{-1}	1, 3 ≫ 2, 4
FcγRIII	60–70	FcγRIIIA	10^{7} M^{-1}	1, 3 > 2, 4
	50–80	FcγRIIIB	<10^{7} M^{-1}	1, 3 ≫ 2, 4

IgG, immunoglobulin; MW, molecular weight.

FcγRIIb isoforms are important negative regulators of ITAM-dependent activation and establish the threshold for effector cell activation. Inert when self-aggregated, inhibitory FcγRIIb abolishes cellular signals when co-ligated with stimulatory receptors. The ITIM motif (V/IxYxxL), contained in a 13-amino acid sequence present in the intracytoplasmic domain of both FcγRIIb1 and FcγRIIb2, is essential for the negative regulatory properties of FcγRIIb and other inhibitory receptors (reviewed in (149 ,150 ,151)). Like ITAMs, ITIMs are phosphorylated by protein tyrosine kinases and then recruit SH2-containing cytoplasmic molecules. Inhibitory function requires the recruitment of phosphatases to the phosphorylated ITIM. Although the protein tyrosine phosphatases SHP-1 and SHP-2 bind to FcγRIIb-phosphorylated ITIM motifs, the inositol polyphosphate 5′-phosphatase SHIP has been shown to be preferentially recruited to FcγRIIb and appears to play the predominant role in FcγRIIb-mediated inhibition by preventing Ca^{2+} influx (152 ,153 ,154).

Since most cells express more than one FcγR isoform, it is likely that antigen-antibody complexes coaggregate more

than one type of receptor. Coclustering of stimulatory FcγR represents a mechanism where different FcγR cooperate to amplify signals and produce more efficient activation of effector cells. FcγR that act synergistically transphosphorylate each other, leading to activation of tyrosine kinases and downstream substrates and initiation of $[Ca^{2+}]i$ transients, with subsequent cytoskeletal changes and transcriptional activation. In contrast, the regulation of ITAM-dependent cell activation by inhibitory FcγR is a mechanism for negative cooperation. Coclustering of inhibitory FcγRIIb with ITAM-bearing FcγR prevents the influx of extracellular Ca^{2+} and attenuates effector cell activation (149 ,150). By providing activated protein tyrosine kinases to phosphorylate the ITIM of FcγRIIb, stimulatory FcγRs play a role in their own inhibition. In cells that express both stimulatory and inhibitory receptors for IgG, the relative levels of these two types of receptors determine the state of cell activation after interaction with immune complexes.

FcγR-Mediated Effector Functions

The multivalent interaction of phagocytes with immune complexes leads to internalization of the complex, generation of reactive oxygen intermediates, and release of inflammatory mediators, including prostaglandins, leukotrienes, hydrolytic enzymes, and cytokines (e.g., IFN-γ, interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], and so on) (155 ,156 ,157 ,158 ,159). The role of individual isoforms of FγcRs in initiating these effector functions has been investigated in experiments using anti-FcγR monoclonal antibodies, cell lines with limited expression of FcγRs, and transfectants. There is significant overlap among the biologic activities mediated by each family of FcγR, and the relative contribution of each receptor family depends on the nature of the ligand, the state of phagocyte activation, and the effector function being assessed.

Binding and internalization are the most important effector functions for immune complex clearance. Experiments using erythrocytes coated with Fab fragments of anti-FcγR monoclonal antibodies show that in cultured human monocytes (a model system for fixed-tissue macrophages), FcγRI, FcγRIIa, and FcγRIIIa mediate phagocytosis (160). FcγRI is a key receptor on monocytes involved in the binding of IgG anti-(Rh)D-sensitized erythrocytes and small complexes in vitro (161). A role for FcγRIIIa is evident from studies showing that blockade of FcγRIII by infusion of anti-FcγRIII monoclonal antibody in humans and nonhuman primates inhibits clearance IgG anti-D-sensitized erythrocytes as well as clearance of soluble and erythrocyte-bound DNA/anti-DNA immune complexes, but to a lesser extent (Fig. 12-2) (47 ,162 ,163). Studies revealing an altered distribution of the allelic variants of FcγRIIa and FcγRIIIa in patients with lupus nephritis compared to control subjects without SLE highlight the importance of these receptors as inherited risk factors in the pathogenesis of SLE, presumably related to altered immune complex handling associated with the genotypes (discussed later). Taken together, these studies underscore the crucial role of each FcγR family in particulate and soluble immune complex handling by mononuclear phagocytes.

On neutrophils, while FcγRIIa mediates phagocytosis, the capacity of FcγRIIIb to independently mediate internalization is minimal (160 ,164 ,165). Although FcγRIIIb, the glycosyl phosphatidylinositol-anchored molecule, independently generates intracellular signals and initiates several other effector functions, it is conceptualized by some as a trap for circulating immune complexes that focuses IgG ligand for more efficient recognition by other FcγR species on neutrophils (91 ,160 ,166). Recent studies suggest a far more significant role for FcγRIIIb in triggering neutrophil responses—as a potentiator of other receptors on the cell. Crosslinking of FcγRIIIb enhances the amount of FcγRIIa-specific internalization, and coligation of FcγRII and FcγRIIIb results in a synergistic phagocytic response: internalization that is greater than the sum of the FcγRII and FcγRIIIb (165 ,166 ,167). This synergistic capacity of FcγRIIIb also enables complement receptor-mediated phagocytosis.

Regarding the intact phagocyte, the mechanisms triggering individual receptor-mediated function as well as engagement of multiple families of receptors in an interactive framework must be considered. In this context, studies of the ligation of individual receptor species (discussed earlier) demonstrate the functional potential of a given receptor. Alternatively, engagement of two receptors may lead to quantitatively, and perhaps qualitatively, distinct cell functions in relation to the engagement of either receptor alone. With engagement of mononuclear phagocyte FcγRs by immune complex, there are both heterotypic and homotypic receptor clusters, resulting in intracellular interactions between the signals that are generated by each receptor family. In monocytes, FcγRI and FcγRIIa cooperate in triggering activation, presumably by heterotypic clustering. In neutrophils, FcγRIIIb acts synergistically with FcγRIIa to mediate phagocytosis (166 ,167). Coaggregation of activating FcγR with inhibitory FcγRIIb alters the threshold and magnitude of effector functions. Interactions between FcγRs and other leukocyte receptors modulate FcγR-initiated functions; for example, engagement of complement receptor type 3 enhances FcγR-mediated phagocytosis by monocytes. In the interaction of immune complexes with leukocytes, effector function capacity is a consequence of positive and negative cooperation among FcγR families and between FcγRs and other receptors, all of which may be simultaneously engaged.

Cytokines elaborated during an immune response alter FcγR expression and functional capacity. For example, IFN-γ and G-CSF upregulate FcγRI on monocytes and induce its expression on polymorphonuclear cells (PMNs), whereas IL-4 inhibits the expression of all ITAM-bearing FcγR (168 ,169 ,170). Granulocyte-macrophage CSF (GM-CSF) specifically increases FcγRIIa, and transforming growth factor-β (TGF-β) increases FcγRIIIa (170). In contrast to their effects on stimulatory receptors, IFN-γ decreases and IL-4 increases the expression of the inhibitory receptor, FcγRIIb2, on human monocytes (171). It has been proposed that FcγRIIb functions to modulate inflammatory response by establishing the threshold

of immune complex-stimulated activation of macrophages. That IFN-γ (a prototypic T-helper-1 [Th1] cytokine) and IL-4 (a prototypic Th2 cytokine) differentially regulate the expression of FcγR isoforms with opposite functions provides a mechanism for regulation of activating and inhibitory signals delivered by FcγRs on phagocytes. Cytokines released within an inflammatory milieu thus act in an autocrine and paracrine manner to modulate effector cell function.

Inherited Differences in FcγRs

Mechanisms for the diversity of FcγR within an individual were discussed earlier. Inherited or acquired differences in FcγR structure, expression, or function provide the basis for differences in FcγR function between individuals, and these differences may contribute to disease susceptibility and pathogenesis. Heritable differences include gene deletions, promoter polymorphisms, and allelic forms. Individuals with the rare deficiency of FcγRIa1 are free of clinical disease, circulating immune complexes, and increased susceptibility to infection (172). The initial report of FcγRIIIb deficiency in a patient with SLE focused attention of the genetics of FcγR and immune complex disease (173). Defects in the expression of FcγRIIIb as a consequence of alterations at the genomic level have been identified in multiple unrelated individuals completely lacking FcγRIIIB alleles (174 ,175 ,176), but they have no consistent clinical pattern of increased infections or immune complex-mediated diseases (177). Although a second report of SLE in FcγRIIIb deficiency raises the possibility that this receptor functions in immune complex handling, perhaps as a carrier for circulating complexes, more extensive population and family studies are required (178).

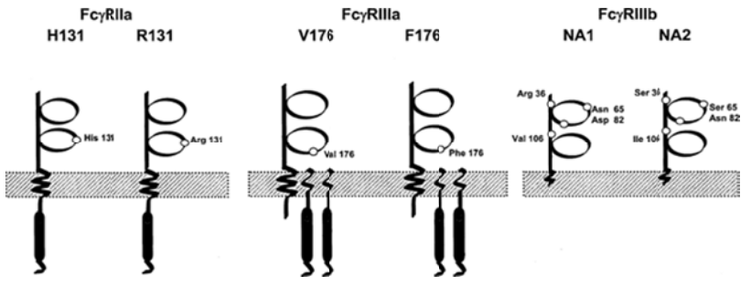

Figure 12-6. Allelic variants of activating human FcγR. Left: The FcγRIIa polymorphism is a consequence of an arginine (R131) to histidine (H131) substitution at amino acid position 131 in the extracellular domain, which causes differences in binding affinity for human IgG2 and C-reactive protein (CRP). Middle: The FcγRIIIa polymorphism is the consequence of a valine (V176) to phenylalanine (F176) substitution at position 176, leading to changes in binding affinity for human IgG1 and IgG3. Right: The NA1 and NA2 polymorphism of FcγRIIIb reflects four amino acid substitutions with consequent differences in N-linked glycosylation sites and quantitative differences in phagocytic function. (Adapted from Salmon JE, Pricop L. Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. *Arthritis Rheum* 2001;44:739–750, with permission.)

The concept that the balance of stimulatory and inhibitory FcγR is a determinant of the susceptibility to and severity of immune complex-induced inflammatory disease has been validated by data from murine models (12 ,13 ,14). Descriptions of deletions in the promoter region of FcγRIIB in all major autoimmune-prone mice strains have underscored these findings. These deletions are associated with a reduction in the expression and function of the inhibitory FcγRIIb on macrophages and activated B cells, defects that would be expected to promote autoimmunity (179 ,180 ,181). Furthermore, mice with targeted deletions of FcγRIIB develop autoantibodies and autoimmune glomerulonephritis in a strain-dependent fashion, related specifically to deficiency of FcγRIIb on B cells (182). As yet, there have been no reports of deficiency of inhibitory FcγRIIb function in antibody-mediated human disease. However, it is possible that differences in the relative expression of ITAM- and ITIM-containing FcγR influence an individual's risk of developing autoimmune disease. Indeed, polymorphisms in the promoter region of FcγRIIB which alter receptor expression are associated with alterations in cell function and enhanced autoimmunity (123 ,183).

Genetically determined alterations in FcγR structure provide a basis for inherited predisposition to disease. Allelic variants of human FcγR profoundly influence phagocyte biologic activity. Single amino acid substitutions within the extracellular domains of some stimulatory FcγR alter the capacity of these receptors to bind IgG and have been associated with risk for and phenotype of autoimmune and infectious disease (Fig. 12-6). Allelic polymorphisms have been identified in four FcγR family members: FcγRIIa, FcγRIIb, FcγRIIIa, and FcγRIIIb. Inherited variants of FcγR have differential binding avidity for different IgG subclasses.

FcγRIIa, expressed on mononuclear phagocytes, neutrophils, and platelets, has two codominantly expressed alleles, H131 and R131, which differ at amino acid position 131 in the extracellular domain (histidine and arginine, respectively), an area which strongly influences ligand binding (Fig. 12-6). The allelic variants differ substantially in their ability to bind human IgG2 (98 ,99 ,118 ,184). FcγRIIa-H131 is the high binding allele and R131 is low binding, while heterozygotes have intermediate function. Because IgG2 is a poor activator of the classical complement pathway, FcγRIIa-H131 is essential for handling IgG2 immune complexes. Even with model immune complexes containing IgG2 in combination with other IgG subclasses, there is differential handling in PMNs from homozygous individuals related to host FcγRIIA genotype (11). The genotype distribution of FcγRIIA in Caucasian and African American populations is approximately 25% homozygous for H131, 50% heterozygous, and 25% homozygous for R131. Among Asians the frequency of the R131 allele is much lower, and less than 10% of the population is homozygous for R131 (reviewed in (185)).

FcγRIIa has substantial clinical importance for host defense against infection with encapsulated bacteria known to elicit IgG2 responses, such as *Neisseria meningitidis*, *Hemophilus influenzae*, and *Streptococcus pneumoniae* (186 ,187 ,188). There is an increased frequency of homozygosity for FcγRIIa-R131 among otherwise healthy children who suffer from recurrent respiratory tract infections or fulminant meningococcal sepsis. FcγRIIa-R131 has also been shown to be a risk factor for invasive pneumococcal infection in patients with SLE (189). Like IgG2, CRP binds to several encapsulated bacteria. The recent report of a reciprocal relationship between the binding affinities of IgG2 and CRP for FcγRIIa suggests a mechanism for partial protection from invasive infection in individuals homozygous for FcγRIIa-R131 (145). That FcγRIIa is the main receptor for CRP raises the possibility that handling of nucleosomes bound to CRP may also be influenced by allelic polymorphisms (146).

FcγRIIIa, expressed on mononuclear phagocytes and NK cells, also displays codominantly expressed biallelic variants, F176 and V176, which differ in one amino acid at position 176 in the extracellular domain (phenylalanine or valine, respectively) (100 ,134 ,135) (Fig. 12-6). FcγRIIIa alleles differ in IgG1 and IgG3 binding; V176 homozygotes bind IgG1 and IgG3 more avidly than F176. These differences in IgG binding have implications for antibody-mediated immune surveillance, (antibody-dependent cell-mediated lytotoxicity toxicity [ADCC]), antibody-mediated host defense against pathogens and autoimmune disease. The distribution of genotypes of FcγRIIIA in disease-free Caucasian and African-American populations has been reported to be 40% to 50% homozygous F176, 40% to 50% heterozygous, and 8% to 18% homozygous V176 (185).

Two common allelic variants of FcγRIIIb, a receptor exclusively expressed on neutrophils, have been characterized and shown to alter neutrophil function. The allotypes, known as neutrophil antigen (NA) 1 and NA2, were identified as a consequence of their involvement in blood transfusion reactions and alloimmune neutropenias. They differ by five nucleotides, which results in a substitution of four amino acids in the membrane-distant first extracellular domain (133) (Fig. 12-6). Although binding of IgG does not seem to be affected, the NA1 and NA2 allelic forms do have different levels of quantitative function (99 ,167 ,190 ,191). Neutrophils obtained from NA1 homozygous donors have a more robust FcγR-mediated phagocytic response as compared to cells from NA2 donors, despite equivalent receptor density (190 ,191). Functional differences between the NA1 and NA2 alleles appear to have clinical significance. Homozygous NA1 individuals may be more resistant to bacterial infection, especially when FcγRIIa cannot be effectively engaged, as suggested by the finding of increased *Neisseria meningitidis* infection among hosts with complement component 6 or 8 deficiency who are homozygous for FcγRIIIb-NA2 and FcγRIIa-R131 (192).

Polymorphisms in FcγRIIb that alter signaling and variations in the promoter region that alter gene expression have recently been described. A single amino acid substitution in the transmembrane domain of FcγRIIb, replacement of 232 isoleucine with threonine (Fcγ RIIb232 I/T), leads to attenuated function (193). Fcγ RIIb-T232 is excluded from lipid rafts and is thus unable to inhibit activatory receptors resulting in the unopposed proinflammatory signaling thought to promote SLE (194 ,195). In addition to its expressions of mononuclear phagocytes and dendritic cells, FcγRIIb is present on B cells and plays crucial role in the maintenance of self-tolerance. Less potent inhibition of B cells receptor signaling associated with Fcγ RIIb-T232 alleles may favor autoimmunity and thus contribute to susceptibility for lupus. Single nucleotide polymorphisms in the promoter of FcγRIIB have also been identified which correlate with altered gene transcription and surface expression of Fcγ RIIb (123 ,183 ,196).

Studies of the allelic polymorphisms of FcγR demonstrate functional consequences of subtle structural differences, and these models suggest the potential impact of the rich structural diversity in FcγRs on immune complex handling.

Abnormalities in Fcγ Receptors in SLE

FcγR saturation by circulating immune complexes with decreased receptor availability was initially proposed as a potential mechanism for defective FcγR-mediated clearance (64). The complexity of FcγR structure was not appreciated at the time of these studies, however. Support for this hypothesis was derived from in vitro induction of loss of surface FcγRs in monocytes by culture with immune complexes (197 ,198) and in vivo production of mononuclear phagocyte blockade by infusion of immune complexes (4). Even so, several different studies of blood monocytes from patients with SLE demonstrated an increase rather than the predicted decrease in FcγR binding (199 ,200 ,201). In experiments quantitating the binding of monomeric IgG, oligomeric IgG, and IgG-sensitized erythrocytes to monocytes, there was an increase in the levels of binding in patients with SLE compared with the levels in controls. The observed increase in FcγR binding correlated with SLE disease activity in some studies and thus was highest in the population most likely to

have the greatest abnormality in FcγR-mediated clearance (199). In other studies, FcγR binding directly correlated with levels of immune complexes (202). Whether the increased binding capacity is specific to one class of FcγR or generalized is not known. The basis for the observed increase in patients with SLE is speculative, but this increase may result from exposure to cytokines and cellular activation (143 ,202 ,203).

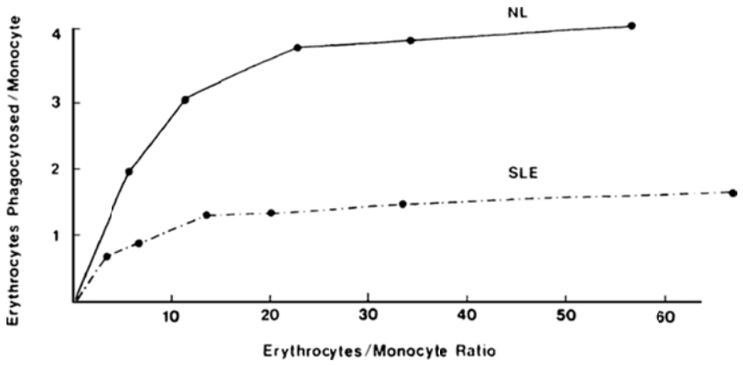

Figure 12-7. In vitro phagocytosis of 51Cr-labeled IgG sensitized erythrocytes (EAs) by monocytes from normal volunteers ("-") and patients with SLE ("- • - ") was measured at various ratios of erythrocytes to monocytes. Phagocytic capacity was dependent on the ratio of erythrocytes to monocytes in the incubation mixture. At all ratios, the patients with SLE had lower FcγR-mediated internalization. (From Salmon JE, Kimberly RP, Gibofsky A, et al. Defective mononuclear phagocyte function in systemic lupus erythematosus: dissociation of Fc receptor-ligand binding and internalization. *J Immunol* 1984;133:2525–2531, with permission.)

Despite enhanced binding of FcγR ligand in vitro, FcγR-mediated phagocytosis of IgG-sensitized erythrocytes was markedly impaired in monocytes derived from patients with SLE (201) (Fig. 12-7). The defect in phagocytosis in vitro was most profound in those patients with the most significantly impaired in vivo mononuclear phagocyte system clearance. These observations, along with those from other studies demonstrating a correlation between in vivo clearance and in vitro FcγR phagocytosis, support the role of defective phagocytosis as an important component of altered FcγR-mediated clearance (77 ,86). The dissociation of FcγR-mediated binding and FcγR-mediated internalization suggests relative receptor-effector uncoupling. Because the probes used in these studies could not discriminate among the FcγR expressed on monocytes, the contribution of a given class (or classes) of FcγR to this dysfunction is not yet known.

The net FcγR-mediated phagocytic capacity in SLE is a result of at least two factors. The first is inherited, such as that associated with allelic polymorphisms of FcγR; the second is disease acquired and may be related to disease activity. It has been hypothesized that low binding FcγR alleles are susceptibility factors for SLE. Association studies indicate that FcγRIIa-R131 and FcγRIIIa-F176 alleles are highly enriched in some groups of patients with SLE (204 ,205 ,206 ,207 ,208 ,209). In the initial studies with African Americans, a group with a high prevalence and severity of SLE, an association between FcγRIIA alleles and lupus nephritis was described (204). There was an increased frequency of FcγRIIA-R131 gene in the patients with SLE and a corresponding decrease in FcγRIIa-H131, the only human FcγR with the ability to efficiently recognize human IgG2 in black patients with lupus (Fig. 12-6). The skewing in FcγRIIA gene distribution was most marked in the nephritis group as compared with control subjects without SLE. The H131/H131 genotype was uncommon among patients with nephritis, suggesting that FcγRIIA-H131 plays a protective role in the homozygous state, an idea that is supported by in vitro experiments showing that the FcγRIIa-R131 gene product is characterized by deficient IgG2 handling in both homozygotes and heterozygotes. Thus, it appears that FcγRIIa-R131/R131 homozygotes and R131/H131 heterozygotes, who have the potential for less-efficient immune complex clearance, are at a greater risk for immune complex deposition. Similar results have been observed for FcγRIIIA. There is a strong association of low binding F176/F176 genotype (Fig. 12-6) with SLE, especially nephritis, and a corresponding under-representation of the homozygous high binding V176/V176 genotype (100). As a consequence of these studies, FcγR were considered the first non-major histocompatibility complex (MHC) genes associated with SLE. Indeed, there is evidence from a genome-wide scan of linkage of the region of the FcγR gene cluster on chromosome 1 with SLE (210).

Although homozygosity for low binding alleles at either the FcγRIIA or the FcγRIIIA locus can lead to impaired binding of immune complexes, an association between low binding alleles of FcγR and lupus, and especially lupus nephritis, has not been demonstrated in all ethnic groups studied (100 ,204 ,211 ,212 ,213). Nonetheless, one meta-analysis of studies seeking to establish a relationship between SLE and FcγR variants showed that FcγRIIa-R131 is associated with SLE, especially in African Americans, and that FcγRIIIa-F176 is associated with SLE in Caucasians and in other groups (182). More recent meta-analyses (comprising more than 3000 patients) suggested that FcγRIIa confers a risk for SLE, but not for nephritis and that the population attributable fractions of SLE cases because of FcγRIIa-R131 were 13%, 40%, and 24%, in subjects of European, African, and Asian descent, respectively (214). In contrast, FcγRIIIa low binding alleles confer a risk for lupus nephritis in these populations, but is not a significant susceptibility factor in the absence of nephritis (215). Even within ethnic groups where associations have been demonstrated, there have been conflicting results. Such lack of uniformity has been ascribed to population admixture, lack of appropriate internal controls, differences in disease phenotype, and the confounding influence of other inherited susceptibility factors.

An alternative explanation for these inconsistencies is that the role of specific loci varies with the qualitative nature of the immune response. Differences in the IgG subclass of pathogenic autoantibodies have a profound effect on the relative importance of FcγR alleles in disease. For example, in the presence of anti-C1q antibodies, which correlate with severe renal disease and are largely of the IgG2 subclass, FcγRIIA genes appear to play a crucial role in determining disease severity (216 ,217). Two studies have found that FcγRIIa-R131 alleles were associated with renal disease among Caucasian lupus patients with anti-C1q antibodies, whereas analysis of

the population as a whole revealed no significant difference in the frequencies of FcγRIIa-R131 and -H131 alleles compared with controls (216 ,217). Indeed, IgG2 is a predominant IgG subclass found in glomeruli of patients with proliferative nephritis. In a recent study, the frequency of genotypes containing the low-binding IgG2 allele, FcγRIIa-R131, was significantly greater than expected in patients with class III or class IV nephritis and in patients with intense IgG2 deposition. CRP, a ligand with particular affinity for FcγRIIa-R131, was consistently present in the renal immune deposits of lupus nephritis specimens (218). Thus, with precisely defined phenotypes, FcγRIIa variants have been identified as disease modifiers, in this example, conferring inherited risk for nephritis.

The finding that other autoantibodies associated with nephritis, specifically antidouble-stranded DNA (dsDNA) and antinucleosome antibodies, are predominantly IgG1 and IgG3 supports the importance of FcγRIIIA variants as disease modifying genes (219 ,220). For both FcγRIIa and FcγRIIIa, optimal handling of pathogenic immune complexes is provided only in the homozygous state for high binding alleles; heterozygotes at either locus have intermediate-binding capacity. Low affinity for IgG2-containing immune complexes by FcγRIIa-R131 and for IgG1 and IgG3 by FcγRIIIa-F176 results in impaired removal of circulating immune complexes, increased tissue deposition, and accelerated organ damage. An alternative explanation for the association of FcγRIIA and FcγRIIIA with lupus is that these genes are in linkage disequilbrium with other candidate proteins. The fact that IgG2 and IgG3 subclasses are present in immune deposits of proliferative glomerulonephritis, however, supports a role for both FcγR genes in conferring risk for SLE and the possibility that these receptors act additively in the pathogenesis of disease (221 ,222 ,223).

The relative importance of interactions between alleles and the potential role of linkage disequilibrium between two FcγR genes within the same cluster on chromosome 1q21-23 is not yet established. However, in a cohort of Hispanic patients with a high prevalence of lupus nephritis there was selection for haplotypes containing both FcγRIIa-R131 and FcγRIIIa-F176 (224). Given that the distance between any of the low affinity FcγR genes is less than 300 kilobases (kb), linkage disequilibrium of haplotypes might be expected (94 ,95). Studies in multiplex SLE pedigrees have demonstrated linkage disequilibirum between adjacent genes within the FcγR cluster on chromosome 1 (225). Furthermore, subtle variations in genes controlling immune function, which may have little or no effect in the general population, can assume greater significance in individuals with autoimmune diatheses.

Allelic polymorphisms in inhibitory FcγRIIb have also been associated with SLE in population studies. The FcγRIIb232I/T substitution in the transmembrane domain affects the localization and function of FcγRIIb and the less inhibiting 232T/T genotype occurs more commonly in Japanese, Thai and Chinese lupus patients than in ethnically similar controls, pointing toward FcγRIIB as a common susceptibility gene in the Asian populations (193 ,226 ,227). There is no evidence of skewing of the distribution of these alleles in European Americans of African American SLE patients (228 ,229). Promoter variants have also been described for FcγRIIB. An uncommon polymorphism (G-C substitution at position –343 relative to the start of transcription) leading to decreased FcγRIIb expression and function in activated B cells has been associated with SLE in Caucasians (196). According to the linkage disequilibrium analysis and conditional tests of association within the FcγR cluster, the association of FcγRIIB with SLE does not represent disequilibrium with FcγRIIA or FcγRIIA (123).

The physiology of stimulatory and inhibitory FcγR alleles provides a new framework within which the interplay between humoral immune response and host genotype may be defined and heritable risk factors for disease susceptibility and disease severity may be identified.

In addition to FcγR dysfunction, there is impaired phagocytosis of other probes in SLE monocytes. Internalization of apoptotic cells is decreased in SLE which may promote autoimmunity (230). Additionally, reduced uptake of nonopsonized particles and complement-opsonized particles has been clearly demonstrated in SLE monocytes (196 ,231). Thus, as predicted by the in vivo clearance studies, there are bipartite defects in internalization by SLE monocytes, dysfunction of FcγRs and complement mechanisms (67 ,68).

Collectively, in vivo clearance data and in vitro monocyte data indicate that FcγRs play a central role in immune complex handling. Regarding conditions with decreased complement-dependent immune complex uptake by fixed tissue macrophages, such as hypocomplementemia or deficiency in erythrocyte CR1 receptors, both of which are seen in SLE, it has been proposed that FcγR-mediated clearance mechanisms can handle both complement- and noncomplement-fixing complexes. In contrast, even in the face of intact complement mechanisms, immune complexes that do not fix complement, or that fix complement poorly (e.g., such as IgG2 containing complexes), are cleared less efficiently if there is abnormal FcγR-mediated function (Fig. 12-1). In this context, the observations that normal subjects with HLA haplotypes associated with SLE handle an erythrocyte-bound FcγR ligand probe less efficiently and that allelic variants of FcγR have distinct functional capacities provide a compelling argument that certain individuals may be at higher risk for the development of immune complex disease (232). In contrast to the complement system, the defect in FcγR function in SLE is not associated with decreased receptor number or saturation, but rather with a dissociation of receptor-ligand binding and internalization superimposed on possible inherited differences in FcγR function (199 ,200 ,201).

Strategies for Modulating FcγR-Mediated Immune Complex Clearance

The emerging picture of the extensive structural diversity of human FcγRs, the importance of FcγRs in immune complex clearance, and the evidence for FcγR dysfunction in SLE presents the opportunity for novel treatment strategies.

A variety of cytokines can regulate total receptor expression, modulate relative isoform predominance, and modulate receptor function (115). For example, in vivo and in vitro studies have shown that IFN-γ and G-CSF upregulate FcγRI expression (108 ,115 ,233 ,234) and that TGF-ß, IFN-γ, and M-CSF upregulate FcγRIIIa on monocytes (170 ,235) while IL-4 and IL-13 downregulate expression of all three classes of stimulatory FcγRs (189 ,236). In contrast, IL-4 increases the expression of FcγRIIb2 on monocytes (171). The identification and utilization of cytokines that increase the ratio of expression of inhibitory and stimulatory FcγR represents a new approach for the treatment of autoimmune diseases. Similarly, targeted pharmacologic manipulation of protein kinases or phosphatases may yield effective treatments.

Steroid hormones also have major effects on FcγR expression and function. Estrogens augment and progesterones inhibit (237 ,238), but steroid effects may vary with the level of activation of the mononuclear system (239). For example, glucocorticoids enhance the IFN-γ-induced augmentation of monocyte FcγR function, whereas alone they are inhibitory for normal donors (240 ,241 ,242 ,243 ,244). Interestingly, for monocytes from patients with SLE, which may be primed in vivo with IFN-γ (245 ,246 ,247), glucocorticoids enhance function. Patients with active SLE who are treated with high-dose intravenous pulse methylprednisolone have improved clearance of IgG-sensitized autologous erythrocytes, enhanced monocyte FcγR expression and phagocytosis, and decreased levels of circulating immune complexes (203). These data raise the possibility that pharmacologic therapies can act synergistically with endogenous cytokines to achieve the desired outcome.

Endogenous and pharmacologic agents also modify FcγR function either directly or as consequence of interacting with cytokines to alter net FcγR function (203 ,248). Released at sites of tissue injury, adenosine interacts with two classes of cell surface receptors on macrophages. Occupancy of high-affinity adenosine A1 receptors enhances FcγR-mediated internalization, whereas ligation of low-affinity adenosine A2 receptor is inhibitory (249). The rapid and potent modulation of FcγR-mediated function suggests that adenosine is an important local regulator of the inflammatory response, and that receptor-specific adenosine analogues may provide novel therapies for immune complex disease.

The complement split product C5a, which is generated at sites of immune complex-triggered inflammation, upregulates activating FcγRIIIa expression and downregulates inhibitory FcγRIIb expression on macrophages, providing a mechanism beyond its anaphylatoxin activity, to augment immune-mediated tissue damage (250). By altering the balance of activation/inhibitory FcγR, C5a, TNF, and IFN-γ lower the threshold of activation of effector cells. In contrast, the antiinflammatory property of intravenous gamma globulin (IVIG), a therapy for some autoimmune diseases, induces increased surface expression of FcγRIIb on macrophages in mouse models of antibody-mediated tissue injury. Disruption of inhibitory FcγRIIb either by genetic deletion or with a blocking monoclonal antibody reversed the therapeutic effect of IVIG (251). Taken together these studies indicate that modulation of inhibitory signaling, thereby raising the threshold required for immune complexes to trigger activation, is a potent therapeutic strategy for attenuating autoantibody–triggered inflammatory diseases.

Soluble FcγR may be novel anti-inflammatory agents. Circulating forms of FcγRII and FcγRIII are present in normal individuals (136 ,137). In animal models, infusion of soluble FcγR inhibits immune complex-mediated activation of phagocytes by blocking access to FcγR on effector cells (252). Circulating FcγR have also been shown to suppress IgG production by B cells and may therefore be immunosuppressive (253). With the development of soluble TNF receptors and soluble complement receptors as therapeutic modalities, one can envision the use of soluble FcγRs to block antibody-mediated tissue injury (254 ,255). The unique properties of different FcγRs—affinity and IgG subclass preference—could be exploited to target specific pathogenic antibodies with specific soluble FcγR variants.

With our increasing recognition of the role of FcγRs in the pathophysiology of SLE and such a range of receptor-modulating agents, successful therapeutic intervention will be feasible and form the basis of further advances in the treatment of SLE.

Summary

- Properties of antigens and antibodies are important in determining the fate and pathogenic potential of immune complexes.
- Efficiency of mononuclear phagocyte system immune complex clearance depends on the function of Fcγ receptors (receptors recognizing the Fc region of immunoglobulin) and the complement receptors.
- SLE is characterized by impaired Fcγ receptor-dependent and complement-dependent mononuclear phagocyte function resulting in inadequate clearance and tissue deposition of immune complexes.
- Genetic variations in Fcg receptors influence antibody binding and pathologic effects of immune complexes.

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Chapter 13

Complement and Systemic Lupus Erythematosus

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Historical Overview

The complement system is arguably linked more intimately to systemic lupus erythematosus (SLE) than to any other human disease. This association has been recognized for decades and, until recently, was viewed as inexplicably paradoxical. Two seemingly irreconcilable early observations formed the foundation for this conundrum. First, in 1951, Vaughan et al. were the first to assay serum complement in cases of SLE (1). They determined from four cases that diminished CH_{50} correlated with disease activity. Elliott and Mathieson confirmed this and noted complement depression to be particularly associated with “albuminuria” (2). Lange et al. discovered complement to be diminished in virtually all cases of acute, but not in chronic, glomerulonephritis, while low complement was characteristic of SLE whether or not there was renal involvement (3). Schur and Sandson, in the largest study to that time, found CH_{50} to be below 50% of normal in 24% of patients with active SLE, and in 46% of those with renal involvement, but in only 4% with inactive disease (4). Schur suggested that complement levels were of particular value in following and evaluating patients with SLE, especially those with nephritis. These seminal observations were followed by a large body of work from many laboratories demonstrating that complement activation, reflected by diminished serum levels of C3, C4, and CH_{50}, plays a major role in the tissue inflammation and organ damage that result from lupus pathogenesis.

Seemingly paradoxical to these findings was a second set of observations that demonstrated a strong association between hereditary homozygous deficiency of the classical pathway components and development of SLE (5 ,6 ,7). In fact, inherited complement deficiency is still recognized as conferring the greatest known risk for development of SLE. Thus, for decades this paradox has been pondered. How is it that complement deficiency may be causative in SLE, yet activation of this same inflammatory cascade is detrimental in patients who already have the disease?

Discoveries made during the past several years have begun to explain this perplexing link between complement and SLE and have concomitantly identified potential strategies and opportunities for mining the complement system for lupus genes, biomarkers, and therapeutics. In this chapter we will review the biology of the complement system in relation to SLE, summarize common methods for measurement of complement, revisit the utility of complement assays in clinical management of SLE, and consider the potential for targeting the complement system for therapeutic intervention.

Biology of the Complement System

The complement system comprises more than 30 plasma and membrane-bound proteins that form three distinct pathways (classical, alternative, and lectin) designed to protect against invading pathogens (8 ,9 ,10 ,11) (Fig. 13-1). Many of the complement proteins exist in plasma as functionally inactive pro-proteins until appropriate events trigger their activation. Once activated, the proteins within each pathway undergo a cascade of sequential serine protease-mediated cleavage events, release biologically active fragments, and self-assemble into multimolecular complexes. In general, activation of the complement system can be viewed as a two-stage process. The first stage, unique to each of the three activation pathways, involves the early complement components that lead to the formation of the C3 convertases. The second stage, common to all three pathways once they converge, results in the formation of a lytic complex consisting of the terminal complement components (Fig. 13-1).

Complement Activation Pathways

The classical pathway of complement activation, which is responsible for executing a major effector mechanism of antibody-mediated immune responses, is thought to play an important role in SLE pathogenesis. There are five proteins specific to activation of the classical pathway: C1q, C1r, C1s, C4, and C2 (Fig. 13-1). Activation of this pathway begins when C1q binds to the Fc portion of IgG (particularly IgG1 and IgG3) or IgM molecules that are bound to an antigen. The binding of C1q to an antigen-antibody complex (immune complex) leads to activation of C1r (a serine protease), which, in turn, leads to activation of C1s (also a serine protease). C1s enzymatically cleaves the other two classical pathway proteins, C4 and C2, generating and releasing two small soluble

polypeptides, C4a and C2b. At the same time, this proteolytic cleavage leads to the formation of a surface-bound bimolecular complex, C4b2a, which functions as an enzyme and is referred to as the classical pathway C3 convertase. Recent studies have shown that in addition to immune complexes, a variety of other molecules can also initiate activation of the classical pathway by interacting with C1q. These include C-reactive protein (12), amyloid P component (13), ß-amyloid protein (14 ,15), and DNA (16).

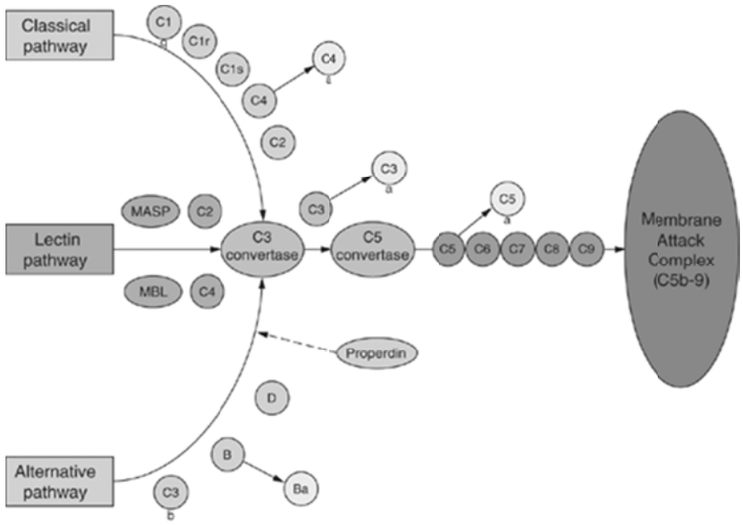

Figure 13-1. Overview of the complement system and activation pathways.

Activation of the alternative pathway is not dependent on antibodies or other specific recognition molecules. Three plasma proteins are unique to the alternative pathway: Factor B, Factor D, and properdin (Fig. 13-1). Normally, native C3 molecules undergo a so-called “C3 tickover” process, a continuous, low-rate hydrolysis of the thioester group that generates iC3* (hydrolyzed C3) and subsequently C3b fragments (17). A fraction of these spontaneously generated C3b fragments may covalently attach to the surface of microbial pathogens and host cells via thioester bonds. The bound C3b molecules are capable of binding Factor B. Once bound, Factor B is cleaved into Ba and Bb fragments by Factor D (a serine protease). While the small, soluble Ba fragment diffuses away from the activation site, the Bb fragment remains associated with C3b. Similar to the C4b2a complex in the classical pathway, the surface-bound C3bBb complex serves as the alternative pathway C3 convertase. The C3bBb complexes, if bound to mammalian cells, will be rapidly degraded by several regulatory proteins, thereby preventing self-damage of the host cells and tissue. However, the C3bBb complexes associated with microbial pathogens, which do not generally express these regulatory proteins, will remain intact and can be further stabilized by the binding of properdin.

The lectin pathway shares several components with the classical pathway (Fig. 13-1). Initiation of the lectin pathway is mediated through the binding of mannose-binding lectin (MBL; also known as mannose-binding protein [MBP]) to a variety of repetitive carbohydrate moieties such as mannose, *N*-acetyl-D-glucosamine, and *N*-acetyl-mannosamine, which are abundantly present on a variety of microorganisms (18 ,19). MBL, a plasma protein composed of a collagen-like region and a carbohydrate-binding domain, is structurally similar to C1q. As in the case of the C1qC1rC1s complex, MBL forms complexes in the plasma with other proteins such as mannose-binding protein-associated serine proteases (MASPs) (20 ,21 ,22). Under physiologic conditions, MBL does not bind to mammalian cells, probably because these cells lack mannose residues on their surface. Once bound to microbial pathogens, MASPs can cleave C4 and initiate the complement cascade. Alternatively, MBL, in place of C1q, may trigger the activation cascade by activating C1r and C1s. At this point, the lectin pathway intersects with the classical pathway and a C3 convertase, i.e., the C4b2a complex, is eventually generated.

C3 convertases, generated during the first stage of complement activation, cleave C3, the central and most abundant component of the complement system. This proteolytic cleavage gives rise to a smaller C3a fragment and a larger C3b fragment. The C3b molecules associate with C4b2a or with C3bBb complexes to form the classical and alternative C5 convertases. The C5 convertases cleave C5 and initiate activation and assembly of the terminal components, C5, C6, C7, C8, and C9, into the membrane attack complex (MAC; C5b-9) on the surface of foreign pathogens.

Effector Functions of Complement

The complement system is traditionally thought to have four biological functions in protecting against invasion by pathogens: (1) opsonization, (2) activation of

inflammation, (3) clearance of immune complexes, and (4) osmotic lysis of invading microorganisms (8 ,9 ,10 ,11). During SLE pathogenesis, complement activation and its inflammatory consequences are generated by self-antigens and autoimmune complexes rather than by foreign microbes.

The soluble proteolytic fragments, C3a, C4a, and C5a, are highly potent pro-inflammatory molecules. They attract and activate leukocytes by binding to specific receptors (e.g., C5a receptor) expressed on those cells. The larger fragments, C3b, C4b, and their derivatives (e.g., iC3b and iC4b), can remain bound to the surface of microbial pathogens (or autoantigens) and facilitate recognition and uptake of the opsonized particles by phagocytic cells. This function is mediated through the binding of these complement-derived fragments to complement receptor 1 (CR1) (for C3b and C4b), CR3 (for iC3b), and CR4 (for iC3b) expressed on phagocytes.

The binding of C4b and C3b to immune complexes also prevents their aggregation into insoluble complexes and enhances their clearance. The clearance of C3b/C4b-opsonized immune complexes is mediated by erythrocytes that express CR1 and are capable of transporting immune complexes to macrophages of the reticuloendothelial system in the spleen and liver (23 ,24). In addition, C3b/C4b-opsonized immune complexes may bind to B lymphocytes, monocytes, and neutrophils. Phagocytosis of opsonized immune complexes by neutrophils and monocytes is often accompanied by release of lysosomal enzymes. Finally, the C5b-9 MACs may perturb the osmotic equilibrium and/or disrupt the integrity of the surface membrane of target cells, thereby causing lysis of these cells.

For several decades, the role of the complement system was thought to be limited to these four effector functions. However, there has been a recent explosion in discovery of additional roles for complement, particularly as link between innate and adaptive immunity (25 ,26).

Regulators of Complement Activation and Complement Receptors

In humans and other mammals, complement activation is controlled by a redundant family of regulatory proteins to ensure that this effective machinery is not inappropriately activated by host cells and tissues (Table 13-1). To control the potent consequence of complement activation, soluble or cell-surface regulatory molecules need to act at multiple steps of the activation pathways using different mechanisms, functioning as proteolytic enzymes, cofactors for proteolytic enzymes, or competitive inhibitors of multi-molecular convertases (Fig. 13-2).

Receptors for proteolytic fragments of complement proteins (e.g., C3b, C4b, iC3b, C3d) and, in some circumstances, for complement proteins with altered conformation (e.g., C1q) are expressed by a wide spectrum of cells and serve pivotal roles in executing many of the effector functions of complement described above. Recent studies have led to the identification of at least four receptors for C1q, cC1qR (calreticulin; a collectin receptor), gC1qR, C1qRp, and CR1 (CD35) (27 ,28 ,29 ,30). Binding of C1q-opsonized immune complexes to endothelial cells via C1q receptors has been reported to induce expression of adhesion molecules on endothelial cells and thus enhance leukocyte binding/extravasation (31). On other cell types, C1q binding, presumably via distinct receptors, has been shown to enhance phagocytosis, increase generation of reactive oxygen intermediates, and activate platelets (32 ,33 ,34).

CR1 (CD35) and CR2 (CD21), two major receptors for C3- and C4-derived fragments, belong to the “Regulators of Complement Activation (RCA)” family (35 ,36). CR1 is widely expressed by erythrocytes, neutrophils, monocytes/macrophages, B lymphocytes, some T lymphocytes, and glomerular podocytes (37 ,38 ,39). CR1 binds primarily C3b and C4b. One important function of CR1 expressed on erythrocytes is to bind and clear immune complexes (23 ,24).CR1 also plays a role in regulation of complement activation by serving as a cofactor for Factor I, which is responsible for cleaving C3b and C4b to iC3b and iC4b (40 ,41). CR2 is expressed mainly on B lymphocytes, activated T lymphocytes, and follicular dendritic cells, and binds primarily iC3b, C3dg, and C3d (42 ,43 ,44 ,45 ,46). CR2, together with its cognate complement ligands, is a critical link between the innate and adaptive immune systems (26 ,47). For example, coligation of CR2 (as part of the CD19/CD21/CD81 B cell coreceptor complex) and B cell receptors on the surface of B lymphocytes via the binding of C3d-decorated immune complexes or antigens enhances B cell activation, proliferation, and antibody production (47 ,48 ,49). Antigens and immune complexes opsonized by C3-derived fragments can be retained in the germinal centers of secondary lymphoid follicles via binding to CR2 expressed on follicular dendritic cells (43 ,44); the retained antigens provide essential signals for survival and affinity maturation of B cells as well as for generation of memory B cells (50).

CR3 and CR4 belong to the ß2 integrin family and are composed of two subunits: a common ß chain (CD18) and a specific α chain (CD11b in CR3 and CD11c in CR4). These receptors are expressed on phagocytic cells (e.g., neutrophils, monocytes, and macrophages), antigen-presenting cells (e.g., dendritic cells), and follicular dendritic cells (51 ,52). CR3 and CR4 not only play important roles in phagocytic removal of C3-opsonized pathogens, but also participate in mediating adhesion of mononuclear phagocytes to endothelial cells (53 ,54 ,55).

Complement and SLE

The involvement of complement in the etiopathogenesis of SLE has been scrutinized over the past several decades. Suffice it to say that the role of complement in SLE is both complex and paradoxically intriguing. On the one hand, activation of the complement system is thought to play an important role in tissue inflammation/damage in SLE as a consequence of tissue deposition of immune complexes formed by autoantigens and autoantibodies (56 ,57 ,58). On the other hand, a hereditary deficiency of a component of the

classical pathway (C1, C2, or C4) has been associated with the development of SLE (5 ,6 ,7 ,59 ,60 ,61). These seemingly discordant roles for complement may be reconciled by studies performed during the past several years. Those studies have demonstrated that, while the complement system plays a role in maintaining immune tolerance to prevent the development of SLE (59 ,61 ,62), it also participates in tissue-destructive inflammatory processes once SLE is established in a patient (56 ,57).

Table 13-1: Components of the Human Complement System

Effector Protein	Function/Pathway Involved	Mr (kD)
C1q	Recognition, binding/classical	450 (a six-subunit bundle)
C1r	Serine protease/classical	85
C1s	Serine protease/classical	85
C4*	Serine protease (C4b); anaphylatoxin (C4a)/classical	205 (a 3-chain, αβγ, complex)
C2	Serine protease (C2a); small fragment with kinin-activity (C2b)/classical	102
C3**	Membrane binding, opsonization (C3b); anaphylatoxin (C3a)/terminal	190 (a 2-chain, αß, complex)
C5	MAC component (C5b), anaphylatoxin (C5a)/terminal	190 (a 2-chain, αß, complex)
C6	MAC component/terminal	110
C7	MAC component/terminal	100
C8	MAC component/terminal	150 (a 3-chain, αβγ, complex)
C9	MAC component/terminal	70
Factor B	Serine protease/alternative	90
Factor D	Serine protease/alternative	24
Properdin	Stabilizing C3bBb complexes/alternative	55 (monomers); 110, 165, 220, or higher (oligomers)
MBL	Recognition, binding/lectin	200–400 (2–4 subunits with three 32 KD chains each)
MASP-1	Serine protease/lectin	100
MASP-2	Serine protease/lectin	76
Soluble Regulatory Protein	**Function**	**Mr (kD)**
C1-inhibitor (C1-INH)	Removing activated C1r and C1s from the C1 complex	105
C4-binding protein (C4bp)	Displacing C4b in the C4b2a complex; cofactor for factor I	570 (a 7-subunit complex)
Factor H	Displacing Bb in the C3bBb complex; cofactor for factor I	160
Factor I	Serine protease cleaving C3b and C4b	88
Clusterin	Preventing insertion of soluble C5b-7 complexes into cell membranes	70
S protein (vitronectin)	Preventing insertion of soluble C5b-7 complexes into cell membranes	84
Carboxypeptidase N	Inactivating anaphylatoxins	280 (a multi-subunit complex)
Membrane-Bound Regulatory Protein	**Function**	**Mr (kD)**
CD35 (CR1†)	Binding C3b and C4b; cofactor for factor I	160–250 (4 isoforms)
CD46 (MCP‡)	Promoting C3b and C4b inactivation by factor I	45–70 (different glycosylation forms)
CD55 (DAF§)	Accelerating decay of the C3bBb and C4b2a complexes	70
CD59 (protectin; H19)	Preventing C9 incorporation into the MAC in a homologous restriction manner	18–20
Complement Receptor	**Structure/Mr (kD)**	**Complement Ligand(s){ParaMarks}**
CR1 (CD35)	Single chain; 190–280¶	C3b; C4b; iC3b; C1q
CR2 (CD21)	Single chain; 140–145	C3dg/C3d; iC3b;
CR3 (CD11b/CD18)	2-chain,α/ß; 170/95	iC3b
CR4 (CD11c/CD18)	2-chain, α/ß; 150/95	iC3b
cC1qR (calreticulin)	Single chain; 60	C1q (collagenous tail); MBL
gC1qR	Tetramer; 33/subunit	C1q (globular head)
C1qRP	Single chain; 126	C1q (collagenous tail)
Complement Receptor	**Structure/Mr (kD)**	**Complement Ligand(s){ParaMarks}**
C3a receptor	Single chain; 50?	C3a
C5a receptor (CD88)	Single chain; 50	C5a

*Serum concentration range considered normal: 20–50 mg/dL
**Serum concentration range considered normal: 55–120 mg/dL
†Complement receptor 1
‡Membrane cofactor protein
§Decay accelerating factor
{ParaMarks}Noncomplement ligands (e.g., Epstin-Barr virus for CR2 and fibrinogen for CR3 and CR4) not listed.
¶Four allotypes with different numbers of SCR and displaying distinct Mr under reducing condition: CR1-A (220 kD), CR1-B (250 kD), CR1-C (190 kD), and CR1-D (280 kD).

Immune Complex Abnormalities, Complement Activation, and SLE

Considerable evidence has indicated that many of the clinical manifestations and pathology in patients with SLE can be attributed to immune complex abnormalities (e.g., decreased solubility and impaired disposal of immune complexes) and consequent complement activation. In patients with SLE, decreased serum levels of C3 and C4 (because of genetic or acquired factors, or both) may not permit sufficient binding of C3 and C4 fragments to the antigen-antibody lattice and thereby prevent the formation of small, soluble immune complexes (63 ,64). Furthermore, reduced levels of CR1 on erythrocytes, frequently detected in patients with SLE, may lead to impaired binding, processing, and transporting of immune complexes to phagocytes of the reticuloendothelial system (see further discussion below). Consequently, abnormally large quantities of immune complexes are likely to circulate for prolonged periods of time and form insoluble aggregates that may deposit in various tissues. Deposited immune complexes do not seem to cause tissue damage directly but provide ample binding sites for complement components. The ensuing activation of the complement system causes the release of various mediators, promotes cellular infiltration and interaction, and culminates in tissue damage. The vascular endothelium and glomerular basement membrane appear to be highly susceptible to this mode of inflammatory damage. This pathogenic sequence provides a molecular basis underlying vasculitis and glomerulonephritis, two hallmark manifestations of SLE.

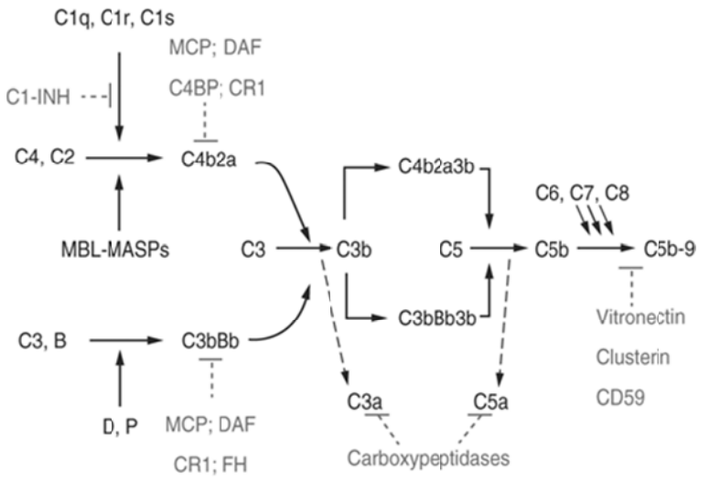

Figure 13-2. Overview of the regulation of complement activation. Regulatory proteins and their action sites are delineated in gray.

Complement Deficiency and SLE

Hereditary Complement Deficiency

Hereditary complement deficiency in humans has been reported for almost every component of the complement system (60 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82). Although the overall incidence of hereditary complement deficiency is low in the general population, a deficiency of any complement component is significantly associated with specific human diseases (Table 13-2). The clinical manifestations associated with the hereditary deficiency of individual complement components vary widely. Patients with homozygous deficiency of the early components of the classical pathway, C1, C4, and C2, are particularly at risk for development of SLE (6 ,7 ,60 ,65 ,66 ,67 ,68 ,75 ,78 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94).

In humans, there are two isotypes of C4, C4A and C4B (95 ,96 ,97). Complete C4 deficiency (homozygous deficiency of both C4A and C4B) is extremely rare. It was first reported in 1974 in a patient who manifested an acute SLE-like disease (5), and a total of 24 cases have since been reported (7). However, homozygous deficiency of C4A alone and heterozygous deficiency of C4A and/or C4B is relatively frequent (7 ,66 ,98). Increased incidence of deficiency of C4A and, less commonly, of C4B, has been reported in patients with SLE (83 ,88 ,89 ,90 ,91 ,92 ,99 ,100 ,101). A review of clinical cases of SLE

associated with complement deficiency revealed a hierarchical correlation between the position of the deficient component within the classical pathway and the prevalence of SLE. It has been estimated that SLE occurs in approximately 90%, 80%, and 30% of patients deficient in C1q, C4, and C2, respectively (7) (Table 13-3). This risk for SLE in complement deficient individuals is greater than concordance of this disease in monozygotic twins (102). This observation indicates that the classical complement pathway loci encode “lupus genes.”

Table 13-2: Complement Component Deficiencies and Associated Diseases

Deficient Component	Functional Defects	Associated Diseases
C1	Impaired clearance of immune complexes and apoptotic cells	SLE*,**; glomerulonephritis; bacterial infections
C2	Impaired clearance of immune complexes	SLE*,**; glomerulonephritis; bacterial infections
C4	Impaired clearance of immune complexes	SLE*,**; glomerulonephritis; scleroderma, Sjogren syndrome; bacterial infections
C3	Impaired opsonization; impaired clearance of apoptotic cells	Bacterial infections*,†; SLE‡
C5	Impaired chemotaxis; absent lytic activity	Bacterial infections†
C6	Absent lytic activity	Bacterial infections†
C7	Absent lytic activity	Bacterial infections†
C8	Absent lytic activity	Bacterial infections†
C9	Impaired lytic activity	Bacterial infections†
Properdin	Impaired alternative pathway activation	Bacterial infections†
MBL	Impaired lectin pathway activation	Bacterial and viral infections*; SLE
C1-inhibitor	Excessive C2 and kininogen activation	Hereditary angioedema*; SLE
Factor H	Excessive alternative pathway activation	Hemolytic uremic syndrome; bacterial infections
Factor I	Excessive alternative pathway activation	Bacterial infections

*Predominant phenotype

**Risk hierarchy for developing SLE: C1 deficiency (~90%) >C4 deficiency (~80%) >C2 deficiency (~30%)

†Most frequently infections with encapsulated bacteria, especially *Neisseria meningitidis*

‡SLE occasionally reported for patients with homozygous deficiency

Clinically, patients with SLE associated with homozygous C1q or C1r/s deficiency usually present with symptomatic disease at an early age (younger than 20), have severe disease with prominent cutaneous manifestations, and do not exhibit the usual female predilection (see (7) for a comprehensive review). Similarly, SLE associated with homozygous C4 deficiency often occurs at an early age and manifests cutaneous lesions more frequently than renal disease (7). Patients with SLE and homozygous C2 deficiency also feature less renal involvement but have more cutaneous involvement (especially photosensitivity) and arthralgia. Serologically, the prevalence of ANA and anti-dsDNA antibodies is often lower in patients with complement deficiency-associated SLE than in patients with idiopathic SLE, but the presence of anti-Ro antibodies appears to be common in SLE associated with C2 or C4 deficiency (7).

Table 13-3: Homozygous Complement Deficiency and SLE

Deficient Component	No. of Patients Reported	No. of Patients with SLE (and SLE-like disease)	Prevalence of SLE (and SLE-like disease) (%)	Sex Ratio (F-to-M)	Reference(s)
C1q	42	39	93	1.3:1	(7)
C1r/s	14	8	57	1.7:1	(7)
C4	26	21	81	2:1	(7,99)
C2	77	24	32	7:1	(7)
C3	24	4	16	3:1	(7,106,107)

In contrast to the high incidence of SLE and SLE-like disease in patients with homozygous deficiency of the classical pathway components, patients with homozygous C3 deficiency seldom develop SLE. Of the reported 24 cases of C3

deficiency, only 4 patients were described to have SLE-like disease and all were negative for ANA (7 ,103 ,104 ,105 ,106 ,107). As for terminal complement components, there have been occasional case reports of patients with SLE and C6 deficiency (76 ,108), C7 deficiency (77), C8 deficiency (72), and C9 deficiency (80). However, the predominant phenotype of homozygous deficiencies of terminal complement components is recurrent infections (Table 13-2).

MBL, the initiating component of the lectin pathway, is structurally and functionally homologous to C1q (18 ,19). Polymorphisms in the promoter and coding regions of the MBL gene that lead to altered serum levels of MBL or encode defective MBL proteins incapable of activating the complement system have been reported (109). Like C1q deficiency, MBL deficiency has been associated with increased susceptibility to SLE (110). Increased frequencies of MBL gene variants encode defective MBL protein (because of changes in amino acid 54 and 57) have been found in SLE patients of different ethnic origins (111 ,112 ,113 ,114). Associations between low serum levels of MBL and SLE have also been reported (112 ,115 ,116). Clinically, MBL deficiency in patients with SLE appears to increase their susceptibility to infections (117).

Acquired Complement Deficiency

Complement deficiency may also occur as an acquired phenomenon. When complement is increasingly consumed during heightened active states of an underlying disease, acquired deficiency can occur and usually involves multiple components simultaneously. For example, patients with hereditary deficiency of C1-inhibitor (C1-INH) suffer from episodes of angioedema (Table 13-2). In these patients, C1-INH deficiency causes unregulated activation of the kininogen system and the complement system (C1r and C1s directly and C4 and C2 consequently). As a result of the unchecked complement activation, patients with C1-INH deficiency have reduced serum levels of C4 and C2. Interestingly, development of SLE in patients with C1-INH-deficiency has been reported (118 ,119 ,120 ,121). These reports not only echo the association between hereditary complement deficiency and SLE described above, but also underscore the notion that early components of the complement classical pathway play a protective role against development of SLE.

Table 13-4: Phenotypes of Mice Deficient in Complement or Complement Receptors

Mouse Model	Phenotype	References
C1q-/- (on 129/B6 hybrid background)	Accumulation of apoptotic bodies in glomeruli; spontaneous development of autoantibodies against nuclear antigens; development of glomerulonephritis; impaired clearance of apoptotic cells in inflamed and noninflamed peritoneum	(129,130,131)
C1q-/-/*lpr*	Similar to the above strain; development of accelerated, severe autoimmune disease	(131)
C4-/- (on 129/B6 hybrid background)	Development of antinuclear autoantibodies; impaired clearance of apoptotic cells by macrophages in inflamed peritoneum	(130,132,133)
C4-/-/*lpr*	Similar to the above strain; development of glomerulonephritis, lymphadenopathy, and splenomegaly	(135,136)
C3-/-	Increased susceptibility to bacterial infections	(134,135)
C3-/-/C4-/-/*lpr*	Similar to those of C4-/-/*lpr* mice	(135)
C2-/-/Factor B-/-	No development of spontaneous autoimmunity	(279)
C1q-/-/C2-/-/Factor B-/-	Development of antinuclear autoantibodies and glomerulonephritis	(280)
Cr2-/-	Abnormal T cell–dependent antibody response; no spontaneous autoimmunity	(185,186)
Cr2-/-/*lpr*	Development of antinuclear autoantibodies, anti-dsDNA autoantibodies, and glomerulonephritis;	(136,187)

In patients with SLE, autoantibodies against complement components have also been found to cause acquired complement deficiency. For example, C3 nephritic factor, an autoantibody capable of stabilizing the alternative pathway C3 convertase BbC3b, can cause consumption of complement proteins via unregulated activation of the alternative and terminal pathways (122 ,123). Another autoantibody reactive with the first complement component, C1q, has been detected at increased frequencies in patients with SLE (124 ,125). A significant portion of patients with SLE also develop functional C1q deficiency secondary to the presence of anti-C1q antibodies (7). Although the pathophysiologic role of anti-C1q in SLE is largely unknown, its presence in patients with SLE has been associated with lupus nephritis (126 ,127 ,128).

Animal Models of Complement Deficiency

Clinical studies of patients with SLE and complement deficiency strongly suggest that early components of the classical pathway play a physiologic role in protecting against the development of SLE, a role that seems to be independent of C3. The observed association between early complement component deficiency and susceptibility of SLE in humans has been corroborated by recent animal studies. Several laboratories have generated mice deficient in individual complement proteins and complement receptors using gene-targeting techniques (Table 13-4). It has been reported

that C1q-knockout (C1q-/-) and C4-knockout (C4-/-) mice on the129/C57BL6 (B6) hybrid genetic background spontaneously developed higher levels of antinuclear autoantibodies (ANA) than strain-matched wild-type mice (129 ,130 ,131 ,132 ,133). Moreover, C1q-/- mice developed histopathologic changes resembling glomerulonephritis by 7 to 8 months of age (129 ,131). The development of autoimmune disease in C1q-/- and C4-/- mice appeared to be dependent on the genetic background of the knockout mice. Neither C1q-/- mice and C4-/- mice on the B6 background nor those on the 129 background exhibited autoimmune phenotypes (131 ,132). C1q-/- mice back-crossed onto the MRL/*lpr* (a mouse model of SLE) background developed accelerated, severe autoimmune disease (131). These findings together suggest that susceptibility genes other than C1q or C4 are required for the expression of SLE-like disease in mice.

In contrast to C1q-/- and C4-/- mice, C3-knockout (C3-/-) mice on the 129/B6 hybrid background were not found to develop autoimmune phenotypes (132 ,134 ,135). In a recent study, *lpr* mice deficient in C4, C3, or both were generated by cross-breeding and were examined for the development of autoimmune phenotype/disease (135 ,136). Autoimmune disease, characterized by marked splenomegaly, lymphadenopathy, high titers of ANA and anti-dsDNA, and glomerulonephritis, were noted to develop in C4-/-/*lpr* as severely as in C4-/-/C3-/-/*lpr* mice, but the disease did not occur in C3-/-/*lpr* mice. These findings suggest that deficiency of C4, but not C3, predisposes to the development of SLE in mice.

In summary, the results of animal studies, in accord with the clinical observations in humans, suggest that the early components of the complement classical pathway, particularly C1 and C4, play a protective role against the development of SLE. The animal studies have also provided insightful clues to the potential mechanisms linking complement deficiency and SLE, which will be discussed in the next section.

Possible Mechanisms Underlying the Complement Deficiency-SLE Association

Currently, there are three non-mutually exclusive hypotheses explaining the intriguing clinical association between complement deficiency and SLE. The first hypothetical mechanism envisions that impaired clearance of immune complexes in the absence of early complement components may trigger/augment the development of SLE. It is interesting to note that of the two isotypes of human C4, C4A has predominantly been implicated in the binding and solubilization of immune complexes (137 ,138 ,139). Consequently, it is not unexpected that the prevalence of C4A deficiency is reportedly higher in SLE patients than in the general population (83 ,89 ,99). Several studies have demonstrated abnormal processing of immune complexes in SLE patients (140 ,141 ,142 ,143). These studies showed that the initial clearance of immune complexes was impaired in the spleen, supporting the concept that impaired clearance of immune complexes may contribute to the development of SLE in the context of complement deficiency.

In 1996, a second hypothetical mechanism was proposed to explain the link between complement deficiency and development of SLE (144 ,145). This hypothesis was based upon the discovery that C1q can bind directly to apoptotic keratinocytes. Subsequent observations in support of this hypothesis demonstrated that endothelial cells and monocytes that are undergoing apoptosis also bind C1q (146) (Fig. 13-3), and this binding can subsequently trigger activation and deposition of C4 and C3 on these apoptotic cells (147 ,148). Thus, apoptotic cells and blebs become opsonized and can be effectively taken up by phagocytic cells via a complement receptor-mediated mechanism (147 ,148). During apoptosis, normally hidden intracellular constituents are often biochemically modified and redistributed/segregated into surface blebs of dying cells (149 ,150 ,151). Impaired clearance of apoptotic cells because of complement deficiency may lead to persistence of such “altered-self” constituents, which may be recognized by the immune system, breach immune tolerance, and trigger autoimmune responses (152). Taken together, these studies suggest that complement is involved in facilitating the clearance of autoantigen-containing apoptotic bodies and therefore plays a pivotal role in maintaining immune tolerance (7 ,59 ,61 ,153).

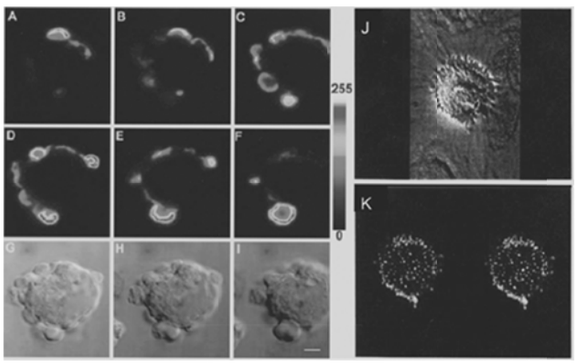

Figure 13-3. (See color plate.) Binding of C1q to apoptotic endothelial cells and keratinocytes. Panels **A–F,** Confocal analysis of C1q present on surface blebs of a human umbilical vein endothelial cell undergoing UVB-induced apoptosis. Shown are six consecutive cross-sections through the cell stained for the presence of C1q by indirect immunofluorescence. The fluorescence intensity is represented by a color scale, with white being the highest intensity and black the lowest. **G–I**, Different interference contrast images of panels **D–F** are shown to visualized morphology of the entire cells and apoptotic blebs. **J** and **K**, Confocal analysis of bound C1q on apoptotic human keratinocytes. Shown are a merged phase and fluorescence image of a single plane of an apoptotic keratinocyte **(J)** and a stereo image of a Z-series of several planes though a single apoptotic keratinocyte **(K)**. (Modified from

Korb LC, Ahearn JM. C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes. Complement deficiency and systemic lupus erythematosus revisited. *J Immunol* 1997;158:4525–4528;

and

Navratil JS, et al. The globular heads of C1q specifically recognize surface blebs of apoptotic vascular endothelial cells. *J Immunol* 2001; 166:3231–3239;

Using a mouse model, Botto et al. were first to report accumulation of apoptotic cells in the kidneys and spontaneous development of autoimmune responses to nuclear autoantigens and glomerulonephritis in the absence of C1q (129). Subsequently, Taylor et al. reported similar, but less severe, defects in the clearance of apoptotic cells and spontaneous autoantibody production in C4-deficient mice (130). Results from these animal studies suggest that clearance of apoptotic cells is impaired or delayed in the absence of C1q, and less so in the absence of C4 (Table 13-4). Likewise, the persistence of apoptotic cells may lead to development of autoimmunity and tissue damage in humans. Reduced phagocytic activity of neutrophils, monocytes, and macrophages of SLE patients has previously been observed (154 ,155 ,156). Specifically, a reduced capacity of SLE-derived macrophages to phagocytose apoptotic cells was reported by Hermann et al. (157). Moreover, impaired clearance of iC3b-opsonized apoptotic cells in vitro has been reported using monocyte-derived macrophages prepared from SLE patients (158). Evidence has also been generated in vivo to support impaired clearance of apoptotic cells in human SLE. Bermann et al. reported an abnormal accumulation of apoptotic cells, accompanied by a significantly decreased number of tangible body macrophages (cells responsible for removing apoptotic nuclei), in the germinal centers of lymph nodes in a small subset of SLE patients (159). Collectively, data from both animal and human studies not only substantiate the observed hierarchical correlations between the deficiency of C1, C4, or C2 and the risks for developing SLE, but also provide a strong mechanistic basis linking complement deficiency and SLE.

The third hypothetical mechanism relates to the capacity of complement to determine activation thresholds of B and T lymphocytes, suggesting that complement deficiency may alter the normal mechanism of negative selection of self-reactive lymphocytes (26 ,62 ,136 ,160 ,161). Since coligation of *CR2* and BCR augments B cell activation by decreasing the threshold to antigenic stimulation (47), it has been postulated that self-antigens not opsonized by C4b or C3b are unlikely to trigger sufficient activation of self-reactive B cells, and, as a result, these cells may escape negative selection. The escaped cells may become activated once encountering relevant autoantigens in the periphery, and thus breach self-tolerance to autoantigens.

CR1 Deficiency in SLE

Several investigators have reported reduced levels of CR1 expressed on erythrocytes of patients with SLE (162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174). Whether this deficiency in erythrocyte-CR1 (E-CR1) is genetically determined, acquired, or both has been controversial. Earlier studies performed in the 1980s have led some investigators to conclude that erythrocyte CR1 deficiency is inherited (163 ,165 ,166 ,167). Subsequent studies by other investigators using SLE patients of various ethnic backgrounds, however, have suggested that erythrocyte CR1 deficiency is an acquired phenomenon (162 ,164 ,168 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176). In some studies, reduced E-CR1 levels were shown to correlate with disease activity (162 ,168 ,170 ,174). These conflicting results may originate, in part, from differences in experimental methods and ethnic populations used in different studies.

To elucidate the molecular basis of the observed differential erythrocyte CR1 expression in SLE patients versus healthy individuals, Wilson and colleagues examined E-CR1 levels in SLE patients and their relatives (165). They found that a significant number of patients' relatives had reduced E-CR1 levels, and three patterns of high, intermediate, and low E-CR1 levels could be identified in both SLE patients and controls. Family studies indicated that E-CR1 levels were genetically regulated in a biallelic codominant manner. Subsequently, the same investigators, using DNA probes for the CR1 gene and restriction fragment length polymorphism (RFLP) studies, demonstrated the presence of two HindIII-digested CR1 gene fragments, 7.4 kb and 6.9 kb in length, respectively (177). It was shown that the 7.4 kb fragment (H allele) correlated with high E-CR1 levels, whereas the 6.9 kb fragment (L allele) was associated with low E-CR1 levels. A heterozygous 7.4 kb/6.9kb (H/L) pattern correlated with intermediate E-CR1 levels. These results are consistent with the originally proposed biallelic codominant fashion of inherited E-CR1 expression (165). It should be pointed out, however, that E-CR1 levels were considerably lower in SLE patients than in healthy individuals with the matched HindIII RFLP pattern, suggesting that additional genetic or nongenetic factors may influence E-CR1 expression in SLE patients (166). Although it was initially postulated that increased prevalence of the HindIII L/L genotype may be associated with SLE, subsequent studies have not supported this theory. Several studies have reported that gene frequencies of HindIII H and L alleles do not differ significantly between SLE patients and normal controls (162 ,169 ,172 ,173 ,174), suggesting that the L/L genotype does not increase susceptibility to SLE.

Other lines of evidence that support the possibility of acquired low E-CR1 levels in SLE patients include: (1) normal erythrocytes transfused into SLE patients lost significant amounts of CR1 within a few days after transfusion (178); (2) CR1 levels on reticulocytes (the youngest form of erythrocytes) of SLE patients were equivalent to those on reticulocytes of normal individuals (179); and (3) significantly greater loss of E-CR1 in the peripheral circulation was observed in SLE patients than in normal controls (179).

In summary, experimental and clinical studies strongly indicate that both genetic and acquired factors contribute to the observed deficiency of erythrocyte CR1 in SLE patients. However, the precise nature of these factors remains to be elucidated.

Abnormal Expression of CR2 and CR3 in SLE

Relative deficiency of *CR2* has been observed in patients with SLE. Expression of *CR2* on lymphocytes was found to be approximately 50% to 60% lower in patients with SLE than in healthy controls (180 ,181 ,182). It is unclear whether decreased *CR2* expression correlates with increased activity of SLE and, if so, whether it is a cause or a result. Animal

studies, nevertheless, have shown that decreases in CR1/CR2 expression preceded the development of clinically apparent disease in MRL/*lpr* mice (183). This finding suggests that decreased CR1/CR2 expression may be involved in the initiation or progression of autoimmune disease.

The role of CR2 in the development and pathogenesis of SLE has been investigated using gene-knockout mice. In mice, CR2 and CR1 are encoded by the same gene through differential splicing of the RNA transcripts (184). Therefore, CR2-deficient (Cr2-/-) mice generated by gene-targeting techniques are also deficient in CR1. Initial studies have shown that Cr2-/-mice on the 129/B6 hybrid background did not develop an autoimmune phenotype (185 ,186). However, when back-crossed with B6/*lpr* mice (a mouse model of SLE), Cr2-/-/*lpr* mice on the 129/B6 hybrid background exhibited more aggressive disease at earlier time points than did B6/*lpr* mice (136), featuring marked splenomegaly and lymphadenopathy, increased ANA and anti-dsDNA titers, and increased immune complex deposition in renal glomeruli. In comparison, Cr2-/-/*lpr* mice on the B6 background developed autoantibodies but not other manifestations (187). Recent studies of SLE susceptibility genes using the NZM2410 mouse model further support an important role of CR2 in the development of autoimmune phenotypes (188). Boackle et al. recently identified that Cr2 is a candidate gene within the NZM2410 Sle1c locus (189 ,190). These investigators showed that B6 mice congenic for the Sle1c locus expressed dysfunctional CR1 and CR2 proteins, which are encoded by a defective Cr2 gene with a single nucleotide mutation. Although these mice developed autoantibodies to chromatin, they did not develop glomerulonephritis. Collectively, these results indicate that the Cr2 gene alone is insufficient to induce expression of a full spectrum of autoimmune phenotype/disease in lupus-prone mice, which apparently requires the contribution of additional SLE susceptibility genes.

With respect to CR3, reduced expression of CR3 on lymphocytes of patients with active SLE has previously been reported (191 ,192). Clinical manifestations of these patients included prominent cutaneous changes associated with immune vasculitis and panniculitis, arthritis, serositis, and nephritis. Although congenital deficiency of CR3 (known as “leukocyte adhesion deficiency”) is associated with increased susceptibility to pyogenic bacterial infections (193 ,194), the pathophysiologic significance of decreased CR3 expression in SLE remains to be elucidated. In contrast to the reported decreased CR3 levels on lymphocytes, one study showed increased levels of CR3 on neutrophils of patients with SLE (195). The highest neutrophil CR3 levels were detected in patients with the most severe disease, and in some patients the increased CR3 levels on neutrophils returned to normal when disease flares subsided. Based on these findings, it was postulated that neutrophils of patients with active SLE increase surface expression of CR3 in response to complement activation and these activated neutrophils may contribute to endothelial injury in SLE (195).

Measurement of Complement

During clinical inflammatory states in which complement activation occurs, e.g., flares of SLE, complement proteins would presumably be consumed at a rate proportional to activity of the disease. Thus, measuring complement activation may be useful for diagnosing disease, assessing disease activity, and determining response to therapy. Measuring complement activity and individual component levels is also essential for detecting and diagnosing complement deficiency. Conventionally, the complement system is measured by one of two types of assays. Functional assays measure complement-mediated hemolytic activity: CH_{50} (indicative of the activity of the classical pathway) and APH_{50} (indicative of the activity of the alternative pathway). Immunochemical assays measure serum concentrations of individual complement components and their proteolytic fragments (hereafter referred to as “complemented split products” or “complement activation products”).

Measurement of Complement Functional Activity

Assays that measure complement-mediated hemolysis, such as the CH_{50} and APH_{50} assays, are simple quantitative tests for functional complement components in serum or other fluid samples. Because complement activation in SLE is triggered predominantly by immune complexes that active the classical pathway, it is common to check the CH_{50} in patients with SLE. Complement activity is quantified by determining the dilution of a serum (or other fluid sample) required to lyse 50% of sheep erythrocytes sensitized with anti-sheep IgM (for CH_{50} assays) or unsensitized rabbit erythrocytes (for APH_{50} assays) under standard conditions. The reciprocal of this dilution represents complement activity in units per mL of serum. For example, if a 1:160 dilution of a serum sample lyses 50% of erythrocytes, complement activity in that sample is reported as 160 CH_{50} U/mL. Because some complement components are heat-labile, serum samples, if not used immediately, should be stored at –70°C to optimize the preservation of complement proteins in functionally active forms.

Measurement of Complement Proteins

Measurement of serum levels of individual complement components is commonly used to diagnose and assess disease activity in SLE. These tests also help to identify deficiencies of specific complement proteins.

Traditionally, serum is used for complement measurements. As cautioned above for the functional hemolysis assays, serum samples should be handled promptly and carefully to minimize possible degradation of complement proteins. A number of immunochemical methods, which are generally based on the antigen-antibody reactivity between complement components in the test sample and added anticomplement antibodies, are available for such measurement.

The selection of a proper method depends on several factors such as the level of sensitivity required, the availability of specific antibody, the number of samples, and the types of samples. In most clinical immunology laboratories, nephelometry is routinely used to measure complement components that are present at relatively high concentrations in the serum (e.g., C3 and C4). Other components that are usually present at low concentrations (e.g., C1, C2, C5 through C9, Factor B, Factor D, properdin) can be measured using radioimmunodiffusion (RID) or enzyme-linked immunoabsorbent assay (ELISA). When C3 and C4 concentrations are too low to be measured accurately by nephelometry, i.e., <20 mg/dL and <10 mg/dL, respectively, RID is the alternative method of choice. For other body fluids or cell culture supernatants, in which the levels of complement components may be very low, ELISA is the most practical method to use.

Measurement of Complement Split Products

Measurement of serum concentrations of complement components is essentially a static appraisal of an extremely dynamic process that includes activation, consumption, catabolism, and synthesis of these components. Unlike their parental molecules, which are also acute phase proteins (196 ,197), complement split products are generated only when complement activation occurs, and thus acute phase responses alone do not increase their concentrations. Therefore, direct determination of complement split products is thought to reflect more precisely the activation process of complement in vivo and hence the disease activity. Measures of complement split products in the plasma, yielded from activation of the classical pathway (C1rs-C1 inhibitor complex, C4a, and C4d), alternative pathway (Bb and C3bBbP), lectin pathway (C4a and C4d), and terminal pathway (C3a, iC3b, C3d, C5a, and sC5b-9), are currently performed in many clinical immunology laboratories.

When measuring complement split products, plasma, instead of serum, should be used. Plasma (EDTA-anticoagulated) is used to avoid generating complement split products in vitro. Since only low levels of complement split products may be present in the circulation, even after significantly increased complement activation, ELISA and EIA are the most practical methods for their measurement. Complement split products have different, but commonly short, half-lives in the plasma. Some split products, such as C3a, C4a, and C5a, are quickly converted to more stable, less active forms, such as C3a-desArg, C4a-desArg, and C5a-desArg. In comparison, complement products that form multi-molecular complexes usually have a relatively long half-life in the circulation. Examples of the latter include C1rs-C1 inhibitor complexes (products of classical pathway activation), C3bBbP complexes (products of alternative pathway activation), and sC5b-9 (the ultimate product of complement activation). sC5b-9 is the soluble form of MAC that consists of C5b, C6, C7, C8, poly-C9, and the solubilizing protein, protein S.

Complement as a Source of Biomarkers for SLE Diagnosis and Monitoring

Complement Measurement and SLE Activity

Since Vaughan et al. first reported an association between decreased complement proteins and active SLE five decades ago (1), most patients with SLE are commonly monitored by measures of serum C3, serum C4 levels, and complement hemolytic activity (Table 13-5). Numerous studies have been conducted to evaluate the potential utility of these assays in the diagnosis and monitoring of SLE. A succinct review of noteworthy data and precautions is outlined below.

First, although it has generally been thought that decreased levels of complement components reflect activation of the classical and/or alternative pathway and correlate with clinical disease activity, there is still no consensus regarding the actual value of complement measures in SLE monitoring. Some investigators have found CH_{50} as well as serum C4 and C3 levels to be valuable as markers of SLE activity, while others have found them minimally useful (Table 13-6). Evidence in support of the usefulness of CH_{50}, C3 and C4 measurement include the following observations: (1) significantly decreased levels of CH_{50} and serum C3 and C4 have been associated with increased SLE disease activity manifested by active nephritis and extra-renal involvement (4 ,198 ,199 ,200 ,201 ,202 ,203); (2) an increase/decrease in serum C3 levels has coincided significantly with remission/relapse of lupus nephritis (201 ,202 ,203); (3) a decrease in serum C4 levels has been noted to precede clinical exacerbation (199 ,202 ,204); (4) progressive fall of serum C3 or C4 levels may indicate an impending flare of SLE (205); and (5) serum C3 and C4 levels have frequently normalized on resolution of disease flares (199). On the contrary, the following observations argue against the usefulness of conventional complement measurement: (1) serum C4 and C3 levels have been found to remain normal in some patients during disease flares (206 ,207); (2) persistently low C4 levels have been detected in SLE patients with inactive disease (199 ,200 ,208 ,209); (3) decreases in C3 and C4 have not always been accompanied by increases in the split products (e.g., C3a, C3d, and C4d); and (4) the extent of changes in serum C3 and C4 levels do not correlate quantitatively with disease severity (210 ,211 ,212).

Table 13-5: Common Profiles of Complement Measurement in SLE and Other Inflammatory Conditions

Pathway(s) Involved	CH_{50}	APH_{50}	C3	C4	Factor B
Classical	↓	N	↓	↓	N
Alternative	N	↓	↓	N	↓
Classical/Alternative	↓	↓	↓	↓	↓

Table 13-6: Select Studies of Complement Measures in SLE

Complement Component(s)	Study Design	Results/Conclusions
CH_{50}, C1q, c3, C4	Lloy and Schur (199); prospective study following 27 SLE patients through 47 cycles of clinical activity; serologic tests: CH_{50}, C1q, C3, C4, anti-dsDNA, and immune complex (C1q binding activity) levels	CH_{50}, C3, C4 levels lower in patients with active renal disease than in patients with extra-renal involvement; decreasing C4 levels preceded disease flares
CH_{50}, C1q, c3, C4	Valentijn et al. (200); retrospective study using serial serum samples obtained from 33 SLE patients; serological tests: CH_{50}, C3, C4, immune complexes (C1q binding)	Significant correlation between overall disease activity, decreased C3/CH_{50}, and increased immune complex levels; Low sensitivity, specificity, and predictive value; correlation between C3/C4 levels and organ involvement in subgroups of patients
C3, C4	Ricker et al. (201); retrospective study using serial serum samples obtained from 12 SLE patients with severe nephritis; serological tests: C3, C4	Normal C3 levels observed during disease remission; abnormal C3 levels more frequently detected during flares than C4; Higher specificity and sensitivity of C3 than C4 in monitoring SLE disease activity
C1q, C3, C4, C5, C9	Swakk et al. (281); prospective study of 143 patients; serological tests: C1q, C3, C4, C5, C9, anti-dsDNA	Decreased C4, C1q, and C3, in a sequential order, detected in patients with renal exacerbation; decreasing C4 detectable 20–25 weeks before the flare; decreased C1q and C3 detected during but not before the flare
C3	Abrass et al. (210); prospective study following 48 SLE patients; serological tests: C3, anti-dsDNA, immune complexes	C3 and anti-dsDNA neither associated with nor predictive of changes in disease activity
CH_{50}, C3, C4	Morrow et al. (211); prospective follow up of 35 SLE patients; serological tests: CH50, C3, C4, anti-dsDNA, immune complexes	None of the serologic tests reliably distinguished the three clinical groups
C3, C4	Esdaile et al. (205,282); retrospective analysis of serum samples collected from 202 patients; serological tests: C3, C4, anti-dsDNA, immune complexes	All serologic parameters tested poor predictor of SLE flares; some values of patient-based serial measures in association with specific types of flares (e.g., decreased C3 and renal involvement)
C1rs-C1inh, C3d	Sturfelt et al. (213) Serial (at 6- to 8-wk intervals) samples from 33 SLE patients	Increased C1rs-C1inh consistently found during flares; increased C1rs-C1inh detected before flares, especially extra-renal flares; Increased C3d associated with severe disease flares
C1rs-C1inh, C3(Bb)P, C5b-9	Nagy et al. (227) Plasma samples obtained from healthy controls and 65 SLE patients with active and inactive disease	All 3 activation products elevated in SLE patients with inactive disease compared to healthy controls; C3(Bb)P and C5b-9 but not C1rs-C1inh, distinguishing active disease from inactive disease
C3a, C5a	Belmont et al. (215) Plasma samples of 76 SLE patients with severe, moderate, or inactive disease	C3a significantly elevated in patients with severe or moderate disease activity and quantitatively correlated with disease severity; C5a significantly elevated in patients with severe disease activity
C3a, C5a	Hopkins et al. (216) Serial plasma samples from 23 SLE patients (7 pregnant; 5 CNS involvement)	C3a levels significantly higher in patients with a flare than in those with stable disease; Rising C3a levels predictive of disease flares; Highly elevated C3a and C5a in patients with CNS involvement; C3a levels elevated in most pregnant patients
C3a, C4a, iC3b, C5b-9	Porcel et al. (218) Plasma samples of 61 SLE patients (22 inactive disease; 39 active disease; defined by SLEDAI)	C3a, C4a, and C5b-9 significantly elevated in patients with active disease, with a positive correlation with disease activity scores; C5b-9 most sensitive and specific; iC3b not correlated with disease activity
C4d, C3d	Senaldi et al. (214) Plasma samples of 48 SLE patients (11 inactive, 23 mildly active, 14 moderately/severely active)	Elevated C4d levels correlated with disease activity in a linear fashion; C3d levels elevated but not linearly correlated with disease activity
Ba, Bb, C4d, C5b-9	Buyon et al. (221) 380 serial plasma samples from 86 SLE patients with inactive, stable/moderate, or severe disease	Ba levels significantly elevated and positively correlated with disease activity; Elevated C4 and increased Bb predictive of subsequent flares
Bb, C4d, C5b-9	Manzi et al. (222) 21 SLE patients prospectively followed for 1 year	C4d and Bb sensitive indicator of moderate-to-severe disease activity; C4d and Bb sensitive at predicting increasing disease activity
C5b-9	Falk et al. (224) 108 serial plasma samples from 14 SLE patients	C5b-9 levels significantly elevated in SLE patients, and positively correlated with disease activity

*Adapted from Liu CC, Denchenko N, Navratil JS, et al. Mining the complement system for lupus biomarkers. *Clin Appl Immunol Rev* 2005;5:185–206. Copyright 2005, with permission from Elsevier.)

Second, although direct determination of complement split products, compared to conventional complement measurement, should theoretically reflect more precisely the activation process of complement in vivo and thus more specifically clinically active disease, controversy regarding utility of these assays still remains. Studies arguing in favor of the value of these assays have generally shown that plasma concentrations of complement split products, including C1-C1INH complex, C3a, C4a, C5a, C3d, C4d, C5b-9, Ba, and Bb increased before or during clinical exacerbation (206 ,213 ,214 ,215 ,216 ,217 ,218 ,219 ,220 ,221 ,222 ,223 ,224 ,225), and in some cases, the plasma levels correlated strongly with SLE disease activity scores (214 ,215 ,217 ,218 ,219). However, elevated C1-C1INH and C3d levels have been reported not only in almost all clinically ill patients, but also in a significant fraction of patients with quiescent disease, suggesting that plasma C1-C1INH and C3d levels bear little relationship with clinical activity (211 ,226 ,227 ,228 ,229). Moreover, inconsistent results have been reported for the utility of plasma levels of a given complement split product in differentiating patients with different disease activity/severity (218 ,226).

Drawbacks and Problems Associated with Complement Measurement

The discrepant reports regarding the value of measuring serum C4 and C3 to monitor disease activity of chronic inflammatory diseases such as SLE may originate from several factors that particularly confound measurement of C3 and C4 in disease. First, there is a wide range of variation in serum C3 and C4 levels among healthy individuals, and this range overlaps with that observed in patients with different diseases. Second, traditional concentration measurements reflect the presence of C3 and C4 protein entities irrespective of their functional integrity. Third, acute phase responses during inflammation may lead to an increase in C4 and C3 synthesis (196 ,197), which can counterbalance the consumption of these proteins during activation. Fourth, enhanced catabolism (230 ,231 ,232) as well as altered synthesis of C3 and C4 (233 ,234) have been reported to occur in patients with SLE, which clearly can interfere with static measures of serum C3 and C4 levels. Fifth, genetic variations such as partial deficiency of C4, which is commonly present in the general population and in patients with autoimmune diseases (88 ,98 ,99), may result in lower than normal serum C4 levels in some patients because of decreased synthesis rather than increased complement consumption during disease flares. Sixth, tissue deposition of immune complexes may result in complement activation at local sites in patients with certain diseases; such activity may not be faithfully reflected by the levels of complement products in the systemic circulation. Additional concerns should be raised regarding the measurements of complement split products. As mentioned above, many of the split products have an undefined, most likely short, half-life both in vivo and in vitro. Moreover, complement activation can easily occur in vitro after blood sampling. In combination, these

factors may hamper accurate measures of activation products that are derived solely from complement activation occurring in patients.

Complement Measurement and Organ-Specific Involvement in SLE

Given the numerous confounding factors outlined here, it is not surprising that irreconcilable results have prevailed in the research arena of complement and SLE disease activity. However, complement measures may still be informative if they are performed chronologically in the same patient and interpretation is based on the specific genetic and clinical characteristics of the patient. Complement measures in the blood or other body fluid may also be valuable in assessing disease activity or predicting outcome under particular conditions, such as specific organ involvement and pregnancy.

Lupus nephritis is one of the most serious clinical manifestations of SLE. Nephritic flare has been shown to be a predictor of a poor long-term outcome in SLE patients (235). Measurements of complement components and activation products in the plasma or in the urine may be a useful tool for evaluating the extent of active inflammation in the kidneys. SLE patients with renal involvement were found to more frequently have markedly reduced serum levels of C3 and C4 than patients with extra-renal involvement only (199 ,200). SLE patients with normal C3 and C4 levels were rarely found to have active nephritis (199 ,200). Therefore, the absence of a low C3 or C4 level in a patient with SLE may help to exclude the possibility of ongoing renal disease. Low C3 and C4 levels may also be helpful in predicting long term outcome in SLE, because low C3 levels have been reported to be predictive of persistently active glomerular disease (236) and associated with end stage renal disease (237). In addition to low C3 and C4, very low levels of serum C1q were detected in SLE patients with, but not in those without, active renal disease (199). In patients who had lupus nephritis requiring intense treatment, persistently low C1q levels before and after treatment have been shown to be indicative of continuously progressive damages in the kidneys and hence a poor outcome (238).

Because it seems likely that C3d generated in the kidney at sites of immune complex deposition would pass into the urine, measurement of C3d in the urine has been pursued as a test for specific and accurate estimation of inflammation in the kidney. Kelly et al. (239) and Manzi et al. (222) have reported the detection of C3d in the urine in SLE patients with acute nephritis and in patients without evidence of renal involvement. These results suggest that urinary C3d may also come from nonrenal origins and thus it may not be viewed as a specific marker of acute nephritis or a prognostic indicator of renal disease. Nevertheless, in the study by Manzi et al. (222) urinary C3d was shown to be better than serum C3, plasma C4d, Bb, and C5b-9 in distinguishing patients with acute lupus nephritis from those without such disease activity. Recently, Negi et al. reported that C3d levels were elevated in the urine of patients with active disease, more so in patients with active lupus nephritis (0.87 AU/mL) than in patients with active extra-renal disease (0.31 AU/mL) or in patients with inactive lupus nephritis (0.06 AU/mL) (240). Taken together, these results suggest that increased levels of urinary C3d may reflect active SLE, particularly active lupus nephritis.

In addition to renal disease, low serum C3 and C4 levels have been associated with hematological manifestations. Ho and colleagues reported that decreases in C3 were associated with concurrent decreases in the platelet counts, white blood cell counts, and hematocrit (241). However, they also noted that decreased complement levels were not consistently associated with SLE flares, as had previously been found by other investigators.

Complement levels and CNS disease in SLE has also been investigated. Hopkins et al. reported that plasma C3a levels increased in SLE patients during a disease flare, and were particularly high in 5 patients who had acute CNS dysfunction (216). In 4 of these 5 patients, significantly elevated plasma C5a levels were also detected. Subsequently, Rother et al. reported that SLE patients with CNS involvement had significantly higher levels of plasma C3d than did patients without CNS involvement (223).

Finally, several studies have examined levels of complement proteins in other body fluids. Such investigations have included analyses of synovial fluid (242), pleural fluid (243 ,244), pericardial fluid (245 ,246), and cerebrospinal fluid (CSF) (247 ,248 ,249). Generally, these studies have been too limited to provide conclusive indications regarding the utility of these assays.

Measurement of Cell-Bound Complement Activation Products

Recent reports have explored the hypothesis that cell-bound complement activation products (CB-CAPs) may serve as biomarkers for SLE diagnosis and monitoring (250 ,251). This hypothesis was based upon the following rationale. First, serum C3 and C4 levels have no diagnostic utility and limited monitoring utility, and the inherent flaws in these measurements has been well documented as described above. Second, measurement of soluble complement activation products has been shown to have utility in certain clinical situations; however these assays have not replaced measurement of serum C3 and C4 in clinical practice. Furthermore, the half-lives of these fragments in the circulation is likely to be short. Third, cell surface receptors for complement activation products are present on all circulating cells and may confound accurate and reliable measurement of the soluble activation products. Fourth, C3- and C4-derived complement activation products are capable of covalent attachment to cell surfaces via thioester bonds, and this property of the peptides may increase longevity in the circulation. Fifth, C4-derived complement activation products are known to be present on surfaces of normal erythrocytes, although the physiologic significance of this phenomenon is unknown (252 ,253). Sixth, CB-CAPS on specific cell types might reflect additional disease information by unique

cellular properties such as the life span of erythrocytes and reticulocyte.

Using a flow cytometric assay, Manzi et al. conducted a cross-sectional study to examine erythrocyte-bound C4d (E-C4d) levels in patients with SLE, patients with other inflammatory and immune-mediated diseases, and healthy controls (250). E-CR1 was determined simultaneously in light of the previous reported association of low levels E-CR1 in SLE. The results from this study showed that: (1) significantly higher E-C4d levels occur in patients with SLE than in patients with other diseases and healthy individuals, and (2) abnormally high E-C4d levels, in conjunction with abnormally low E-CR1 levels, is a pattern with high diagnostic sensitivity and specificity for SLE, as compared with healthy individuals (81% sensitive; 91% specific) and patients with other inflammatory diseases (72% sensitive; 79% specific), respectively (250). During investigation of the diagnostic utility of E-C4d for SLE, these investigators also observed significant longitudinal fluctuation of E-C4d with individual patients. This discovery suggested that E-C4d levels might correlate with disease activity in SLE.

Erythrocytes develop from hematopoietic stem cells in the bone marrow and emerge as reticulocytes. Reticulocytes maintain distinct phenotypic features for 1 to 2 days before fully maturing into erythrocytes. Reticulocytes, if released into the peripheral circulation during an active disease state, may immediately be exposed to and bind C4-derived fragments generated from activation of the complement system. Because erythrocytes, which have a life span of approximately 120 days, may bind and retain activation-derived C4d throughout their lifetime, the E-C4d level is likely to be the cumulative result of complement activation and disease activity over a 120-day period. Because of the brief transitional stage of reticulocytes, the levels of C4d attached to reticulocytes (R-C4d) are more likely the result of ongoing activation instead of past events. Therefore, it was hypothesized that R-C4d levels, as opposed to E-C4d levels, may reflect more effectively and precisely the current disease activity in a given SLE patient at a specific point in time, thereby serving as “instant messengers” of SLE disease activity.

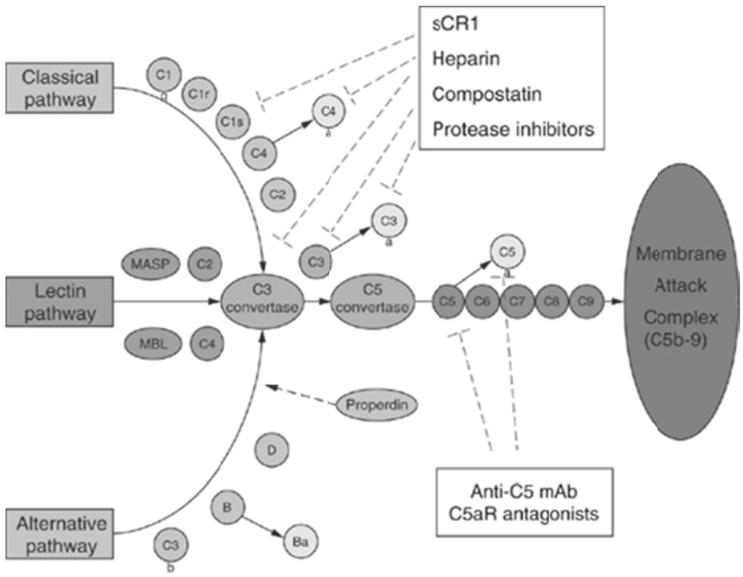

Figure 13-4. Anticomplement therapeutics and potential target molecules

Initial studies indicate that: (1) a wide range of R-C4d is detected in SLE patients, but not in patients with other diseases and healthy controls, (2) the mean R-C4d level of SLE patients is significantly higher than that of patients with other diseases or healthy controls, and (3) R-C4d levels fluctuate and correlate with clinical disease activity as measured using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus Activity Measurement (SLAM) (251). These findings suggest that C4d-bearing reticulocytes may serve as a biomarker for SLE disease activity, providing clues to current and perhaps impending disease flares in patients with SLE.

Anticomplement Therapeutics for SLE

The fundamental role of complement activation in SLE pathogenesis has led naturally to exploration of the complement system as a target for therapeutic intervention. To date, a variety of reagents that inhibit or modulate complement activation at different steps of the cascade have been developed (254). These reagents can be classified into two broad categories: (1) inhibitors of the early steps of complement activation and (2) inhibitors of the terminal pathway that do not interfere with early activation events (254 ,255) (Fig. 13-4). Examples of the first group include: soluble CR1 (sCR1;

capable of regulating the generation of C3/C4 fragments and C3 convertases) (256 ,257 ,258 ,259 ,260), heparin (a polyanionic glycosamine; capable of binding/inhibiting C1, inhibiting C1q binding to immune complexes, blocking C3 convertase formation, and interfering MAC assembly) (261 ,262 ,263 ,264), compstatin (a synthetic peptide capable of binding C3 and preventing its proteolytic cleavage), and protease inhibitors (265 ,266). Prominent among the second group are anti-C5 monoclonal antibodies (mAbs) that can bind C5, block its cleavage and formation of C5a, and abrogate MAC assembly (267 ,268 ,269 ,270 ,271). Synthetic antagonists of C5a receptors also belong to the second group and have been exploited to block the anaphylactic and chemotactic effects of C5a (272 ,273 ,274). Considering that C3b opsonization of pathogens and immune complexes is crucial for host defense and for prevention of immune complex-associated adverse reactions, it is reasonable to postulate that inhibitors of complement activation at a downstream step, such as C5 cleavage, will have therapeutic effects for patients with inflammatory diseases but will less likely increase the risk for infection in these patients.

Eculizumab, a humanized anti-C5 mAb, has recently been studied in the NZB/W F1 mouse model of SLE, and has been shown to improve significantly renal disease and increase survival of treated mice (271). A Phase I clinical trial of Eculizumab in patients with SLE concluded that Eculizumab was safe and well tolerated, without significant adverse effects (275). Heparin, traditionally used as an anticoagulant and known to inhibit complement activation, has recently been demonstrated to prevent antiphospholipid antibody/complement-induced fetal loss in a murine model (264). This seminal observation suggests that heparin at “sub-therapeutic” (nonanticoagulating) doses may be beneficial in pathological situations where excess complement activation is unfavorable, such as ischemia/reperfusion injury, antiphospholipid antibody syndrome, and lupus nephritis.

Conclusion

In 1948, Hargraves reported discovery of the LE cell (276), although the origin and significance of the structure were unknown at the time. For decades the LE cell was investigated scientifically and used for diagnosis and management of patients with SLE, although its clinical utility eventually fell out of favor.

Despite the historical introduction to this chapter, it is now apparent that discovery of the LE cell is likely to have been the first observation to link complement and SLE. Shortly after Hargraves discovered the LE cell, it was determined that in vitro generation of LE cells is dependent on complement activation (277 ,278). Fifty years later, the LE cell is recognized as a neutrophil that has engulfed the remnants of apoptosis, thus linking complement, apoptosis, and SLE.

So consider the LE cell as a lupus biomarker relic and an early icon of the disease. Perhaps it should instruct us to forge ahead with microarrays, proteomics, molecular signatures, and knockout mouse models of lupus, but also to carry with us and occasionally revisit more simple observations of the past. The complement system holds at least one important clue to the mystery of lupus, and recent progress is cause for optimism that a solution to the puzzle may be within reach.

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Chapter 14

Mechanisms of Acute Inflammation and Vascular Injury in Systemic Lupus Erythematosus

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Inflammation in systemic lupus erythematosus (SLE) is mediated by a complex interaction of the cellular and humoral components of the immune system. In contrast to the host immune response to infection that begins with a known inflammatory trigger, the etiologies of connective tissue diseases such as SLE remain undefined. In the absence of known etiologies, treatment strategies rely on the dissection of inflammatory processes into component parts while keeping in mind the complex interaction of these components. This chapter reviews inflammatory mediators released from cells—neutrophils, monocytes, and platelets—as well as humoral activators of these cells, such as chemoattractants, complement components and immune complexes. A particular focus is placed on the pathogenic mechanisms by which inflammatory cells and mediators provoke vascular injury in SLE. Additionally, since endothelial injury and subsequent alteration of endothelial adhesiveness and permeability to leukocytes and platelets are principal mechanisms of both the inflammatory states that characterize exacerbated SLE as well as atherosclerosis, we discuss the relationship between atherosclerosis and autoimmunity.

The Role of Circulating Phagocytic Cells and Platelets in Immune Injury

The activation of phagocytic cells and platelets in response to chemoattractants, immune complexes, and other stimuli provokes the release of inflammatory mediators that account for diverse manifestations of inflammation in autoimmune disease (Table 14-1). The major effector cells and their functions are discussed in the following sections.

Neutrophils

Neutrophils, together with monocyte/macrophages, are the body's “professional phagocytes.” While in the circulation neutrophils are maintained in a resting state, with their wide array of cytotoxic mediators either stored in cytoplasmic granules or separated into plasma membrane and cytosolic compartments. Activation of the cell may be triggered by the engagement of particles, such as invading microorganisms or in the case of autoimmune disease, in response to soluble stimuli that engage specific cell surface receptors (1).

Directed Migration or Chemotaxis

In inflammation, the initial step of neutrophil activation requires movement toward a target. Such directed migration, or chemotaxis, occurs along a chemical gradient originating at the target. Well-described chemoattractants include bacterial products such as formulated peptides, and activated complement components such as C5a. These chemoattractants interact with specific surface receptors that have seven hydrophobic transmembrane domains (2). Ligand/receptor interactions lead to conformational changes of the receptor that allow it to interact with, and activate, heterotrimeric G (guanosine triphosphate [GTP] binding) proteins. Activation of C proteins results in the generation of an intracellular signal that triggers chemotaxis and related functional responses.

Chemoattractants for neutrophils include leukotriene B4, platelet-activating factor, interleukin-8, and the complement split product C5a. At low concentrations, and when distributed in a gradient, these molecules lead neutrophils to migrate toward regions of higher concentrations. At higher concentrations, however (i.e., at the bacterial source of the gradient), these same molecules cause neutrophils to cease migration and become activated, as described below.

Phagocytosis

Following directed migration along a chemoattractant gradient, phagocytosis is initiated by the attachment or binding of target particles to the neutrophil surface. Attachment is followed by enclosure of the particle within a plasma

membrane pouch. Upon closure this pouch becomes a vacuole in the cytoplasm, termed a phagosome. Fusion of the respective membranes permits entry of lysosomal contents into the phagosome and the shielded enzymatic degradation of ingested material, an important aspect of the scavenging function of both neutrophils and macrophages. Thus, phagocytosis can deliver a microbial prey to a sequestered compartment in which the noxious action of host cytotoxins (e.g., degradative enzymes) can be confined.

Table 14-1: Pro-Inflammatory Mediators Released By Neutrophils, Macrophages, and Platelets

Secretory products produced by neutrophils and macrophages
Reactive oxygen intermediates (e.g., superoxide anion)
Proteolytic enzymes
Reactive nitrogen intermediates (e.g., nitric oxide)
Bioactive lipids
Cyclooxygenase products: prostaglandin E2 (PGE2), prostaglandin F2a prostacyclin, thromboxane
Lipoxygenase products: monohydroxyeicosatetraenoic acids, dihydroxyeicosatetraenoic acids, leukotrienes B4, C, D and E*
Platelet-activating factors
(1 O-alkyl-2-acetyl-sn-glycerlyl-3-phosphorylcholine)
Chemokines (e.g. interleukin-8)
Secretory products produced by macrophages
Polypeptide hormones
Interleukin lα and 1β (collectively. IL-1)
Tumor necrosis factor-α (cachectin, TNF)
Interferon-α
Platelet-derived growth factor(s)
Transforming growth factor-β
ß-endorphin
Complement (C) components
Classic path: C1, C4, C2, C3, C5
Alternative path: factor B factor D, properdin
Coagulation factors
Intrinsic path: IX, X. V. prothrombin
Extrinsic path: VII
Surface activities: tissue factor, prothrombinase
Prothrombolytic activity: plasminogen activator inhibitors, plasmin inhibitors
Secretory products produced by platelet
Plasminogen
α2-plasmin inhibitor
Platelet-derived growth factor
Platelet factor IV
Transforming growth factors α and β
Serotonin
Adenosine diphosphate
Thromboxane A2
12 hydroxytetraenoic acid

*Not produced by neutrophils.

Although designed primarily to ingest invading microorganisms, macrophages and neutrophils can be triggered by opsonins to release cytotoxic products in the absence of phagocytosis. This occurs in autoimmune disease where antibodies activate the complement system and where soluble immune complexes with covalently bound (i)C3b can engage neutrophil Fc and complement receptors. While the host has multiple inhibitory proteins in extracellular biologic fluids that inactivate released products, such protective mechanisms can be overcome with resultant tissue injury as observed in necrotizing vasculitis, arthritis, and glomerulonephritis (3).

Release of Toxic Proteolytic Enzymes

Neutrophils contain two morphologically distinct granules (specific and neutrophilic) that contain proteases, which under normal circumstances are sequestered within the granule and the phagolysosome, presenting no threat to the host. However the extracellular release of granule contents may promote inflammation and damage at tissue sites. Phagocytosis is not necessary for neutrophil degranulation, which may be provoked by soluble stimuli (e.g., C5a, interleukin-8). Degranulation is augmented when neutrophils encounter stimuli deposited on a surface: lysosomal release unfolds by a process of reverse endocytosis, or what has been called “frustrated phagocytosis.” This exuberant release of lysosomal enzymes from neutrophils may be relevant to the pathogenesis of tissue injury in diseases characterized by the deposition of immune complexes on cell surfaces or on such extracellular surfaces as vascular basement membranes or articular cartilage (4).

In glomerulonephritis, neutrophil-derived proteases can be demonstrated in urine and are involved in the degradation of the extracellular matrix proteins of the glomerular basement membrane (GBM) and mesangium. The principal proteases that promote GBM degradation appear to be serine proteases, elastase, and cathepsin G, as well as neutrophil collagenase (5). In addition to proteases, neutrophils also release cationic proteins with bactericidal activity, such as lysozyme, bactericidal/permeability increasing factor (BPI), and the defensins, which increase glomerular permeability by neutralizing the anionic components of the GBM.

Production of Toxic Oxygen Radicals

The activation of phagocytic leukocytes results in the production of oxygen free radicals, such as superoxide anion, which have potent microbicidal activity. When released extracellularly, oxygen derived free radicals cause tissue injury and irreversible modification of macromolecules (6 ,7). Superoxide anion is produced by the addition of an extra electron to molecular oxygen by a multiprotein complex that requires membrane-bound cytochrome b558 and key cytosolic proteins (including a ras-related low molecular weight GTP-binding protein). The multiprotein complex is assembled at the plasma membrane in response to cell activation. Stimulated neutrophils also produce hydrogen peroxide, hydroxyl radicals, and possibly singlet oxygen.

Some of the most toxic oxygen metabolites produced by neutrophils are generated by the myeloperoxidase (MPO) hydrogen peroxide-halide system. MPO is a highly cationic enzyme present in the azurophilic granules that catalyzes the reaction of hydrogen peroxide with a halide such as chloride to form hypohalous acids (e.g., hypochlorous acid). These products are capable of killing a variety of microorganisms as well as mammalian cells. There is evidence that the MPO-hydrogen-peroxide halide system plays a significant role in phagocyte-mediated injury in glomerulonephritis (5).

Macrophages

Macrophages as Secretory Cells in Inflammation

Macrophages play multiple roles in inflammation and immunity, serving as both antigen-presenting cells (APCs) that drive adaptive immune responses, as well as primary inflammatory cells in chronic disease. Tissue macrophages derive from circulating monocytes, and differentiate and become activated upon exiting the vasculature. Although the process of macrophage adhesion to, and diapedesis through, blood vessels is similar in many respects to that of neutrophils, the macrophage responds to a different set of chemotactic factors, including the chemokines RANTES, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1a and -1b and the growth factors transforming growth factor (TGF)-ß and platelet-derived growth factor (PDGF) (8). Macrophages/monocytes play an important role in immune-mediated tissue injury particularly in nephritis (9). These cells express the three major classes of Fc receptors as well as ß1, ß2, and ß3 integrins, which facilitate phagocytosis of opsonized particles, intercellular adhesion, and adhesion to extracellular matrix proteins. Recruitment requires the expression of adhesion molecules on activated vascular endothelium (e.g., intercellular adhesion molecule-I [ICAM1] vascular cell adhesion molecule [VCAM1]), which are recognized by counter-ligands of the circulating monocyte (e.g., LFA1, VIA4). When monocytes emigrate into tissues they can be transformed into activated macrophages following exposure to cytokines such as interferon-γ (IFN-γ), IL-l, and tumor necrosis factor (TNF-α), Macrophage activation results in an increase in cell size, increased synthesis of proteolytic enzymes, and the secretion of a variety of inflammatory products.

Release of Inflammatory Mediators and Protease

Through ligation of specific surface receptors, macrophages may be activated by IFN-γ, complement components, immune complexes, LPS, and cytokines such as IL-1ß, TNF-α, and IL-6 (8). Once activated, macrophages secrete a variety of pro-inflammatory products, including proteases (e.g., lysozyme, elastase and collagenase), inflammatory cytokines (IL-1ß, TNF-α, IL-6, IL-12, and others), chemokines (including IL-8, MCP-1, MCP-2, RANTES, and others), and several complement cascade proteins (C1, C2, C3, and C4) (8). Indeed, a vigorous acute-phase response, driven by cytokine production and characterized by an increased erythrocyte sedimentation rate and high serum levels of acute phase reactants, is typical of diseases accompanied by extensive macrophage involvement. Activated macrophages also produce reactive oxygen species, inducible nitric oxide, and a number of eicosanoids (primarily PGE2, but also TxA2, prostacyclin, LTB4, and PAF) (10). In addition, activated macrophages produce procoagulant factors such as tissue factor and plasmin inhibitor (11). The procoagulant products include tissue factor (identified as a receptor for factor VII) factor × activator, prothrombin activator, and vitamin K–dependent clotting factors II, VII, IX, and X. Monocytes in rheumatic disease patients display a higher procoagulant producing activity than normal, likely a result of exposure to cytokines and cleavage products of complement. Increased procoagulant activity may contribute to fibrin deposition at sites of inflammation and has been implicated in the formation of crescent formation in glomerulonephritis. Whether such increased macrophage procoagulant activity observed in these conditions also promotes a systemic “hypercoagulable” state in SLE remains unknown.

Production of Cytokines

Macrophages also secrete a variety of polypeptide hormones that regulate immune function and inflammation as well as wound healing and repair (12). Macrophages, for example, produce three cytokines, IL-lα., IL-1ß, and TNF-α, which not only have overlapping functions, but also are capable of inducing each others' release by macrophages themselves (6). Macrophages also produce the cytokine neutrophil-activating peptidel/IL-8, which is a potent neutrophil chemoattractant. The production of IL-8, induced by IL-lα, IL-1ß, and TNF-α has been described in a variety of tissues, including alveolar macrophages renal mesangial cells, and psoriatic skin lesions (13). 1L-8 is now known to be a member of a family of macrophage inflammatory proteins (MIPs) or chemokines.

Nitric Oxide as a Modulator of Inflammation

Macrophages are also among the cellular sources of reactive nitrogen intermediates such as nitric oxide (NO). NO, although originally identified as a product of endothelial cells, which accounts for “endothelium-derived relaxation factor” activity, is now appreciated to be a highly reactive molecule with diverse biologic functions. The exposure of macrophages to cytokines (e.g., IL-1b, IFN-γ) markedly increases NO production. Activities of NO that may be important in the inflammatory response include vasodilation and its capacity to react with superoxide anion to form

toxic peroxynitrite compounds. Consistent with a pro-inflammatory role in SLE, the inhibition of NO synthesis has been demonstrated to reduce the severity of glomerulonephritis in Medical Research Laboratory lymphoproliferative (MRL/pr) mice (14). In human SLE, serum nitrite is elevated during active disease and levels correlated with both SLEDAI and titers of antibodies to double-stranded DNA (15).

NO, constitutively produced by vascular endothelium, may exert anti-inflammatory properties (16). For example, NO inhibits neutrophil and platelet aggregation, inhibits the adhesion of neutrophils to endothelial cells, inhibits neutrophil oxidant production, and thereby may serve as a “Teflon coat” for blood vessel walls against cellular injury (17). Thus, the role of NO in inflammation is complex. The pro or anti-inflammatory properties will depend on (a) whether NO is constitutive (and therefore physiologic) or induced (usually pathologic) and (b) the potential for the formation of toxic derivatives such as peroxynitrite, and (c) the adaptive responses of target cells.

Platelets

Platelets as Inflammatory Cells

Platelets, derived from marrow megakaryocytes, are involved in hemostasis, wound healing, and cellular responses to injury (18 ,19). In addition to its important hemostatic function, the platelet may also act as an inflammatory effector cell. In addition to containing procoagulant factors such as Von Willebrand factor, fibrinogen, and factor V, platelet granules also contain inflammatory mediators, including chemokines (such as RANTES, MCP-3, MIP-1α, and platelet factor 4), growth factors (such as platelet-derived growth factor and transforming growth factor-B), histamine, and serotonin (20). Signaling molecules up regulated on the activated platelet include P-selectin, which adheres to and activates neutrophils, and CD40L, which induces inflammatory phenotypes in many cell lines, including the endothelium (21). Upon activation, platelets also rapidly produce eicosanoids, such as thromboxane A_2, from membrane-derived arachidonic acid, and other lipid membrane-derived inflammatory products, such as platelet-activating factor. Platelets both participate in cell-cell interactions and release micro particles that contribute to leukocyte-leukocyte and leukocyte–endothelial cell interactions; these interactions play a role in inflammation as well. Platelet activation at sites of tissue injury is induced by such hemostatic factors as thrombin, adenosine diphosphate, arachidonate derivates, and exposed subendothelial collagen. There is evidence of platelet activation in immunologically mediated diseases such as asthma, cold urticaria, scleroderma, and SLE (21 ,22 ,23 ,24).

Platelets in Glomerulonephritis

Platelets have been identified in the glomeruli of patients with SLE nephritis where they are believed to play a particularly important role (25). Urinary thromboxane levels are elevated in patients with active lupus nephritis, a finding that has several implications: first, it is a sign of abnormal platelet aggregation in the microvasculature with the potential for thrombosis and endothelial injury; second, the vasoconstrictive properties of TxA_2, would be expected to decrease glomerular filtration rate (GFR) and renal blood flow; and third, the release of growth factors and other mediators by activated platelets could aggravate the proliferative glomerular lesion. Studies of the administration of specific TxA_2 antagonists have shown promise in the improvement of both GFR and renal blood flow in SLE nephritis (26).

Platelet activation in lupus glomerulonephritis has been attributed to a variety of substances, including immune complexes, activated complement components (including C3a and the membrane attack complex, C5b9), PAF, and vasopressin. Additionally, neutrophils may also be able to activate platelets via the release of oxidants and proteases. Platelets may aggravate immune injury in glomerulonephritis via several mechanisms, which include (a) promoting thrombosis; (b) reducing GFR through the production of thromboxane and other vasoactive substances; and (c) releasing products that activate macrophages, neutrophils, and glomerular mesangial cells (5).

Endothelial Cell Activation

In order to localize to extravascular tissue, leukocytes must first exit the vasculature and migrate to the appropriate extravascular space. These processes require a complex sequence of events, beginning with the (micro) vascular response to injury. In response to a bacterial infection or other insult, arterioles vasodilate and endothelial cells contract, exposing the basement membrane; blood flow slows and plasma extravasates. In turn, leukocytes concentrate and gain increased contact with the endothelium. Endothelial cells, activated by mediators such as IL-1ß and TNF-α produced by tissue macrophages, DC, and fibroblasts, express surface molecules that contact and bind leukocytes, permitting their egress from the vasculature (9). Different groups of adhesion molecules sequentially mediate leukocyte rolling, tight adhesion, and passage through the vascular wall, or diapedesis. The first step, leukocyte rolling, occurs via the interactions of the adhesion molecule E-selectin on endothelial cells and L-selectin on leukocytes with mucin-like sialylated glycoproteins on leukocytes and endothelial cells, respectively (27). Selectins are expressed constitutively on both leukocytes and endothelium, and their interactions are strong but short-lived, with the result that a percentage of leukocytes are transiently adherent, or marginated, to the endothelium at all times, moving along with a tumbleweed-like progression. At sites of inflammation, following exposure to stimuli such as IL-1ß and TNF-α, the expression of E-selectin on endothelial cells increases markedly, leading to an increase in the percentage of leukocytes that marginate and roll along the vessel margins. The next step, leukocyte tight adhesion, results from the interaction of leukocyte adhesion molecules known

as integrins (e.g., CD11a/CD18 or LFA-1, CD11b/CD18 or CR3, α4β1 or VLA-4) with counter-ligands on endothelial cells (e.g., the cellular adhesion molecules [CAMs], ICAM-1, and VCAM-1) (28). This process requires de novo expression of CAMs on endothelium and activation of pre-existing integrins on leukocytes, both in response to inflammatory mediators. Important integrin-ligand pairing characterize specific cell-cell adhesion and contribute to the distribution and homing of leukocytes; common integrin-ligand pairs include CD11a/CD18 with ICAM-1 (lymphocytes-endothelium), CD11b/CD18 with ICAM-1 (neutrophils-endothelium) and VLA-4 with VCAM-1 (monocytes-endothelium). Finally, transmigration of leukocytes though the endothelium and basement membrane is mediated by chemokines such as MCP-1, C5a, and IL-8 (29).

Table 14-2: Pathologic and Clinical Spectrum of Vascular Injury on Systemic Lupus Erythematosus (SLE) A

Pathology	Pathogenesis	Clinical Phenomenon
Capillaritis*	Immune complex deposition	**Glomerulonephritis, pulmonary alveolar hemorrhage
Vasculitis*	Activation of complement, neutrophils, and endothelium Modeled by Arthus lesion	†Cutaneous purpura, polyarteritis nodosa-like systemic and cerebral vasculitis
Leukothrombosis	Intravascular activation of complement, neutrophils, and vascular endothelium Modeled by Shwartzman lesion	Widespread vascular injury, hypoxia, cerebral dysfunction, SIRS
Thrombosis	Antibodies to anionic phospholipid-protein complexes interact with endothelial cells, platelets, or coagulation factors Modeled by APS	Arterial and venous thrombosis, fetal wastage, thrombocytopenia, pulmonary hypertension
	Disseminated intravascular platelet aggregation	TTP
Atherosclerosis	Activated endothelium, increased endothelial cell adhesion molecules, increased tissue factor, decreased 27-hydroxylase	MI, CVA

*Capillaritis or microvascular angiitis and lupus vasculitis share a similar pathogenesis but are associated with different clinical phenomena, designated here as ** and †.
APS, antiphospholipid syndrome; CVA, cardiovascular accident; MI, myocardial infarction; SIRS, systemic inflammatory response syndrome; TTP, thrombotic thrombocytopenic purpura.

The Activation of Complement and Inflammatory Cells in the Development of Vascular Injury in SLE

SLE is the autoimmune disease that best exemplifies the consequences of the systemic generation of humoral inflammatory mediators and the activation of the cellular constituents of inflammation as outlined above. Immune complex formation, episodic complement activation, the recruitment of stimulated leukocytes and platelets into tissues, and endothelial cell activation produce vascular injury during SLE exacerbations. Vascular disease in SLE can be classified into two broad categories: inflammatory and thrombotic. The former may be associated with local deposition of immune complexes or result from leukocyte-endothelial cell interactions in the absence of immune complex deposition, and the latter is almost invariably associated with circulating antiphospholipid antibodies (Table 14-2).

Inflammatory Vascular Disease

In most instances, inflammatory vascular disease in SLE involves complement activation and resultant cell-mediated tissue injury.

Complement Cascade

Three distinct complement cascades—the classical, alternative, and lectin pathways—differ in their mechanisms of target recognition and initial activation, but converge at the C3 convertase (30). Many of the complement plasma proteins are zymogens, proteases that become active upon undergoing proteolytic cleavage; thus, these proteins generally circulate in a dormant state. Once active, many of these molecules participate in cleavage or activation of subsequent components. Thus, each successive enzymatic step results in rapid and dramatic amplification. In response to

this potential for explosive activation, a number of complement regulatory molecules exist to dampen the cascades. Given the importance of complement activation to rheumatic disease, a more detailed understanding of the three complement cascades is important for both researchers and practicing rheumatologists.

Classical Pathway

Activation of the classical pathway occurs when the C1q component of the multimeric complement 1 complex (C1) binds to multiple Fc portions of IgG or IgM antibodies complexed to antigen. When at least two of the six globular heads of C1q have bound, the remaining components of the C1 complex (two molecules each of C1r and C1s) undergo a conformational change, which results in the cleavage of soluble C4 and C2 to C4a and C4b and C2a and C2b, respectively (31). C4b and C2b combine to form C4b2b (the C3 convertase of the classical pathway), that cleaves C3 to C3a and C3b. C3b combines with C4b2b to form C4b2b3b (C5 convertase), which in turn cleaves C5 to C5b and C5a (32). Subsequent assembly of the C5b-9 membrane attack complex (MAC) can form a pore in the cell membrane of some pathogens, leading to their death. Additionally, C3b molecules serve as opsonins, which are covalently attached to a single bacterium's surface, facilitating its phagocytosis by macrophages and neutrophils.

Mannose-Binding Lectin Pathway

The mannose-binding lectin pathway is a primitive version of the classical cascade, activated by bacterial surface mannose residues. Since these residues are not accessible on vertebrate cells, the lectin-binding pathway distinguishes “self” from “other” on the basis of a generic pattern, rather than a specific antigen; as such, it represents a bridge between innate and acquired immunity. When the C1q-like mannose-binding lectin (MBL) component of the MBL complex (analogous in form and function to the C1 complex) binds mannose on a pathogen, it activates the mannose-associated serine proteases MASP-1 and MASP-2 (akin to C1r and C1s); thereafter, the MBL complex functions as does activated C1, cleaving C4 and C2 to form C3 convertase (33).

Alternative Pathway

The alternative pathway is unique in that it does not require a pathogen-binding protein for its activation; rather, it is initiated by the spontaneous hydrolysis of C3 in plasma. C3·H20 binds the alternative pathway component factor B, which is then converted by the associated factor D into Ba and Bb. The C3·H20-Bb complex that results is an alternate C3 convertase, and converts fluid-phase C3 into C3a and C3b, the latter of which is quickly inactivated by hydrolysis unless it deposits upon a cell surface. Once covalently bound to a cell surface (or indeed, any surface), however, C3b binds factor B; factor D cleaves factor B, resulting in the formation of the C3 convertase, C3bBb, which is equivalent to the C3 convertase^{C4b2b} of the classical and lectin pathways (32). Others and we have demonstrated that during the course of active SLE, complement is activated via both the classical and alternative pathways, and may be useful in distinguishing disease flare from preeclampsia in pregnant lupus patients (35).

C3a and C5a—Anaphylatoxins

The release of the soluble fragments C3a and C5a, also known as anaphylatoxins, accelerates the inflammatory response in multiple ways: anaphylatoxins engage receptors on mast cells and basophils, triggering release of vasoactive mediators such as histamine and the cysteinyl leukotrienes LTC4 and LTD4. C5a and C3a induce vasodilation and increased capillary permeability; upregulate adhesion molecule expression on leukocytes and endothelial cells; and cause smooth muscle contraction. C5a in particular is also a powerful chemoattractant for, and activator of, neutrophils, and can stimulate both neutrophil degranulation and the respiratory burst (36). Since plasma C3a is a reflection of complement degradation, while plasma levels of total C3 reflect degradation and synthesis of C3, an acute phase protein, measurements of C3a may be more sensitive and specific biomarker of lupus disease activity than C3 (37).

Complement Regulation

When C3bBb binds to host cells, the host complement-regulatory proteins complement receptor 1 (CR1) and decay-accelerating factor (DAF) rapidly bind C3b and displace Bb. CR1 and DAF, in combination with the membrane cofactor protein (MCP), also catalyze the cleavage of C3b by the plasma protease factor I to produce inactive C3b. Bacterial surfaces lack these proteins and instead permit binding of properdin, which stabilizes rather than degrades the alternative C3 convertase.

Other proteins have also evolved to inhibit complement activation. These include fluid-phase proteins, such as C1 inhibitor and the C3 and C5 convertase inhibitors, C4-binding protein and factor H, as well as membrane-bound proteins such as the C3 and C5 convertase inhibitors, and CD59, a glycolipid that prevents insertion of the MAC into plasma membranes (38).

Complement in SLE

Patients with specific complement deficiencies are especially prone to SLE; approximately 90% of C1q-null, 75% of C4-null, and up to 30% of C2-null individuals develop lupus. Why complement deficiency predisposes to autoimmune disease is not entirely clear, but may result from inefficient clearing of apoptotic cells and immune complexes, resulting in either inappropriate exposure to self-antigens or in immune complex deposition (39).

Complement activation plays an important role in the tissue injury observed in many of the manifestations of SLE, including that because of immune complex (IC) deposition, non-IC dependent neutrophil-mediated vasculopathy, as well as injury because of antiphospholipid antibodies. The renewed interest in complement in inflammatory disease has led to the development of pharmacologic complement inhibitors that are currently in development, including humanized monoclonal C5 antibody and a solubilized CR1 molecule, which prevents activation of the C3 convertase (40).

Immune Complex Deposition-Dependent Vascular Inflammation

Vasculitis in SLE is most commonly a result of the local deposition of immune complexes, particularly those containing antibodies to DNA (anti-DNA), in blood vessel walls (41 ,42). This lesion is best modeled by the Arthus reaction and experimental serum sickness. Maurice Arthus reported in 1903 that the repeated cutaneous injection of horse serum into a group of rabbits produced inflammatory reactions characterized by intense polymorphonuclear leukocyte infiltration, hemorrhage, and sometimes necrosis (43). It is now known that this reaction is due the formation of antigen antibody complexes in the vicinity of blood vessel walls, complement activation, and the generation of anaphylatoxins (e.g., C4a, C3a, C5a) and chemotaxins (C5a) (44 ,45). The resulting infiltration of vessel walls by polymorphonuclear leukocytes leads histologically to leukocytoclastic vasculitis and the release of lysosomal enzymes and oxygen radicals to tissue injury (42).

Modifications of the classic active Arthus reaction include immunization by intravenous rather than cutaneous injection of the antigen as well as the local passive Arthus reaction (e.g., simultaneous cutaneous injection of antigen and antibody), direct passive Arthus reaction (e.g., passive transfer of preformed antibody by the intravenous injection of serum from another immunized rabbit), and the reverse passive Arthus reaction (e.g., antibody injected cutaneously and antigen injected intravenously) (46). The necessary elements of these reactions have been examined and require antigen, precipitating antibody, intact complement pathway, and neutrophils (47). One study suggests that the inflammatory response to immune complexes also requires cell-bound Fc receptors with subsequent amplification by cellular mediators and complement (48). A role for C5a receptors has also been established since C5aR blockade abrogates this response (49).

Immune complex disease in SLE can also be modeled by the acute experimental serum sickness or the glomerulonephritis that accompanies chronic serum sickness. In the acute serum sickness model, the injection of antigen is followed by an immune response, with the generation of antibody and clearance of the antigen by the cells of the reticulo-endothelial system. However, during the period of accelerated decay of the antigen the presence of circulating immune complexes produces inflammatory injury involving arteries, glomeruli, and joints (50). Chronic serum sickness requires usually at least 5 weeks of repeated injection of intravenous antigen. The resulting periods of antigen excess leads to circulating immune complexes and glomerulonephritis histologically similar to that observed in patients with SLE (Fig. 14-1) (51).

A major consequence of immune complex deposition in SLE is the activation of complement. The consumption of complement components and their deposition in tissue is reflected by a decrease in serum levels of C3 and C4 in most patients with active disease (52). However, since the synthesis of both C3 and C4 increases during periods of disease activity, the serum levels of these proteins may be normal despite accelerated consumption (53). Conversely, chronically depressed levels of individual complement components, because of decreased synthesis, hereditary deficiencies, or increased extra vascular distribution of complement proteins, has been reported in SLE (54). The decreased serum complement levels in these patients may lead to the mistaken conclusion that excessive complement activation is ongoing. To define more precisely the role of complement activation with respect to clinical disease activity of SLE, circulating levels of complement degradation products during periods of active and inactive disease have been measured (55 ,56 ,57 ,58 ,59 ,60). Levels of plasma C3a, Ba, and the serum complement attack complex, SC5b9, were each shown to be more sensitive indicators of disease activity than either total C3 or C4 level (55 ,56 ,57 ,58 ,60). Elevations of plasma C3a levels may precede other serologic or clinical evidence of an impending disease flare (60). The combination of elevated C3a and anti-dsDNA, as pathogenically anti-dsDNA/DNA immune complexes are complement fixing and more likely to incite tissue injury, can predict flare and may identify patients responsive to preemptive short-term, moderate dose steroid treatment (61).

The pathologic consequences of immune complex deposition in SLE include both a microvascular angiitis (e.g., glomerulonephritis or pulmonary capillaritis with or without pulmonary hemorrhage) and necrotizing vasculitis (e.g., cutaneous purpura, polyarteritis nodosa—like systemic, cerebral).

Immune Complex "Independent" Vascular Injury

Some patients with SLE have small vessel disease and inflammatory vasculopathy in the absence of local immune complex deposition, particularly those patients with central nervous system (CNS) involvement (57 ,62 ,63). Several lines of investigation now suggest yet another mechanism for this complement mediated vascular injury in SLE, one not dependent on immune complex deposition (64 ,65 ,66 ,67 ,68 ,69). This mechanism is best modeled experimentally by the Shwartzman phenomenon. The local Shwartzman lesion requires a preparatory intradermal injection of endotoxin, which is followed in 4 to 18 hours by the intravenous injection of endotoxin (70 ,71). This results in the intravascular

activation of complement triggering the release of anaphylatoxins such as C3a and C5a into the circulation (72). The split products attract and activate inflammatory cells, such as neutrophils and platelets, causing them to aggregate, to adhere to vascular endothelium, to occlude small vessels, and to release toxic mediators. Activation of complement thus leads to an occlusive vasculopathy that may also result in widespread ischemic injury (72).

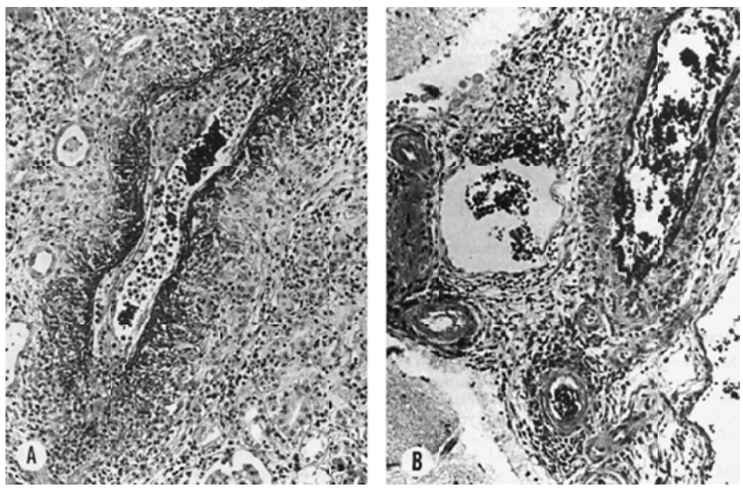

Figure 14-1. A, Polyarteritis-type lupus vasculitis with fibrinoid necrosis in the kidney of a patient with systemic lupus erythematosus (SLE). **B,** Polyarteritis-type lupus vasculitis with fibrinoid necrosis in the brain of a patient with SLE. (H&E, × 160.)

The Shwartzman phenomenon was originally described as a model of meningococcal sepsis but it is now recognized that cytokines such as ILl and TNF-α can substitute for endotoxin (Fig. 14-2) (62). Such agents stimulate the up regulation on the endothelial cell surface of ICAM1 and E-selectin, which are the counterreceprors for the neutrophil adhesion molecule CD 11b/CD 1 8 and SialylLewis X, respectively (74). It had long been established that complement activation in plasma stimulates circulating neutrophils to produce the local Shwartzman lesion, but it has been recognized that the preparatory phase represents a time of ICAM I and Eselectin up regulation (71). The importance of this local endothelial cell activation is supported by the capacity of antibodies to ICAM 1 and E-selectin administered intravenously to prevent the development of the experimental lesion (71).

The episodic, uncontrolled activation of complement proteins is a characteristic feature of SLE. Disease exacerbations are typically accompanied by decreases in total C3 and C4 values in association with elevations in plasma of the biologically active complement split products, C3a desArg and C5a desArg (57 ,58 ,75 ,76). During periods of disease flare, circulating neutrophils are activated to increase their adhesiveness to vascular endothelium, as indicated by the up regulation of the surface ß-2-integrin CD 11b/CD 18 (complement receptor 3) (66 ,67). One study demonstrated that the surface expression of three distinct endothelial cell adhesion molecules, Eselectin, VCAM 1, and ICAM1, is also up regulated in patients with SLE (Fig. 14-3) (63). Endothelial cell activation was most marked in patients with disease exacerbations characterized by significant elevations of plasma C3a desArg, and the activation reversed with improvement in disease activity (63). In these studies, endothelial cell adhesion molecule up regulation was observed in otherwise histologically normal skin and was notable for the absence of local immune complex deposition (63). These data suggest that excessive complement activation in association with primed endothelial cells can induce neutrophil endothelial cell adhesion and predispose to leuko-occlusive vasculopathy during SLE disease flares. This pathogenic mechanism may be of particular relevance to vascular beds, which lack the fenestrations that permit the trapping of circulating immune complexes. Such an example is the CNS, where the blood–brain barrier can prevent the access of circulating immune complexes to the

perivascular tissues. But in the setting of widespread endothelial cell activation, exuberant systemic complement activation can promote diffuse microvascular injury in the absence of immune complex deposition and produce the most common pathologic finding of CNS lupus, microinfarction (Fig. 14-4) (75 ,79 ,80 ,81 ,82).

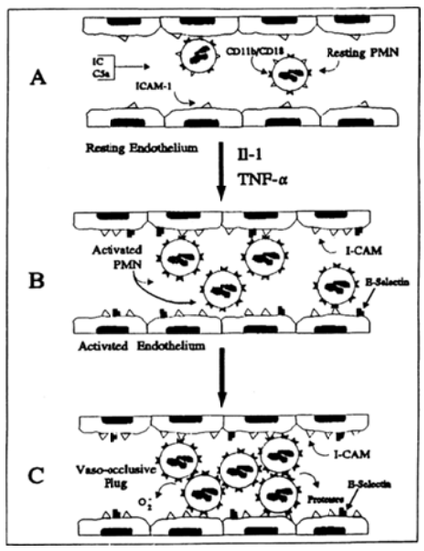

Figure 14-2. Schematic presentation of the Schwartzman phenomenon. **A,** Stimulation of polymorphonuclear leukocytes (PMNs) via engagement of C5a or Fc receptor leads to activation of CD11b/CD18, promoting homotypic and heterotypic aggregation. IC, immune complexes. **B,** Endothelial cell activation by cytokines, such as interlukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), leads to upregulation of constitutive intercellular adhesion molecule-1 (ICAM-1) and induces the expression of E-selectin. **C,** The co-occurrence of PMN and endothelial cell activation results in leukothrombosis and vasoocclusive plugs.

Similar pathologic events may also be present in the mesenteric circulation and produce features of SLE enteritis (62) or produce pulmonary leukosequestration and acute, reversible hypoxemia during disease exacerbations (83).

A role for complement activation and the Shwartzman phenomenon in the setting where antineuronal antibodies may mediate neuropsychiatric lupus is supported by experimental data. Several groups have reported on the prevalence of anti-dsDNA antibodies that cross-react with an epitope on the glutamate/N-methyl-D-aspartate (NMDA) receptor subunit NR2A, which is highly expressed in the human brain, and suggested that their excitotoxic effects can mediate CNS manifestations of lupus (84 ,85). Diamond has studied this mechanism in an animal model immunized by antigen to produce these antibodies, but observed that no neuronal damage occurred until breakdown of the blood-brain barrier occurs. Only following the administration of LPS, the prototypical inducer of the preparatory signals required for the Shwartzman phenomenon, did these antibodies gain access to the brain and it become possible to demonstrate neuronal death with resulting cognitive dysfunction (86). It is likely that some SLE patients with CNS involvement experience a brain capillary leak syndrome or “acute cerebral distress syndrome” resulting either only in reversible leukoencephalopathy or, in the presence of autoantibodies with specificity for brain antigens, the potential for neuronal death (87).

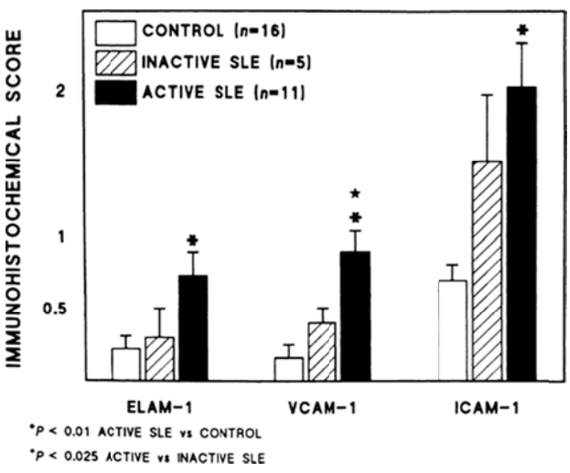

Figure 14-3. Systemic lupus erythematosus (SLE) disease exacerbation is accompanied by endothelial cell upregulation of three adhesion molecules: E-selectin (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1). The mean expression of all three adhesion molecules is significantly greater in patients with active SLE versus healthy controls as well as patients with inactive SLE.

The systemic inflammatory response syndrome (SIRS) is a reaction characterized by widespread inflammation primarily affecting vascular endothelium (88). The same cascade of mediators involved in the Shwartzman phenomenon has been invoked in SIRS (89 ,90). The main endogenous mediators of SIRS include TNF-α and Ill (89). A prominent role for PAF vasodilator prostaglandins, complement activation, and upregulation of adhesion molecules on leukocytes and endothelial cells has also been identified (89). A consequence of SIRS is multiple organ failure (MOF) or multiple organ dysfunction syndrome (MODS) with manifestations that include catecholamine unresponsive hypotension, decreased myocardial contractility, cerebral dysfunction, and adult respiratory distress syndrome

(ARDS). Interestingly, mAbs directed against the leukocyte integrin CD 18 can ameliorate the lung injury in experimental models of SIRS (91).

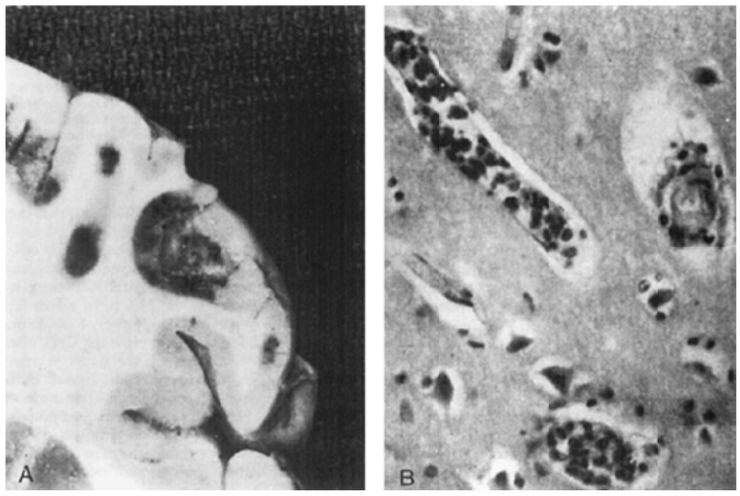

Figure 14-4. Specimen of brain obtained postmortem from a patient with fatal exacerbation of neuropsychiatric lupus without antiphospholipid antibodies. **A,** The frontal lobe reveals multiple small cortical infarcts. **B,** High magnification reveals leukothrombosis, with occlusion of small blood vessels by leukocyte aggregates, as well as acute ischemic changes of the neurons. (Photo courtesy of Dr. Nelson Torre, Buffalo, NY.)

It is now recognized that SIRS may arise both from sepsis and noninfectious causes, such as immune-mediated organ injury (88). Serious exacerbations of SLE can produce a syndrome that is indistinguishable from SIRS and can be accompanied by MOF. Therefore, the Shwartzman phenomenon may serve as a model for both SIRS and the multiorgan dysfunction that can accompany acute lupus crisis.

Inflammatory vasculopathy independent of immune complex deposition may also occur as a result of antineutrophil cytoplasmic antibodies (ANCAs) or lymphocyte responses. A pathogenic role for ANCAs in vasculitis has been suggested by the demonstration of activation of poly-morphonuclear leukocytes and enhanced adhesion to endothelial cells by antibodies to proteinase 3 or myeloperoxidase (92). Lymphocytes directly reacting to antigen may release cytokines that result in tissue damage and a mononuclear inflammatory disease (93). These mechanisms of vascular injury are more typical, however, of Wegener's granulomatosis and Sjögren's syndrome, respectively, than of SLE.

Thrombotic Noninflammatory Vascular Disease

Antiphospholipid Antibody Syndrome

The presence of antibodies to negatively charged phospholipids is associated with recurrent arterial and venous thrombosis, thrombocytopenia, and fetal wastage (94 ,95). Antiphospholipid antibody syndrome (APS) requires the demonstration of an antiphospholipid antibody (e.g., biologic false-positive Venereal Disease Research Laboratory [VDRL] test, lupus anticoagulant, anticardiolipin antibodies) and thrombotic phenomena. It is known that the presence in serum of this family of autoantibodies, perhaps operating through cofactors (e.g., ß2-glycoprotein-1 or prothrombin) can generate a thrombotic diathesis (96 ,97 ,98 ,99 ,100). The mechanism for this hypercoagulable state has not yet been fully understood, although it appears to involve interactions between the antibodies to anionic phospholipid-protein complexes and antigen targets on platelets (101 ,102), endothelial cells (103 ,104 ,105 ,106), or components of the coagulation cascade (107 ,108). Experimental evidence suggests

that increased platelet aggregation, altered endothelial cell function (e.g., decreased prostacyclin or increased thrombomodulin production), or disturbed function of clotting factors (e.g., decreased protein C activation by thrombomodulin, decreased protein S function, as well as decreased prekallikrein and fibrinolytic activity) may explain the predisposition to thrombosis. The role of cytokines in the primary APS or in APS secondary to SLE requires clarification (109). Evidence that antiphospholipid antibodies can activate endothelial cells and increase expression of adhesion molecules suggests another mechanism for the thrombophilia (110).

A critical role for complement and neutrophils in APS, especially in the circumstance of placental vasculopathy that underlies fetal loss, is supported by experimental studies where antibodies that block C5a-polymorphonuclear leukocyte C5a receptor interactions prevent complications in pregnant mice which receive human IgG containing APL antibodies (111 ,112). These findings are further confirmed by experience with using C3 convertase inhibitor complement receptor 1-related gene, gene as well as C3 deficient mice, both of which mitigates fetal loss (113).

A relationship between inflammation and thrombosis in antiphospholipid antibody induced hypercoagulability loss is further supported by the observation that these autoantibodies can induce endothelial cell tissue and monocyte tissue factor (TF) expression. TF, an initiator of the extrinsic coagulation cascade, mRNA and its molecule is increased when murine monoclonal ß2 glycoprotein I dependent anticardiolipin antibodies are incubated with cultured endothelial cells or peripheral blood mononuclear cells (114). That complement activation is proximal to TF production and that blockade of generation of TF can protect pregnancies from aPL antibody induced injury is supported by the finding that in C5aR knock out mice, even after treatment with aPL-IgG, no TF production is observed and the outcomes of their pregnancies are normal (115).

Thrombotic Thrombocytopenia Purpura

SLE exacerbations are infrequently accompanied by secondary thrombotic thrombocytopenia purpura (TTP) with complete or incomplete features of the clinical pentad: fever, microangiopathic hemolytic anemia, thrombocytopenia, renal disease, and neurologic dysfunction (116). In the absence of antiphospholipid antibodies, the characteristic pathology of TTP, eosinophilic hyaline microthrombi, has been identified in patients with SLE. Large multimers of vWF capable of mediating disseminated intravascular platelet aggregation have been demonstrated in patients with chronic, relapsing TTP as well as SLE (117 ,118). A consequence of the endothelial cell activation or injury observed in SLE may be the release of vWF multimers or other mediators of platelet aggregation capable of initiating a TTP-like illness (119). Additionally, evidence suggests that an inhibitor of vWF-cleaving protease (ADAMTS-13) causes the thrombotic microangiopathic hemolytic anemia that characterizes autoimmune associated TTP (120 ,121).

Endothelial Cell Dysfunction and SLE

SLE is increasingly a chronic illness, with rapidly fatal cases now rare and most patients experiencing a long-term disorder with frequent exacerbations and remissions. Therefore, chronic morbidity and late mortality are of greater importance. Urowitz et al. have reported their observations of mortality within the Toronto lupus cohort and described a bimodal distribution of death. Early (within the first year) deaths were most often attributed to active lupus and infection, while late deaths were secondary to atherosclerotic heart disease. Myocardial infarction (MI) accounted for 45% of all deaths. Other studies also document fatal MI in SLE populations but at a significantly lower and constant rate of 3% to 5% of total deaths. In addition to cohort studies of mortality associated with myocardial involvement, the increased prevalence of coronary artery disease in SLE has been documented by case reports and autopsy studies. In 1975, Buckley and colleagues performed autopsies in 36 SLE patients (33 female and mean age of 32 years). Four (11%) experienced MI, while eight (22%) had greater than 50% narrowing in at least one coronary artery. A second autopsy series of 22 patients age 16 to 37 years demonstrated ten (45%) had coronary artery narrowing that exceeded 75%. The Baltimore lupus cohort reported an 8.3% prevalence of cardiovascular events (MI, sudden cardiac death, and angina) in 229 patients. Ginzler et al. from the State University of New York (SUNY) Health Science Center in Brooklyn retrospectively studied 200 patients and found coronary artery disease (CAD) in 30 (15%), MI in 13 (7%), and angina in 24 (12%). Age-specific incidence rates of MI and angina were compared between 498 women with SLE from the University of Pittsburgh Medical Center and 2,208 female controls. Between the ages of 35 and 44 women with lupus were 50 times more likely than controls to experience MI. Most recently studies demonstrate lupus patients on electron beam CT scans have higher calcification scores as compared to controls (Agatston score of 69 vs. 9) and, when controlling for traditional risk factors, increased carotid plaque (37 vs. 15 percent) (122 ,123).

Although coronary artery vasculitis has been described in SLE, it is rare, and the accelerated CAD that is characteristic of SLE is typically otherwise indistinguishable from atherosclerosis that occurs outside the setting of lupus. The pathogenesis of the observed precocious atherosclerosis in SLE includes clustering of traditional risk factors, adverse effects of treatment (e.g., corticosteroids, cyclophosphamide), and vasculopathy that accompanies disease activity (e.g., hypercoagulable state of secondary antiphospholipid syndrome and neutrophil endothelial cell interactions). Endothelial cell injury and dysfunction with subsequent alteration of endothelial adhesiveness and permeability to leukocytes and platelets are principal mechanisms of both the atherosclerosis and the inflammatory state that characterizes SLE.

Endothelial cells line the lumen of all blood vessels and form the interface between the blood and peripheral tissues. Traditionally, the role of endothelial cells was considered to

be that of a gatekeeper. Passive deposition of cholesterol and its metabolites in the artery wall has been thought to be the key step in the pathophysiology of atherosclerosis. In recent years, however, attention has shifted to the role of primary vascular injury. Although many factors cause atherosclerosis, it has become clear now that endothelial cell perturbation and inflammation at the site of vascular injury plays an essential role in the initiation and progression of atherosclerotic disease.

In response to a diverse array of stimuli, activated endothelial cells exhibit a pro-inflammatory phenotype by up regulation of adhesion molecules on their surface, leading to sequential steps in rolling, firm adhesion, and transmigration of leukocytes and monocytes to the site of injury. Formation and accumulation of foam cells in turn promote neointimal proliferation and thinning of endothelium, which result in dysfunction of the endothelial cells, interaction with the platelets, and stimulation of smooth muscle proliferation, leading to fibrous plaque and thrombus formation (124 ,125).

The central role of endothelial cell—leukocyte interaction as an early response in the pathogenesis of atherosclerosis was demonstrated by experiments on the C57BL/6 mice model with homozygous mutations for the ICAM1 gene. This mutation, resulting in a deficiency of endothelial cell ICAM1 expression, is associated with a protective role on the development of atherosclerosis in this animal model (126 ,127). Additionally, SLE, a disease characterized by widespread vascular injury, has a high prevalence of premature atherosclerosis and represents an interesting model to study endothelial perturbation. Table 14-3 gives a summary of the factors altering endothelial cell behavior and specific endothelial responses to these stimuli.

Endothelial Phenotype

Endothelium plays a fundamental role in various physiologic functions, including vasoregulation, hemostasis, inflammation, and adhesion biology. What determines the actual difference between the chronic lesion in atherosclerosis and the acute and sub acute lesions of inflammatory vasculopathy? An understanding of the details of endothelial perturbation in response to stimuli may provide the explanation. Irrespective of the nature of the stimuli (immunogenic or direct mechanical forces), the endothelium responds by up regulating adhesion molecules and recruiting leukocytes, shifting the phenotype to procoagulant by expressing TF or vWF, and/or altering vascular tone.

Prothrombotic Phenotype

By shifting the phenotype to procoagulant in response to stimulation, endothelial cells have a direct role in the mechanism of thrombosis, whether this is at a site of atherosclerotic plaque formation or focal vascular inflammation, or in circumstances characterized by diffuse endothelial activation, such as in SLE, catastrophic antiphospholipid syndrome (CAPS), or SIRS (128 ,129).

Table 14-3: Reviewed Factors Stimulating Endothelial Cells and Endothelial Cells' Phenotypic Responses That Play an Important Role in the Pathogenesis of Atherosclerosis and Inflammatory Vasculopathy

Stimuli	Response
Nonimmunologic	Vascular tone
Mechanical forces: (transmural pressure, tension, hear stress) hypercholesterolemia, oxidized LDL, lysophosphatidylcholine	NO Prostacyclin Endothelin-1
Immunologic	Prothrombotic
Cytokines	Tissue factor
TNF-α	vWF
IL-1 IFN-γ	thrombomodulin
Activated complement products	Pro-adhesive
C3a, C3b, C5a, MAC	ICAM-l
Autoantibodies	VCAM-1
ACL	E-selectin
AECA	
Anti-oxLDL	
CD40/CD40L Interaction	
C1q receptors occupancy	

ACL, AECA, antiendothelial cell antibody; dsDNA, double-stranded DNA; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; LDL, low-density lipoprotein; oxLDL, oxidized LDL; NO, nitric oxide; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor.

Von Willebrand factor (vWF) is a macromolecular protein complex that plays a pivotal role in hemostasis. It functions as a carrier protein for plasma factor VIII, and mediates platelet adhesion to other platelets and to collagen exposed by damaged endothelium. Vascular endothelium is the major source of plasma vWF under physiologic and pathologic conditions. vWF is stored and released from endothelial cell secretory granules, along with its propeptide vWF-to-AgII. Elevated plasma vWF was found in diabetes and other vasculopathies. Its level and the level of propeptide can serve as a marker of endothelial activation (130). A group from Canada (131) demonstrated decreased peripheral dermal staining for endothelial vWF in SIRS patients, as well as in healthy volunteers after TNF-α injection. This was associated with significantly greater plasma vWF levels, indicating degranulation in response to cytokine stimulation that predisposes to formation of platelet microthrombi, lodging in small capillaries, causing areas of local tissue ischemia.

Cultured endothelial cells in vitro express TF in response to a great variety of stimuli (132). The data in vivo are controversial; one group found an absence of expression of TF by endothelium overlying atherosclerotic plaques by in situ

hybridization (133), but a later study reported endothelial expression of TF by using histochemical assessment (134). Solovey et al. (135) demonstrated expression of TF by endothelial cells of sickle cell anemia patients during the acute vaso-oclusive episode, supported by concurrence between TF antigen and messenger RNA (mRNA) expression.

Proadhesive Phenotype

Adhesion of leukocytes to vascular endothelium is one of the earliest events in acute immunogenic and nonimmunogenic inflammation (124). The initial surface expression of adhesion molecules is the common endothelial response not only to a variety of atherogenic stimuli, but also to complement-mediated vascular injury in SLE (immune complex–mediated, as well as immune complex–independent).

Many cells, including endothelial cells (ECs), constitutively express ICAM1, and its expression is up regulated by ILl or TNF-α. VCAM1 is mainly present on ECs activated by ILl, TNF-α, IFN-γ, or 1L4 (136). ECs activated by ILl, TNF-α, or thrombin transiently express E-selectin, thereby mediating endothelial adhesion of neutrophils or memory lymphocytes (120). Expression of these molecules promotes formation of vaso-occlusive plaques, by interaction of endothelium with activated neutrophils, displaying up regulation of ß2-integrin CD11b/CD18. The importance of this interaction was demonstrated in a model of vascular injury underlying thrombotic stroke (138). Investigators used neutrophil depleted or ICAM 1–deficient mice and demonstrated resistance of this model to focal cerebral ischemia and reperfusion injury provoked by experimental intraluminal occlusion of the cerebral artery. Elkon's group (139) demonstrated in MRL/MpJFAS lpr mice that ICAM 1 deficiency results in a striking improvement in survival. Data from several groups analyzing the level of various adhesion molecules in SLE further support the notion that endothelial cells play a central role in systemic inflammatory response by exhibiting their adhesive properties. Immunohistologic examination of nonsun-exposed skin from SLE patients showed up regulation of the surface expression of all three adhesion molecules—E-selectin, ICAM1, and VCAM1—in patients with active SLE and with otherwise histologically normal skin with no evidence of local immune complex deposition (63). Elevation of soluble adhesion molecules (E-selectin, sICAM1, sVCAM1) also has been reported in active SLE (140). A group from France (136) demonstrated increased level of sVCAM1 in patients with primary antiphospholipid syndrome (APLS), SLE-related APLS, or SLE, compared to healthy controls or thrombosis controls.

Stimuli That Activate Endothelial Cells

Factors that alter the functional status of endothelium can be divided into two major categories: nonimmunologic, such as direct mechanical forces, vasoactive mediators, and products of oxidation; and immunologic, such as cytokines, complement, and autoantibodies.

Nonimmunologic

The endothelium experiences three primary mechanical forces: transmural pressure, tension, and shear stress. Sprague et al. (141) have demonstrated flow-mediated modulation of endothelial activation markers, and Nagel et al. (142) reported up regulation of ICAM expression in cultured human vascular cells in response to shear stress.

Recent studies have supported the view that hypercholesterolemia and oxidative stress are important inducers of atheroma formation. One of the actions of oxidized low-density lipoprotein (oxLDL) is to induce endothelial dysfunction (143). Erl et al. (144) demonstrated activation of endothelial cells by oxLDL via distinct endothelial ligands, promoting adhesion of monocytes. It is speculated that one of the mechanisms is a disruption of signal transduction (145). Other mechanisms may be related to the lysophosphatidyl choline (LPC) moiety, one of the active components of oxLDL particles that induces superoxide endothelial cytotoxic effect via peroxinitrite production (145 ,146). Moreover, LPC is a major factor in the antigenicity of oxLDL (147 ,148). Antibodies against oxLDL were demonstrated both in normal, healthy individuals and in atherosclerotic plaques and were found to correlate with atherosclerosis progression (148). The level of oxLDL antibodies has been correlated with titers for aCL in SLE patients (146 ,149). Several groups (149 ,150) have demonstrated cross-reactivity of aPL and oxLDL underlying the link in development of atherosclerosis and immunologic process.

Immunologic

Cytokines are important mediators of endothelial cell activation. Specifically, TNF-α ILl, and macrophage colony-stimulating factor increase binding of LDL to endothelium and increase transcription of the LDL gene (143). TNF-α, IFN-γ, and ILl stimulate adhesion molecule expression on endothelial cells, and few studies have reported increased levels of these cytokines in the circulation during vasculitis (151). The Shwartzman phenomenon, originally described as a model of endotoxemia, currently is being used as a model of the widespread vasculopathy of SLE. It is recognized now that cytokines such as ILl and TNF-α are the preparatory signals of the inflammatory process (63 ,128). These pro-inflammatory cytokines are capable of promoting atherosclerosis by stimulating the adhesive properties of endothelial cells. Accumulation of TNF-α (152) and ILl (153) was demonstrated in atherosclerotic plaques of coronary arteries. It is also important to note that while endothelial cells are activated by cytokines, they also can produce ILl, 1L6, 1L8 and TNF-α while stimulated (151 ,154). These cytokines can act as autocoids to upregulate adhesion molecule expression. Kaplanski et al. (137) demonstrated induction of 1L8 production in a time- and dose-dependent

fashion by thrombin-activated HUVEC; this effect was inhibited by the specific thrombin inhibitor hirudin.

Complement activation that can be either immune complex–dependent or independent plays an essential role in the mechanism of endothelial injury. This can explain the development of widespread vascular injury in SLE, an example of immune-mediated systemic inflammatory response, even without evidence of immune complex deposition in the tissue. Several products of the activated complement system (C3b, iC3b, and C5a) are known to activate endothelial cells in vitro. More recently, Saadi et al. (155) demonstrated that interaction of complement with endothelial cells and the assembly of MAC leads to expression of P-selectin and activation of a protease that cleaves and releases heparan sulfate proteoglycan from the EC surface, and induces up regulation of TF and cyclooxygenase-2 (COX-2). They concluded that activation of porcine aortic and microvascular ECs on MAC exposure involves the intermediate step of ILl release. By using ECs transgenic for human decay accelerating factor that inhibits complement convertase, these authors demonstrated that complement is required for endothelial activation. The presence of sCRi prevented activation of aortic as well as microvascular ECs (156).

Granular deposits of immunoglobulin and complement components were found within atherosclerotic lesions (157), suggesting that complement dependent endothelial activation could play a role in pathogenesis of atherosclerosis.

Antibodies against endothelial cells and cardiolipin were found in a subset of patients with the clinical and angiographic diagnosis of severe premature atherosclerotic peripheral disease (158). Recent research demonstrated that antibodies might contribute to the derangement of functional status of the endothelial cells, rather than simply displaying cell cytotoxicity. It has been reported that both polyclonal as well as monoclonal antiendothelial cell antibodies (AECAs) induce EC activation in vitro. AECAs have been shown to mediate release of vWF, arachidonic acid metabolites, and ETi from endothelial cells, and to induce a pro-inflammatory and procoagulant phenotype of EC. A statistically significant increase in expression of endothelial ICAM1, VCAM1, and E-selectin was demonstrated on human umbilical vein ECs pretreated with AECA positive sera from a scleroderma patient compared to cells pretreated with AECA negative sera (159). Neutralizing antibodies to ILl, but not antibodies to TNE substantially inhibited or blocked this activation, providing further evidence for the autocrine actions of ILl. Del Papa et al. (160) found that AECA IgG from Wegener's granulomatosis patients upregulates the expression of Eselectin, ICAM1, and VCAM1 and induces the secretion of ILl, 1L6, 1L8, and MCP1. Almost identical results were obtained from analysis of AECA positive sera from Takayasu arteritis patients (161). Another proposed mechanism is that binding of AECAs makes negatively charged phospholipids accessible to antiphospholipid antibodies (162), thereby further enhancing the activation of EC.

Cross-reactivity between anti–dsDNA antibodies, cardiolipins, and AECAs has been observed in earlier studies (163 ,164). By using immunofluorescent staining, Simantov et al. (165) were the first to demonstrate expression of cell adhesion molecules, including E-selectin, VCAM1, and ICAM1 by ECs incubated with purified IgG from patients with high titers of anticardiolipin antibodies, even in the absence of clinical or serologic evidence of SLE. They also established that this mechanism is ß2-glycoprotein dependent.

A direct stimulatory effect of anti dsDNA antibodies on endothelium, as indicated by the release of vWF may have a pathogenic role on expression of adhesion molecules (166 ,167). These data can provide a link between SLE and premature atherosclerosis.

The interaction between CD40 and CD40L is another immune-mediated interaction common to both SLE and atherosclerosis that leads to up regulation of adhesion molecules on endothelial cells (147). CD40 is a type 1 member of the TNF receptor super family of proteins, and is present on a wide variety of cells, including vascular endothelial cells. Ligation of this receptor on endothelial cells is known to increase expression of inflammatory adhesion molecules.

Slupsky et al. (168) demonstrated that platelets express the ligand of CD40 within seconds of exposure to agonist, and interact with endothelial cells to participate directly in the induction of an inflammatory response. The same authors also showed that activated platelets induce TF expression on endothelial cells in a CD40/CD40L dependent manner. Moreover, CD40 ligation on ECs down regulates the expression of thrombomodulin and adhesion molecules, further implicating the procoagulant and pro-inflammatory phenotype of ECs (169). In a genetically modified murine model with hypercholesterolemia, blocking antibodies to CD40 reduced atherosclerotic lesion formation (170).

In SLE Clq immune complexes may be a source of arterial injury initiating atherogenesis. Lozada et al. (171) showed that immune complexes stimulate endothelial cells to express adhesion molecules E-selectin, ICAM, and VCAM in the presence of a heat labile complement component Clq. Clq depletion from serum or Clq protein synthesis inhibition on the surface of ECs blocked the expression of adhesive proteins. Moreover, Reiss et al. (172) were able to demonstrate that occupancy of Clq receptors on ECs by immune complexes down regulated mRNA for sterol 27hydroxylase, the enzyme that mediates peripheral cholesterol metabolism, interfering with the capacity of endothelium to convert cholesterol to antiatherogenic metabolites and therefore enhancing atherogenesis.

In summary, we have described factors leading to endothelial perturbation and overlapping features of endothelial response in autoimmune disorders and atherosclerosis. The conversion of the endothelial phenotype to an adhesive and prothrombotic state and interaction of inflammatory mediators with cholesterol metabolism driven by multiple immunologic and nonimmunologic stimuli are the core of the pathophysiologic link between mechanisms of inflammatory vasculopathy, thrombosis, and atherosclerosis.

The Role of Activated Endothelium in SLE

There is a unifying hypothesis to account for the diverse, episodic, and variably distributed (e.g., widespread versus organ restricted; skin, kidney, or CNS) nature of SLE vascular lesions. Inflammatory lesions would require either circulating immune complexes (e.g., DNA/anti-DNA) or significant complement activation of neutrophils, while thrombotic lesions would require antiphospholipid antibodies. An additional biologic factor, however, is required to explain the waxing and waning nature of disease exacerbations and the limitation of lesions to one or few vascular beds. Immune complexes, activated neutrophils, or antiphospholipid antibodies may be necessary but likely are not sufficient to explain vascular pathology in SLE. Since they may be present in the absence of disease activity and can travel throughout the general circulation, they are incapable of explaining the relapsing and focal nature of vascular injury.

New information regarding the activation of ECs may provide the missing feature. The specificity of vascular injury in SLE may depend on the endogenous capacity of vascular endothelium to respond to stimuli. ECs when activated can express E-selectin, increased ICAMi and an inducible form of nitric oxide synthase (iNOS) (63 ,74). However, there is a restriction to this EC capacity; for example, the up regulation of EC adhesion molecules is limited to postcapillary venules (173). It is therefore possible that the episodic and focal nature of vascular pathology in SLE is dependent on the presence or absence as well as nature of EC activation. EC permissiveness for immune complex deposition or in situ formation can result in colitis. Activation of EC adhesion molecules by cytokines, immune complexes, complement components, antiendothelial cell antibodies, or antiphospholipid antibodies can result in leukothrombosis if there is simultaneous activation of neutrophils and complement (110). Abnormal iNOS synthesis may also have a role in permitting trophil EC interaction and the Shwartzman-like lesion. Additionally, EC activation may lead to the expression of membrane-associated coagulation proteins that are the target of antiphospholipid antibodies.

Additional evidence that activated endothelium contributes to the vasculopathy of exacerbated SLE is the finding that disease flare is accompanied by abnormal levels of circulating endothelial cells (CECs). CEC levels are significantly higher in active SLE patients compared to healthy controls and correlate positively with plasma C3a elevations (174). Furthermore, CECs from patients with active SLE express an activated phenotype, staining for tyrosine. Elevated levels of CEC in active SLE represents a marker for endothelial injury and suggests these cells may further potentiate vascular injury by the production of inflammatory and prothrombotic mediators and by engaging in heterotypic aggregation with neutrophil and platelets. In prior studies, the potential for CECs to participate in micro infarction was suggested by studies reported in sickle cell anemia (175) and acute MI (176). In conclusion, the detection of activated CECs in patients with SLE, particularly with higher SLEDAI scores, provides evidence for widespread endothelial injury. Studies are ongoing to determine whether the presence of CECs predicts or is the result of postcapillary vascular damage and micro infarction leading to organ dysfunction or even premature atherosclerosis. That the endothelial cell perturbations that characterize exacerbated SLE can contribute to both is illustrated by the finding that the mean level of soluble EPCR, endothelial cell protein receptor C, which promotes a thrombotic diathesis when shed, is significantly higher in SLE patients than controls (177). The shedding of sEPCR was increased by IFN-γ and IL-1, but blocked by a metalloproteinase inhibitor, suggesting a role for inflammatory cytokines in the induction of the observed increased sEPCR in the plasma of SLE patients (178).

Vascular injury in SLE may require immune complex formation, complement activation, or antiphospholipid antibody production, but we propose that the EC response is responsible for the clinical character of a disease exacerbation by determining the timing and organ distribution of vascular pathology, which includes vasculitis, leukothrombosis, thrombosis, and atherosclerosis.

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Chapter 15
Idiotypes and Idiotype Networks

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History

In 1963, Oudin and Michel (1) proposed the concept of idiotypes (Ids) and anti-idiotypes (anti-Ids) when describing rabbit antibodies that induce antibodies against themselves. In the same year, Kunkel et al. (2) described Ids on human antibodies. In 1974, Jerne (3) suggested that Ids and anti-Ids participate in self-regulatory immune networks. Since then, many investigators have established the importance of Ids in normal immune regulation and in experimental manipulation of cell function and immune responses.

Definitions

Table 15-1 lists terms used in defining Ids and idiotypic networks. Idiotopes are antigenic regions located in the variable regions of immunoglobulin (Ig) molecules or T cell antigen receptors (TCRs). Single Ig molecules can express several different idiotopes; the series of idiotopes is referred to as the idiotype for that molecule. Because the exact structural basis of most idiotopes is unknown, the term idiotype is widely used to describe the entire antigenic region on Igs or TCRs. An antibody produced in response to antigen (Ag) expresses Id and is called Ab1 to define its place in the Id immune network.

Ids are divided into two general classes: private and public. Private Ids are expressed on Igs or TCRs that expand from a single parent clone. Therefore, private Ids define antibodies or T cells that are clonal and relatively specific for a single stimulating Ag. In contrast, public Ids (also called cross-reactive Ids) are expressed on Igs and TCRs deriving from different parental cells. Therefore, Igs and TCRs displaying public Ids bind several different Ags and are likely to appear in many different individuals of the same species. Further, public Ids may be found on antibodies in individuals of different species. For example, public Ids on anti-DNA antibodies derived from certain mouse strains are found on anti-DNA and other Igs in humans (4 ,5).

Because idiotypes are antigenic, they stimulate production of anti-Id antibodies, which are referred to as Ab2. Ab2 molecules are divided into at least two subtypes, depending on whether they mimic Ag (6). Ab2α binds Ab1 but otherwise shares no properties of the initial stimulating Ag. Ab2ß binds Ab1 and behaves in other ways like Ag, and it can stimulate production of more Ab1 and bind to receptors that ordinarily bind Ag. This property was first noted in insulin systems (7). Some Ab2 against antiinsulin Ab1 could bind the insulin receptor on cell surfaces and actually trigger glucose metabolism by those cells. Ab2ß anti-Ids are also called internal image anti-Ids, as suggested by Nisonoff and Lamoyi (8), implying that they share structural similarities with Ag. Some authors refer to them as epibodies or homobodies. Another class of Ab2 is Ab2γ, which contains antiidiotypes that are antigen inhibitable because of steric hindrance with the antigen combining site (9). Ab2ß has been extensively used to generate internal images of infectious pathogens (10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19), tumor antigens (20 ,21 ,22 ,23 ,24), and more recently as mimics of self antigen, for example human interferon (IFN), which has antiviral activity (25). Because Ab2 molecules themselves express Ids, they also stimulate production of anti-anti-Ids (Ab3).

Within the variable region of each Ab1 is a region that binds the epitope of the stimulating Ag. The region on Ab1 that binds Ab2 is called a paratope. If the paratope- and epitope-binding regions of the Ab1 molecule overlap or are located close to each other, binding of either (by epitope of Ag or paratope of Ab2) may inhibit binding of the opposite molecule.

Idiotype Networks

Idiotype networks are complex, and three features are particularly important. First, members of the network regulate each other, thus serving to control immune responses. Second, the network links immune responses to self with immune responses to the external environment. Third, the network links the fetal and newborn repertoires to the adult immune response.

Antigen-Antibody Idiotype Networks

There are at least two major concepts of Id networks. Figure 15-1 illustrates the most widely held view, suggested originally by Jerne (3) and later expanded by him and others (6 ,7 ,8 ,9 ,26 ,27 ,28 ,29). This concept is based on information suggesting that convex epitopes on Ags are bound by concave regions in the Ag-binding groove of Ig molecules, as suggested by x-ray crystallographic studies of an Ag-antibody complex (30). Similarly, convex regions on Ab1 molecules

are bound by concave regions in the binding groove of Ab2; the same process permits binding of Ab3 to Ab2.

Table 15-1: Definitions

1. *Idiotype*. A region on the variable portion of an immunoglobulin molecule or T cell antigen receptor that is antigenic. When present on an antibody molecule, the antibody may be referred to as Ab1.
2. *Idiotype (Id)*. A series of idiotopes on the same molecule.
3. *Private idiotype*. An Id expressed only on the products of a single B- or T cell clone. Antibodies and T cells bearing private Ids will be specific for only one antigen (Ag).
4. *Public idiotype*. An Id expressed on different B- and T cell clones that interrelates them. Antibodies and T cells bearing public Ids are in sum able to recognize multiple different Ags. These also are referred to as cross-reactive or shared Ids.
5. *Antiidiotype*. An antibody directed against the idiotope on immunoglobulin, B cell surface, or T cell antigen receptor. This antibody may be referred to as Ab2.
6. *Anti-Id type 1*. Also called Ab2α, this Ab2 binds to Ab1 but does not otherwise behave like antigen (Ag).
7. *Anti-Id type 2*. Also called Ab2 ß, also called homobody, internal image anti-Id, or epibody. This Ab2 binds Ab1 and behaves in additional ways like Ag. For example, it can bind to receptors for Ag and by so doing can trigger cell activation.
8. *Anti-Anti-Id*. An antibody against Ab2. It also may be referred to as Ab3. Some Ab3 molecules share structural similarities to and behave like Ab1; for example, they may bind the Ag to which the original Ab1 is directed.
9. *Epitope*. The region of an antigen bound by Ab1.
10. *Paratope*. The region of an antibody or T cell receptor bound by anti-Id.
11. *Connectivity*. The property of one Id network influencing the development of another. For example, the Ids on mature immune responses develop in sequence to different Ids, expressed on Ig in fetal mice.
12. *Parallel sets*. A network of Ab1-Ab2-Ab3 that is interactive.
13. *Network antigen*. An Ab2 that can react either with Ag or with Ab1 and therefore can regulate the entire Ag-Ab1-Ab2-Ab3 Id network.

This Id network regulates itself in at least two ways, as shown in Figure 15-1 . First, as discussed earlier, some Ab2 molecules behave like Ag; they stimulate Ab1 production, bind Ag receptors, and can probably trigger cell activation under certain conditions. As illustrated, these Ab2 internal image anti-Ids have sequences or conformations highly similar to those on Ag (31). This property of anti-Id serving as surrogate Ag has been used to isolate and characterize receptors (7) and to stimulate production of neutralizing Ab1, thus using Ab2 as a vaccine (8 ,27). Second, some Ab3 molecules have properties of Ab1, in that they can bind the original Ag. An elegant demonstration of the entire network, from Ag to Ab3, has been reported in a study using the O-specific polysaccharide side chain of Pseudomonas aeruginosa as Ag to raise Ab1 and Ab3 in mice; both Ab1 and Ab3 were opsonizing, protective antibodies that prevented lethal infection (10).

Although experimental results in many Id-anti-Id systems fit the Jerne hypothesis, numerous exceptions have occurred. For example, some Ab2 molecules can serve as surrogate Ags even though their binding to Ab1 is not Ag inhibitable, suggesting that they are not internal image anti-Ids. Such observations have led to another hypothesis of Ag Id-anti-Id interactions, reviewed by Kohler et al. (32). This hypothesis suggests that interactions between Ag, Ab1, Ab2, and so on can occur via side chains/conformations outside the Ag-binding groove of Ig. Some Ab2αs are able to broaden the epitope specificity of the immune response (33). This may be particularly important in situations where a very narrow range of antigenic specificities is recognized by the immune system, for example as in HIV infection. The mechanisms whereby this expansion of response occurs are not clear, but it has been suggested either that Ab2α may bind to both Ab1 and the antigen, thus stabilizing the complex, or that there is formation of a metatope, where new epitopes are revealed through conformational changes that result from the formation of the immune complex (33 ,34). Both hypotheses regarding Id interactions may be correct, and they may occur simultaneously.

The importance of Id networks in SLE has been suggested by several investigators who have demonstrated Ab1 and Ab2 in murine or human SLE; some have detected Ab3 (35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43). In several studies, internal image Ab2 has been shown to bind multiple autoAgs and Ab3 to bind Ab1.

T Cells in Idiotype Networks

The preceding discussion addressed Ag induction of circulating Ab1, Ab2, and Ab3. B lymphocytes secreting the Abs express the same Ids on their surface Ig. In addition, T lymphocytes are involved in Id-anti-Id networks. Several experiments have shown that Ab2 can bind to and, in some cases, activate T cells (44 ,45 ,46). Figure 15-2 illustrates the mechanisms by which B and T-helper (Th) cells interact to activate each other and to upregulate production of Igs bearing certain Ids. Concepts of T cell activation require interaction between antigenic peptides presented by the major histocompatibility complex (MHC) to the TCR of T cells. $CD4^+$ T cells recognize Ag presented by MHC class II; $CD8^+$ T cells recognize Ag presented by MHC class I. Such Ag presentation can be provided by B cells, which can process either surface or cytoplasmic Ig into peptides and present them in surface class I or II molecules (47 ,48 ,49 ,50 ,51 ,52 ,53). Thus, idiopeptides are presented to TCRs. Activation of a Th cell by this method is shown in Figure 15-2 . Ig can probably stimulate both B and T cells; in the reovirus system, a B-cell epitope has been defined in the CDR2 region of the Ig light

chain and a T cell epitope in the CDR2 of the heavy chain (54). Interestingly, in a murine anti-DNA antibody V88, T- and B-cell epitopes were colocated within the same region of the variable region of the heavy chain of immunoglobulin (VH) (55). There is also evidence that idiotypes may interact directly with TCR (56) and thus activate T cells in a non-MHC restricted fashion.

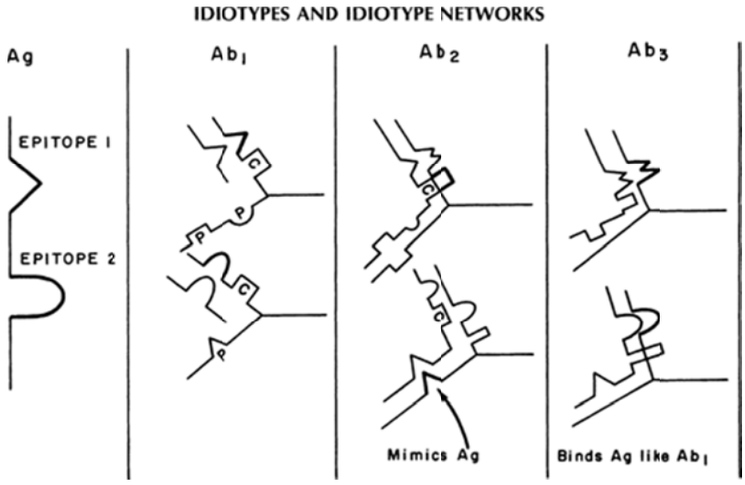

Figure 15-1. The antibody Id network. In the first panel, an antigen (Ag) with two epitopes is shown. In the second panel, two antibodies (Ab1) against the antigen are shown: the top Ab1 recognizes epitope 1, and the bottom Ab1 recognizes epitope 2. Each Ab1 has an idiotope specific to that clone, a private Id (p). Both Ab1s contain an identical sequence or conformational idiotope, designated as a cross-reactive (C) or public Id. Thus, the private Ids identify distinct clonal antibody specific for one Ag, and the public Ids identify related families of antibody that derive from different clones and can recognize different epitopes. In the third panel, two anti-Id antibodies (Ab2) have been induced by Ab1. The upper Ab2 is an alpha type; it binds Ab1 but does not mimic Ag. The lower Ab2 is a beta type; it contains a sequence that mimics an epitope on the original Ag and can behave like Ag (bind receptors for Ag, stimulate Ab1, and so on). It is called Ab2β, internal image anti-Id, or an epibody. In the fourth panel, two antibodies (Ab3) induced by Ab2 are shown. The upper Ab3 binds only Ab2. The lower Ab3 has a sequence or conformation that mimics a sequence/conformation in Ab1; it can bind Ag. Some, but not all, members of the network have the capacity to regulate other members.

Activation of Id-recognizing Th cells is probably necessary for full-scale production of Id-bearing antibodies, as shown in Figure 15-2 . Activation of Th cells releases B-cell growth and differentiation lymphokines, which in combination with cell-cell contact results in B-cell production of Ab1, Ab2, and so on (53).

TCRs can serve as immunogens for developing the Ab1/Ab2/Ab3 network as well as a regulatory T network, as shown in Figure 15-2 . For example, immunization of B10.D2 mice with an anti-Id made against the Id on the TCR of a Th clone specific for Sendai virus was effective in producing virus-specific neutralizing antibody, delayed-type hypersensitivity, cytotoxic T cells, and protection against infection (57). Id-recognizing Th cells may be distinct individual cells, or the recognition of Id may be a property of cells that also can recognize Ag epitopes. In several murine antibody systems, including anti-PC and anti-(T,G)A-L, there is collaboration between Th cells that recognize the Ag and the Id or anti-Id. However, Th cells that specifically enrich Id^+ antibodies have been detected (58).

T cell participation in Id networks is not confined to cells with helper function. Manipulation of the Id network can produce T cells that participate in delayed-type hypersensitivity, suppress Ab1 responses, kill cells expressing surface Ag or Ab2, and suppress proliferation of cells expressing the target Ids on their TCRs (57 ,58 ,59 ,60 ,61). In a pilot clinical trial in multiple sclerosis (MS) patients, antiidiotyic $CD8^+$ T cells were induced following vaccination of patients with irradiated autologous myelin basic protein (MBP)-reactive T cell clones (62). Idiotypic T cells lines were generated from the immunized patients and those reactive against the CDR3 region of the TCR of immunizing MBP-specific clones (63) were able to target and kill autologous MBP-reactive T cells in vitro.

Id-reactive T cells have been identified in both lupus-prone and normal mice (53 ,55 ,64 ,65 ,66 ,67 ,68 ,69 ,70). In the SNF1 model of SLE, $CD4^+$ Id-T cells increase before disease onset

(CD8+ do not), and some CD4+Id-T clones from those mice help production of anti-DNA by syngeneic B cells (71 ,72 ,73). Further, disease can be accelerated by adoptive transfer of CD4+ Id LNF1+ T cell clones in SNF1 mice. Regions of VH in some Id(LN) MAb enriched in positively changed amino acids stimulate these nephritogenic T cells (73). Similarly, CD4+ T cell clones from BALB/c mice immunized with monoclonal antibody (mAb) expressing the 16/6 Id proliferated to that Id and induced autoantibodies and nephritis on transfer to naive BALB/c mice (70). The levels of Id reactive T cells may also vary with disease activity.

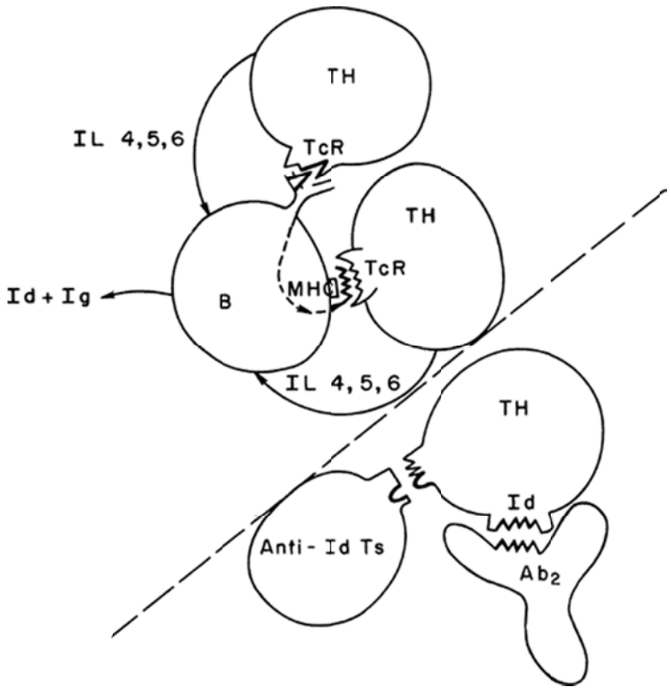

Figure 15-2. The T/B cell Id network. In the upper area, T-helper (Th) cells that augment autoantibody production are shown. They are idiotypic Th cells. The upper Th cell has a T cell receptor (TCR) that recognizes an Id on the surface Ig of an Id+ B cell; the lower Th cell has a TCR that recognizes an idiopeptide processed by the B cell from its surface Ig and presented by a major histocompatibility complex (MHC) class II molecule. Both Id Th cells secrete B cell stimulatory factors (IL-4, -5, and -6) that upregulate production of the Id+ antibody. In the lower area, the Id Th cell recognizes the idiotope on an Ab2 in one region of its TCR; another region of TCR has induced an anti-Id suppressor T cell (Ts), which can suppress the Id Th cell. Thus, both B and T cells participate in cellular and humoral regulatory networks governed by idiotypes.

The Structural Basis of Idiotypes

Apart from a few very well-studied Id systems, for example anti-arsonate CR1A (74 ,75 ,76 ,77) and the T15Id (78 ,79 ,80) associated with the antiphosphocholine response, the structural basis of most Ids has eluded definition. This is partly related to the tools used to define the Id. Classically, most Ids have been defined serologically, using polyclonal antiidiotypic reagents, which has led to problems of reproducibility inherent with such reagents. For many Ids definition has rested solely on whether it is linear (on H or L chain) or conformational (i.e., needs both H and L chain for expression). Delineation of the exact amino acid residues/sequences defining the Id (or their location) has been possible in only a few systems. Some examples are listed below. Within the heavy chain, two amino acids and/or carbohydrates have been defined in the CDR-2 region of the heavy chain (CDR-H2) for antibodies to dextran (81), and D-regions are involved in Id expression in anti-p-arsonate (74 ,75 ,76 ,77) and antigalactan antibodies (82). Id 4B4 found on the Ig of some patients with SLE or Sjögren syndrome (83), Id BH1 on human anticardiolipin antibody (84), and Id cross-reactive idiotype (CRI-EM) described in scleroderma (85) are all associated with the heavy chain. For 16/6Id described in lupus (86 ,87), and related Ids V-88 (88) and WRI-176ß (89), sequences in the FRH2/CDR-H2 (90), CDR-H2/FR-H3 (55 ,91), and CDR-H2 (92 ,93) are reported to carry major idiotopes. Id 9G4, which is expressed by cold agglutinins (94), and by a population of immunoglobulins in SLE (95), is located within the CDR-H1 at positions 23 to 25 as the sequence AVY (94). The trytophan at position 7 in the FR-H1 of 9G4 Id+ heavy chain also contributes to Id expression (96).

Ids associated with the light chain are described in antistreptococcal group A carbohydrate antibodies (97). In lupus autoantibodies the B3Id is located in the CDR-L1 (98); similarly 8.12Id expression is associated with amino acid residues Tyr32 and Asn 33 in CDR-L1 (99 ,100). Id H3 described on anticardiolipin antibodies (101) is also associated with the light chain. Other Ids are conformational. Examples can be found in monoclonal cold agglutinins (102), antithyroglobulin antibodies (103), and Id T15 (78).

Although analysis of the V-region amino acid sequences of Id+~ Igs has greatly facilitated the identification of such residues, mutational evidence confirming their contribution to Id expression has been reported in a very small number of cases (94 ,96 ,100).

An alternative approach to identifying idiotypic determinants has been to use epitope mapping (or pepscan) techniques (104). This method has been used for mapping both B- and T cell idiotopes and has been employed in studies of Id systems in SLE (55 ,64 ,90 ,93). Overlapping peptides of known sequences of VH and/or VL of Id+ Ig are tested against antiidiotypic sera or T cells to determine the sequences of idiotopes contributing to Id expression. In studies of murine and human anti-DNA antibodies expressing public Ids (WRI-176ß, V88, and B3Ids) a large number of B-cell idiotopes were identified using sera from lupus-prone mice and patients with SLE (91 ,93). The idiotopes were located in both the framework regions as well as in the CDRs. Computer models (105 ,106) of the Id+ anti-DNA antibodies revealed that several of the immunodominant sequences identified were located on the surface of the antibody combining site and were therefore accessible, and that others may become accessible upon binding to antigen (105). In addition, the pepscan method detected noncontiguous

epitopes, which in the three-dimensional (3D) binding site structure were situated in close proximity (105) (Kalsi, unpublished data). Descriptions of TCR-related idiotopes are limited, as they require the generation of T cell clones known to participate in idiotypic interactions and a determination of what component of the TCR is responsible for Id expression. However, idiotopes located on the TCR alpha chain CDR3 regions have been identified in murine models of experimental allergic encephalomyelitis (EAE) (107) and in NOD mice models of type 1 diabetes (108). In a recent study of patients with MS, a T cell idiotope was defined in the CDR3 of MBP-reactive TCR (63). Several investigations, however, have concentrated on identifying idiotopes on Id^+ Igs that have the capacity to activate T cells. The earliest such study was reported by Ebling et al. (64). Using T cell proliferation assays, three major sites (p34, p58, and p84) on the VH of nephritogenic murine mAb A6.1 were identified. These idiotopes were reported to modulate disease in young lupus-prone mice. In the murine anti-DNA antibody V88, the immunodominant T cell idiotope was located in the CDR2-FR3 region of the H chain (55). This region also carries at its carboxyl end a major B-cell epitope. Gavalchin and Staines (65 ,73) report on two sequences from the CDR2 and CDR2/FR3 region of the heavy chain of an anti-DNA $IdLNF1^+$ mAb 540, which also carry T cell idiotopes in SNF1 mice.

The definitive proof of the contribution of residues to Id expression (or indeed antigen binding) would derive from studies employing nuclear magnetic resonance or x-ray crystallographic analysis. Very few Ids have been investigated in this manner. To our knowledge there are currently four known crystal structures of idiotype and antiidiotype interaction (109 ,110 ,111 ,112). Mainly these have been generated to determine how closely antiidiotypic antibodies may mimic antigen (112) or not (109 ,110 ,113). In a crystal structure (resolution 1.9 ö) of an antilysozyme Fv fragment and the corresponding antiidiotypic Ab E5.2, Fields et al. (112) reported that essentially the same molecular interactions were observable between the Id and anti-Id as were seen between the antibody and the antigen hen-egg lysozyme (HEL). Thus, mimicry involves similar binding characteristics and is not necessarily a result of exact replication of the antigen. In a more recent study, the structure of the Fab fragment of a mouse anti-anti-idiotypic antibody was solved (114). Fab fragments existed in the crystal as dimers and were found to be self complementary. It is suggested that this may be a means of neutralizing an ongoing idiotypic cascade, and, furthermore, interacting antibodies downstream in the idiotypic cascade may be rendered increasingly similar and self reactive. This is an interesting postulate since it has been found that some autoantibodies may in fact be anti-idiotypic for immunoglobulins (115 ,116).

The composition of an idiotype clearly has a number of components, and the structural feature of the Id which predominates may well depend on the techniques and the tools used in its elucidation. Alongside the structural definition of an Id or a major idiotope there should also be consideration of whether the amino acid residues or sequence being defined as the “structural” idiotope is going to be equivalent in terms of the functional role of the idiotype. For example, when T cell responses to sequences corresponding to the CDR1 and CDR3 regions of a 16/6+ mAb were examined in normal mice, the predominant response in Balb/c mice was to the CDR1 fragment and in SJL and C3H.SW mice to the CDR3 sequence (66). Thus, depending on the strain and therefore the genetic background of the individual, the structural correlate of the major T cell idiotope may be different. Furthermore, since epitope mapping studies show that a number of epitopes within the variable region are “idiotypic,” it is possible that different idiotopes from the same Ig V region have different effects on the immune response.

Idiotypic Regulatory Circuits

Some Ids are regulatory, in that they can be suppressed or upregulated by other members of the circuit and in turn can regulate the other members. Early information regarding regulation suggested that anti-Ids served to suppress expression of their complementary Ids on antibodies to specific Ags (117 ,118). On the other hand, some anti-Ids can upregulate target Ids, even to the extent of activating silent B-cell clones that normally do not express their Id-bearing Ig. For example, BALB/c mice usually do not express the A48Id on their anti-fructosan Abs. However, administration of any of the following can force expression of that Id: (a) A48Id at birth, (b) polyclonal or monoclonal anti-Ids at birth, or (c) keyhole limpet hemacyanian (KLH)-linked polyclonal anti-Id in adults (119). In some systems, certain Ids or anti-Ids are either enhancers or suppressors of Id expression, whereas other Id-bearing mAbs do not regulate the circuit. Furthermore, the nature of the anti-Id (e.g., Ab2ß or γ vs Abα) may determine the ability to regulate idiotypic expression (43).

In many experiments with Ids and anti-Ids, the dose and timing of administration are critical to the results. Generally, administration of small quantities of Ids or anti-Ids upregulates Id expression, whereas administration of large quantities suppresses Id expression. For example, as shown in Figure 15-3 , immunization of young New Zealand black/white (NZB/NZW) F1 (BW) mice with small quantities (200 ng) of anti-IdX (a public Id on anti-DNA in BW mice) in Freund's adjuvant resulted in accelerated appearance of two autoantibodies: IdX^+ anti-DNA, and IdX^+ antihistone (35). In contrast, as shown in Figure 15-4 , repeated administration of large doses of anti-IdX (100 µg every 2 weeks) to adult BW mice suppressed IdX^+ Ig, anti-DNA, and the lupus-like nephritis associated with anti-DNA in this mouse strain (36 ,37 ,120). Thus, the same mAb anti-Id could be used to either enhance or suppress the Id-bearing Ig.

Id-recognizing T cells also can be used to alter immune responses. For example, infusion of T cells that have been sensitized to Ids of $CD4^+$ T cells that recognize alloAgs results in suppression of the ability of Th cells to proliferate to those Ags and prolongs allograft survival (59).

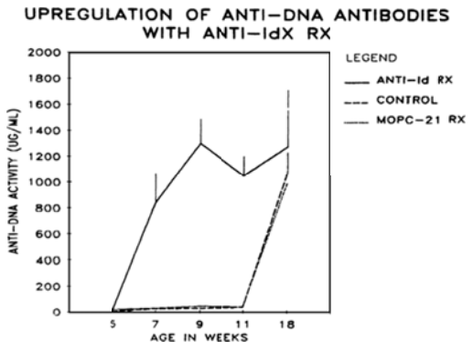

Figure 15-3. Upregulation of Id+ antibodies to DNA by administration of anti-Id antibodies in New Zealand black/white (NZB/NZW) F1 mice. This is an example of an upregulating Id network. Small doses (200 ng) of a monoclonal anti-IdX (made by immunization with an IdX+ antibody to DNA) were injected into young BW mice, and the appearance of anti-DNA in their sera was compared to that of littermates injected with the carrier or with an Id-negative monoclonal antibody (mAb) (MOPC-21). Injections were made at 4 weeks of age. Anti-Id–treated mice developed high titers of antibody to DNA by 7 weeks of age. In the control groups, anti-DNA appeared at 11 weeks (when the mice ordinarily begin to develop antibody); 95 of the anti-DNA in anti-Id–treated mice expressed IdX. The same mice also made IdX+ antihistone antibodies at an earlier age than controls. Thus, the anti-Id activated an Id+ autoantibody network that resulted in production of different autoantibodies expressing the same cross-reactive, public Id. (These experiments were performed by Karen Dunn, MD.)

Although regulatory properties of public Ids can be used to alter in vivo immune responses, the Id network in some systems (e.g., B-cell malignancies) escapes from such regulation (36 ,37 ,121 ,122). One mechanism of escape is somatic mutation of the Id so that it no longer is recognized by anti-Id (122). Because mutation of Ig molecules is common in B cells, whereas it is very uncommon in the TCR of T cells, this escape mechanism probably applies only to Ids on Ig. In NZB/NZW mice treated with anti-Ids, escape of Id^+ antibodies from suppression occurs without evidence of mutation of the Ig (120); this might represent a change in Id-directed T cell help (e.g., a shift from Th1 to Th2 cytokine patterns).

Role of Idiotypes in Maturation of the Immune Response

In adults, the normal B-cell repertoire probably depends on establishment of essential Id networks during fetal life. Studies of B-cell hybridomas obtained from fetal and 1- to 2-week-old normal BALB/c mice have defined early antibody responses in the pre and early immune states (123 ,124). Two properties are clear: (a) many early Ig molecules bind to self Ags, including V regions of Ig; and (b) this pre-immune, natural autoantibody network is highly connected via idiotypy.

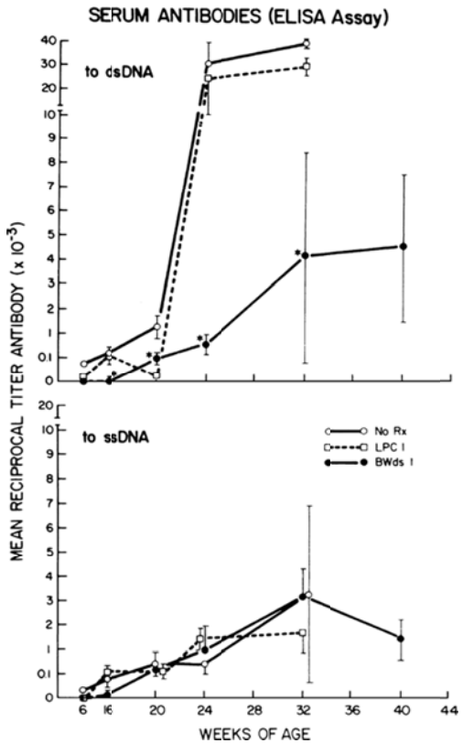

Figure 15-5. Downregulation of autoantibody responses by anti-Id antibodies. The idiotypic system is being studied in these experiments and those shown in Figure 15-3. In this case, high doses of monoclonal IdX (100 µg fortnights), which induce anti-IdX, were given repeatedly to NZB/NZW F1 females beginning at 20 weeks of age. Antibodies to double-stranded DNA (dsDNA) (upper panel) were significantly suppressed in IdX-treated mice (solid line, closed circles) compared with two control groups (open symbols). This is an example of an anti-Id induced by Id downregulating an autoantibody. In contrast, antibodies to single-stranded DNA (ssDNA) were not affected (lower panel). (From ref. 120, with permission.)

Vakil and Kearney (124) have referred to the early multispecific B cell as a superorganizer, influencing many generations of B cells that follow. This early network can be influenced by administration of Id^+ antibodies that do not bind the initiating Ag, so the network is at least partly independent of

Ag and can operate via recognition of Id alone. Any interference with the newborn network (by administration of Ag, Ab1, Ab2, or Ab3) results in impairment of normal antibody responses in adult life, especially if a highly public Id is involved (125). It is interesting that the Id F-423 found in fetal Medical Research Laboratory lymphoproliferative (MRL/lpr) mice occurs on anti–single-stranded DNA (ssDNA) and also appears in adult MRL/lpr and NZB/NZW F1 mice. Administration of IdF-423 accelerates lupus in young MRL/lpr mice and of anti-Id F-423 delays disease onset (126). Similarly, Id D23 on natural autoantibodies of normal mice can be found in the glomerular lesions of NZB/NZW F1 mice with lupus nephritis (127). Thus, some public Ids associated with pathogenic autoantibody repertoires are present in fetal or early postnatal life; SLE might result from their dysregulation.

Several investigators have demonstrated that the maternal idiotypic network has a potent influence in shaping the developing repertoire of the fetus/neonate (124 ,128 ,129 ,130). It has been suggested that to prevent serious infectious diseases of early childhood, immunizations of the mother be utilized to effect immunity in the offspring (130 ,131). Aside from passive immunity there is said to be “immunologic imprinting” by maternal idiotypic networks, which provides molecular information about the external environment and presumably skews development toward those idiotypic networks that would be most useful in dealing with local pathogens (128 ,129). There are a number of examples in the literature reporting the strong association of certain Ids with particular antimicrobial responses (132 ,133 ,134). Furthermore, maternal immunization is reported to alter the kinetics and the quality of the response in successive generations (130 ,135). For example, the F1 generation of mice immunized with respiratory syncytial virus exhibited characteristics of a secondary immune response when challenged with the original immunogen (135). One report (131) has demonstrated the use of engineered Ab2 fragments mimicking the capsular antigen of group B streptococcus for providing immunity transferable to offspring. Thus far, however, anti-Id vaccines remain an interesting but unproven approach to the prevention of infectious diseases in humans.

Idiotypes, Autoimmunity, and SLE

The Role of Idiotypes in Autoimmunity

Id networks play a central role in autoimmune diseases such as SLE. First, most autoantibodies are characterized by public as well as private Ids; therefore, a limited number of networks regulate a substantial proportion of the autoantibody repertoire (88 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147). Second, the Id on any Ab1 that arises may induce internal image Ab2 (epibodies, homobodies), and these internal image anti-Ids can then behave as Ags, thus inducing other Ab1 molecules that react with multiple self-Ags. For example, rabbits immunized with human polyclonal anti-DNA or with monoclonal anti-DNA derived from MRL/lpr mice developed autoantibodies that reacted with DNA, cardiolipin, Sm/RNP, and glomerular extract. All these reactivities were contained in the rabbit anti-Id against the immunizing Ig (38). In fact, some investigators suggest that most autoantibodies are anti-Id responses to antiviral or other protective Ab1 or indeed other autoantibodies (41 ,115 ,148 ,149). Zhang and Reichlin (115) report that some antibodies to Ro and La function as antiidiotypic to anti-dsDNA antibody, mimicking Ag such as p53, and serve to downregulate them. Antibodies able to bind dsDNA antigen, F(ab)2 anti-idiotypes, and idiopeptides from Ids or anti-Ids have also been reported (44 ,55 ,116 ,150 ,151). In susceptible strains of normal mice, certain Id^+ monoclonal anti-DNAs (16/6 and LNF1 for example) or anti-Ids (anti-Id 16/6), when used as immunogens, probably induce a full repertoire of Id network B and T cells, resulting in the appearance of multiple autoantibodies and nephritis (39 ,40 ,42 ,70 ,71 ,72 ,73). Only mice that develop anti-Id responses to Ab1 develop SLE-like disease. (It should be noted that one group of investigators could not repeat the work with Id 16/6 (152).) Third, Id networks are probably defective in patients with active SLE. Anti-Ids are detected in the serum during disease remission but are decreased during periods of disease activity (153 ,154 ,155). In fact, both anti-Ids and Id^+ Ig can be found in human SLE glomerular deposits and may contribute to nephritis (155 ,156 ,157). Ineffective Id networks therefore are among several immunoregulatory abnormalities that contribute to SLE.

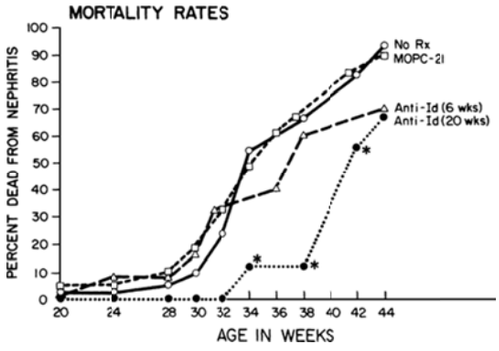

Figure 15-5. Suppression of clinical nephritis in NZB/NZW mice by administration of anti-Id antibody. In these experiments, NZB/NZW F1 females were treated once every 2 weeks with injections of a monoclonal anti-IdX or an Id-negative mAb (MOPC-21). Some littermates were untreated. Mice treated with anti-IdX (dotted line with solid circles) lived approximately 10 weeks longer than other groups. Their production of anti-dsDNA and IdX+ IgG was suppressed during that period but escaped from control. The appearance of IgG anti-DNA in serum was followed within a few weeks by lethal nephritis. Thus, anti-Id therapy was successful, but only as long as the Id network was sensitive to it. (From Hahn BH, Ebling FM. Suppression of murine lupus nephritis by administration of an anti-idiotypic antibody to anti-DNA. *J Immunol* 1984;132(1):187–190, with permission.)

Table 15-2: Mechanisms by Which Idiopathic Networks Participate in the Pathogenesis of SLE

1. Many autoantibodies express public Ids. These Ids may be targets of upregulation, thus keeping the levels of the autoantibodies high.
2. Some public Ids on Ab1 induce anti-Ids (Ab2) that bear internal image of Ags. Those Ab2 molecules in turn induce Ab1 molecules that react with multiple self-Ags. Thus, Id+ anti-DNA can induce Id^+ anti-Sm, Id^+ anticardiolipin, and so on.
3. Certain public Ids are markers of autoantibodies enriched in pathogenic subsets.
4. Idiotypic regulation is skewed toward upregulation of undesirable autoantibodies during periods of disease activity. Restoration of normal Id circuitry might abrogate disease.

Restoration of Id circuitry to normal might suppress active disease, and anti-Ids have been effective in suppressing autoantibody production by murine B cells in vivo (4 ,37 ,38 ,39 ,42 ,126 ,158 ,159 ,160), human B cells in vitro (153), and in patients treated with intravenous immunoglobulin (IVIg) (161). Furthermore, the suppression of target antibodies by administration of anti-Ids or anti-Id T cells, has been highly effective in prolonging survival in murine models of SLE (36 ,73 ,162). Table 15-2 reviews mechanisms by which Ids might participate in the pathogenesis of SLE.

Idiotypes in SLE

Because SLE is considered to be the prototype systemic autoimmune disease, the Id-anti-Id profiles as well as the structure of its most characteristic autoantibody, anti-DNA, have been studied by many investigators (4 ,5 ,35 ,36 ,37 ,38 ,42 ,83 ,86 ,87 ,88 ,99 ,106 ,126 ,127 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,153 ,154 ,155 ,158 ,159 ,160 ,163 ,164 ,165). Although most normal subjects, both human and murine, can make anti-DNA bearing public Ids, and although anti-DNA is part of polyreactive neonatal antibodies, most SLE patients have a different anti-DNA repertoire. For example, when MRL/lpr lupus-prone mice are immunized with Ars-KLH, the anti-Ars response is idiotypically distinct from the Ids of most MRL/lpr autoantibodies (166). In contrast, humans immunized with pneumovax produce anti-PC antibodies bearing Ids typical of anti-DNA in SLE; however, early in the response the Id^+ Ig does not bind DNA (167). Generally, anti-DNA in healthy individuals is composed largely of IgM with low affinity for ssDNA. In contrast, anti-DNA in mice or humans with active SLE is largely IgG with high affinity for both ssDNA and dsDNA. In fact, IgG anti-dsDNA is relatively specific for SLE.

Several laboratories have defined public Ids on anti-DNA antibodies originating either in human or murine systems (Table 15-2). Many such Ids, especially those occurring near the epitope-binding regions of the Ig molecule, occur in both murine and human SLE. Further, they are found in humans with virtually all known connective tissue diseases (most frequently in Sjögren syndrome), in first-degree relatives, and in small proportions of healthy individuals (139 ,168 ,169 ,170). Two groups (171 ,172) have studied Ids in family members of patients with SLE and have found no enrichment in family members or household contacts compared with healthy controls. However, in one study RT-84Id expression was similarly high in patients and their relatives and also occurred more frequently in spouses than in normal healthy controls (170).

Thus, several lines of evidence suggest that many of the public Ids that characterize autoantibodies in SLE are derived from highly conserved germline genes with little or no mutation. That evidence includes the following: (a) presence of the same Id in unrelated people with or without SLE (168 ,170 ,171 ,172), (b) presence of the same Id in mice and humans with SLE (5 ,38 ,143 ,173 ,174), (c) presence of the same Ids in fetal repertoires and natural autoantibodies as on anti-DNA of mature mice with SLE (126 ,127 ,171 ,175), (d) presence of the same idiotypes on different antibodies to self and to nonself (136 ,146 ,176 ,177 ,178 ,179 ,180), (e) ability to induce autoantibodies in normal mice by administering Id from human autoantibodies (42), and (f) inability to correlate the presence or quantity of a nephritogenic Id with MHC class II genes that predispose to nephritis (181). In keeping with this idea that public Ids are constructed from commonly available Ig sequences or conformations, few lupus Ids are confined to anti-DNA; in fact, in healthy individuals the Ids may be found (but rarely on anti-DNA). The 16/6 lupus anti-DNA Id has been found on antibodies to cardiolipin, platelets, cytoskeleton, lymphocytes, brain gangliosides, and mycobacteria (169). With a few exceptions, such as 16/6, 9G4 and 3I, serum levels of the lupus Ids do not correlate well with disease activity, nor do they accurately predict clinical characteristics of a patient (95 ,139).

Sequencing of several Id-bearing anti-DNAs from humans and mice has shown that some are derived from the germline with minimal mutation, whereas others have undergone somatic mutation (see Chapters 18 and 24). These two populations could represent those cases arising from polyclonal activation and those responding to specific antigenic stimulation. Not all anti-DNAs are pathogens; only certain subsets can induce disease directly, especially immune glomerulonephritis (182 ,183). As discussed elsewhere this may be related to the charge, fine specificity, or overall shape of the antigen combining site (184). In studies on antibodies expressing both anti-DNA and anti-F(ab)2 activity, Voss's group (185) showed that the IgG activity was directed toward the hinge region, and suggested that this property may be particularly relevant to immune complex formation and therefore nephritis. Descriptions of unconventional sites within the V regions of some antibodies include the presence of a nucleotide-binding site (186), which may delineate a pathogen in SLE and warrants further investigation.

There is substantial evidence that certain Ids are enriched in pathogenic autoantibody subsets. For example, we found that IdGN2 dominates the Ig in glomeruli of renal biopsy specimens from almost all patients with proliferative histologic forms of lupus nephritis (174); IdGN2 accounted for 28% to 50% of the anti-DNA deposited in glomeruli of patients with diffuse proliferative glomerulonephritis. Other investigators have searched for Id^+ Ig in tissue lesions of mice or humans with active SLE. Ids 16/6, 32/15, 3I, GN2, O-81, RT-72, RT-84, B3, 33C.9, and D5-M have been found in glomerular Ig deposits and/or at the dermal-epidermal junction in patients with SLE (138 ,143 ,144 ,156 ,157 ,169 ,174 ,187) (Table 15-3). It is likely that each of these Ids is enriched in pathogenic antibody subsets. This also is true in animal models of SLE. In NZB × Swiss Webster F1 mice, lupus nephritis with glomerular deposition of IgG anti-DNA, which is highly enriched in a family of Ids designated IdLN (73 ,144). In nephritic NZB/NZW mice, 50% of the glomerular Ig contains IdGN2 and $IdGN1^+$ antibodies. In addition, administration of some (but not all) $IdGN2^+$ monoclonal IgG2 anti-DNA to normal BALB/c mice produces Id^+ Ig deposits in their glomeruli as well as clinical nephritis (183 ,188). In contrast, monoclonal IgG2 anti-DNA bearing another public Id, IdX, does not induce nephritis in normal mice. Ids D23, 3E10, and 4B1 also have been found in glomeruli of mice with lupus (127 ,189 ,190).

As with Ids on antibodies to external Ag, the structural basis of Ids on autoantibodies is known for only a few mAbs. Clearly, the assembly of autoantibodies does not require any special genetic information. They can be constructed from normal germline DNA, with or without somatic mutation;

they can be assembled from different VH and VL gene families, although there may be some bias toward use of certain families; and they can resemble antibodies to external Ag (167 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,188 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199 ,200 ,201 ,202 ,203 ,204 ,205 ,206 ,207). Expression of public Ids can depend on amino acid sequences on the H or L chains, or both, or may be conformational (76 ,81 ,82 ,83 ,86 ,87 ,88 ,97 ,102 ,103 ,163 ,208 ,209 ,210). Finally, the structural characteristics of an antibody that make it pathogenic, perhaps related in part to Id expression, are poorly understood; they may depend on features such as cross-reactivity, charge, and avidity. (See Chapter 24 for a more complete discussion of the characteristics of pathogenic anti-DNA.)

Table 15-3: Public Idiotypes in Patients with SLE

Id	Source	In Tissue	Location	Special Features	Percent of SLE Patients with Id in Serum	Percent of Normals with Id in Serum	References
GN2	Glomerular	++ (G)	H chain	Dominant Id in glomeruli of pts with DPGN	67	13	(139,174)
X	mAb aDNA BW mouse	0	?	Dominant Id on serum IgG of BW mice; anti-Id suppresses disease in mice	42	0	(138,139)
16/6	mAb aDNA	++ (G, S)	H chain germline VH26 or VH4	Immunization of normal mice may induce autoantibody and SLE-like disease	25-28	7	(39,40,86,87) (137,168) (173,243)
3I	Polyclonal aDNA, SLE patient sera	+ (G)	κ L chain	3I+ aDNA enriched in cationic IgG	50	7	(139,198,244)
8.12	Polyclonal aDNA, SLE patient sera	+ (G)	λ L chain V_λII	May be specific for SLE	50	5	(97,99,163,208)
0-81	mAb a-ssDNA SLE patient	++ (G)	?	Enriched in pts with GN; occurs in BW mice	72 (with GN) 19 (without GN)	0	(147,158,206)
NE-1	mAb a-dsDNA	++ (G)	?	Enriched in pts with GN; occurs on a-dsDNA	33 (with GN) 6 (without GN)	0	(158,206)
B3	mAb IgG anti-dsDNA, SLE patient sera	+ in vivo ANA	λ L chain	Associated with arthritis in SLE patients	20	0	(98)
F4	Polyclonal aDNA, SLE patient sera	?	H chain	Expressed only on IgG; enriched in cationic Ig	35	?	(198,245)
H3	Human mAb from SLE PBC	?	λ L chain	Found on aPL; may be specific for SLE	?	?	(101)
RT84	Human mAb from SLE PBC	?	?	Found on aPL and aDNA	19	42	(246)
3E10	mAb aDNA, MRL/lpr mouse	?	Conformational	Found in sera of patients with SLE and GN	75 (without GN)	25 (without GN)	(187)
KIM4.6.3	mAb IgM anti-ssDNA normal human tonsil	?	L chain	Id from normal on aDNA in SLE patients	90	24	(139,142)
134	mAb aDNA, SLE patient sera	?	?	On SLE Ig in serum	42	0	(139,247)
AM	Polyclonal aDNA in SLE patient sera	?	Conformational	On SLE Ig in serum	25	13	(139,248)
BEG-2	mAb aDNA, human fetal liver	?	L chain	Fetal antibody Id found on aDNA in SLE patients	8	7	(139,249)
8EY	mAb aDNA, patients with leprosy	?	?	Id from antibody of patient with infection occurs on aDNA of SLE patients	25	0	(139,250)
A52	mAb aDNA BW mouse	?	Conformational	Id from BW mice found on human aDNA in SLE patients	>50	?	(5)
Y2	mAb aSm MRL/lpr mouse	?	?	Id from MRL/lpr mouse found on aDNA in SLE patients	41	6	(251,252)
4B4	mAb aSm human	?	H chain	Cross-reacts with Sm and p24 gag protein of HIV-1	52	?	(83,253)
D5-R	mAb IgG aDNA, human SLE	0 (G)	L chain	Found only on IgG	20-30	5	(187)
D5-M	mAb aDNA	++ (G)	Conformational	Found only on IgG	20-30	7	(187)
A24	mAb from normal human cord blood in adults with SLE	?	H chain	Newborn has Id/anti-Id network also found in aDNA network	?	?	(254)

GN, glomerulonephritis; aDNA, anti-DNA; aPL, anti-phospholipid; dsDNA, double-stranded DNA; IgG, immunoglobulin G; mAb, monoclonal antibody; MRL/lpr, Medical Research Laboratory lymphoproliferative mice; ssDNA, single-stranded DNA.

Manipulation of Clinical Disease in Mouse Models of SLE by Altering the Idiotypic Network

Id networks have been manipulated by several investigators through administering Ids or anti-Ids in attempts to alter disease in the NZB/NZW, MRL/lpr, and SNF1 mouse models of SLE. In some experiments, autoantibody production was suppressed, nephritis was delayed, and survival was prolonged (36 ,120 ,126 ,158 ,159 ,160 ,162). In our laboratories, IdGN2, commonly found on glomerular Ig in human lupus DPGN and in nephritic NZB/NZW mice, was downregulated in NZB/NZW mice by administration of a closely related anti-Id (36) (Fig. 15-5). Serum levels of IdGN2 were initially suppressed but escaped from control, and mice died of nephritis with deposits of $IdGN2^+$ and $IdGN1^+$ Ig in their glomeruli. Their lives were prolonged a mean of 10 weeks compared to controls treated with an irrelevant murine mAb. There was no evidence of emergence of mutated or unrecognized Ids in the mice with escape nephritis. It may be that the ability of certain Ids to escape from suppression is a feature that partially explains their enrichment in pathogenic antibody subsets. In a study where NOD mice were immunized with 16/6Id, it was reported that instead of developing insulin-dependent diabetes mellitus (IDDM) the animals developed SLE (211). Thus, in susceptible animals, autoimmunity does not go away but simply reemerges under another guise.

Although much work has focused on attempts to suppress the expression of pathogenic Ids in murine lupus, experiments with the 16/6 Id-anti-Id system have shown that lupus and antiphospholipid antibody syndrome can be induced in normal mice by manipulating this Id network (39 ,40 ,42 ,70). Immunization of some normal mice (BALB/c, C3H.SW, AKR, and SJL, but not C57BL/6 or C3H/He) with Ids or anti-Ids resulted in development of circulating Ids and anti-Ids, multiple autoantibodies (including anti-DNA, anti-Sm, and anticardiolipin), leukopenia, and nephritis. The susceptibility of strains to the induction of disease did not seem to correlate with MHC or Ig allotype genes, but it did correlate with the ability to make immune responses within the Id network. That is, susceptible strains immunized with Id or anti-Id responded by producing high-titer anti-Id or Id, respectively; resistant strains did not. This ability depends on characteristics of bone marrow cells, because BALB/c mice chimeric for C57Bl/6 bone marrow are resistant to induction of SLE by Id or anti-Id immunization (212). Clearly, activation of certain Id networks can induce pathogenic reactivity to self.

Recent work on manipulating the idiotypic networks has centered on the use of idiopeptides derived from the V regions of disease-associated autoantibodies. The peptides used have either been selected from the framework and/or CDR regions of the Id^+ Ig or chosen through epitope mapping studies that have shown these to be immunodominant for T- or B-cell responses (53 ,55 ,64 ,65 ,66 ,67 ,68 ,73 ,213 ,214 ,215 ,216 ,217 ,218 ,219 ,220 ,221 ,222). Since idiotypes are able to enhance or suppress the autoimmune response, it is possible that analyzing the effects different components of the Id have on the autoimmune response may assist in determining what aspects of the idiotypic structure are responsible for stimulation or suppression. In this way motifs pertinent to the reestablishment of regulatory Id networks may be defined. For example, peptides containing the motif KFKGK can stimulate $CD4^+$ T cell responses in BW F1 (64) and SNF1 mice (65 ,73). Studies thus far have demonstrated the following: Immunizations of normal healthy mice with VH peptides from the murine monoclonal anti-DNA antibody V88, $16/6Id^+$, and $IdGN^+$ mAb produced symptoms of SLE (66 ,219 ,223). In lupus-prone mice, immunization with idiopeptides of A6.1 VH, IdLNF1 p62–73, 16/6Id, and V88 64–80 accelerated the disease process and reduced survival time (53 ,65 ,66 ,219 ,223). In contrast,

when a CDR3 peptide was used from a D23Id$^+$ murine monoclonal, immunization was found to have protective effects; up to 50% of lupus-prone mice exhibited prolonged survival times (220). In contrast, using high-dose tolerizing regimens, idiopeptides may also be used to tolerize premorbid lupus-prone mice and delay disease onset (66 ,213 ,224). Furthermore, different idiopeptides derived from the same VH may stimulate the production of different cytokine profiles (Th1 vs. Th2) (53). Anti-idiopeptide antibodies are generated in both spontaneous and induced models of SLE, which not only react with the immunizing peptide and the native Id-carrying Mab, but may also have specificity for DNA (55 ,150). Therefore the anti-DNA response may arise as part of another response simply through sharing of idiotypic determinants. The other response may be to an external or internal antigen (eg pneumococcal lipopolysaccharide, EBNA-1, heat shock protein 90, or tumor suppressing molecule p53 (151 ,177 ,215).

Idiotypes in the Treatment of Human SLE

A number of studies have shown that clinical improvement in SLE patients correlates with the presence of circulating antibodies able to interact with Ig variable regions (153 ,154 ,155). As discussed elsewhere in this volume, there is interest in treating selected patients with SLE using intravenous gamma globulin (IVIG). These preparations are generated from pools of serum from up to 45,000 normal individuals (155). It has been demonstrated that the pools contain anti-F(ab)2 activity, some of which is directed against Ids 8.12, 3I, F4, 4B4, and 16/6 (Ids characteristic of SLE autoantibodies), and many authors have suggested that these anti-Ids account for improvement in clinical disease (162 ,225 ,226). In addition IVIg contains natural antibodies to TCR public Ids (227) which could enhance or suppress help or regulation supplied by TCR-Ids.

Harata et al. (147) reported that affinity columns containing two anti-Ids (D1E2 and 1F5) coupled to sepharose were able to remove 25% to 92% of the anti-DNA antibody repertoire from sera of patients with SLE and could also remove Id$^+$ T cells. In a more recent study of five patients with lupus nephritis, Silvestris et al. (161) compared the effects of using anti-Id (F4 and 8.12) IgG (preparation EL-11) purified from IVIg versus the whole IVIg. In the two SLE patients treated with EL-11 intravenously, a significant improvement was noted. In a larger study of 20 SLE patients, Levy et al. (228) reported decreases in disease activity and autoantibody production. Since M-components express Ids associated with SLE, it has been suggested that they may be used as source material for purification of large amounts of antiidiotypic material for clinical use (229). IVIg have been used to treat a variety of diseases and is thought to operate via a number of mechanisms, reviewed elsewhere (230).

Idiotypic vaccination with murine anti-dsDNA antibody (3E10) of patients with inactive SLE and stable nephritis has been tested in a phase I clinical trial (231). Five of the nine subjects developed anti-idiotypic responses to the murine antibody. In a follow-up lasting 2 years, disease exacerbations did not occur.

A peptide (“Edratide”) from the VH region of an Id16.6-positive human anti-DNA is in clinical trials in SLE patients (214 ,215 ,232). In lupus mice, a similar peptide induces regulatory CD4$^+$CD25$^+$ T cells which suppress autoantibody production and nephritis.

Engineered Idiotypes and DNA Vaccines

Recent technologic advances in molecular biology and protein engineering mean that antibody combining sites and therefore idiotypes can be custom built. This can be achieved in a number of ways. First, it is possible to generate single-chain Fv (scFv) fragments that essentially represent the antigen combining site. These consist of the variable region of the heavy chain and the variable region of the light chain joined together with a short flexible segment referred to as the linker. They are produced by using the genes that encode for the VH and VL regions, respectively. The scFv is expressed in bacteria and more recently on the surface of filamentous phage (210 ,233 ,234). Clearly, the antigen combining site can be modified genetically to generate a product with a higher affinity or different specificity, or can even be used to express antigenic determinants that may not be naturally located within the combining site (235 ,236). All this is possible because first, the structure of the antibody-binding site is well conserved and most of the changes that occur in the combining site happen within the CDRs, especially in the CDR3. Second, specific amino acid sequences can be introduced into the CDRs of the V region without altering the overall shape of the combining site. This process is known as CDR grafting. Since B cells are able to process and present their Ig variable regions (47), these “antigenized” Igs (237) can thus be used to elicit highly specific, what are essentially “anti-Id,” responses to infectious agents, for example. Zaghouani et al. (236) have used this approach to produce antibodies to a principal neutralizing determinant derived from the V3 loop of the HIV-1 envelope protein. Earlier reports demonstrated induction of responses to influenza virus (235) and to malaria (238). Xiong et al. (238) demonstrated that as well as CDR-H3, which is the usual site for grafting, CDR-H2 could also be utilized. Furthermore, the introduction of a T cell epitope into the latter loop enhanced the B-cell response for the malaria antigen. This suggests that idiotypic determinants within the variable region may themselves modulate the “anti-idiotypic” response to other determinants within the same site. ScFv can be used as the classic Ab2 antigen mimic. It can also be altered from the wild type to generate a desirable antibody response (235). Features of anti-DNA binding have also been investigated using scFv technology (210 ,239). Recombinant antibodies also feature in investigations and treatment of tumors. CDR grafting in fact has been used in a number of cases to produce humanized antibodies so as to minimize the effects of using murine antibodies directed against tumor antigens (240).

A more recent development is the use of DNA vaccines (241 ,242). Here the DNA encoding for the VH and VL regions itself can be used as an immunogen. The vaccine essentially consists of the genetic material cloned into a bacterial plasmid that is designed to allow expression in eukaryotic cells. The vaccine also contains CpG motifs that can enhance the immune response. The functional protein (Ab2 mimic or Id) when expressed by the vaccinated individual can then elicit immune responses to the antigen, be this infectious agent or tumor antigen. The vaccine can be made effective by conjugating it another component, for example the fragment C of Clostridium tetani (241).

Engineered Ids have not been used in SLE as far as we are aware. However, it is tempting to speculate that public Ids that have been described in lupus may be engineered and used to purify anti-Id reagents for therapy or be used as inoculi singly or linked in a fusion protein with selected cytokines to re-equilibrate dysregulated idiotypic networks.

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Chapter 16

The Importance of Sex Hormones in Systemic Lupus Erythematosus

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Hormones and the immune system interact through a number of pathways (1 ,2) and understanding the interdependence of these systems is important in order to appreciate the pathogenesis of autoimmune disease. This chapter will discuss the influence of gonads on the thymus and vice versa, as well as the effects of estrogens, androgens, and pituitary and hypothalamic hormones on immune responses and autoimmunity. Identification of the stimulating and suppressive properties of individual hormones affords the opportunity to design effective and relatively nontoxic treatments to change hormone concentrations and thereby affect systemic lupus erythematosus.

Gonadal Hormones

Immune System-Reproductive System Interactions

The interdependence of the thymus, ovaries and pituitary-gonadal axis has been demonstrated in female rodents. A number of thymic peptides, including thymulin, regulate T cell maturation, influence pituitary and hypothalamic structure and act as neurotransmitters. In turn, the production of thymulin is controlled by the anterior pituitary hormones, prolactin and growth hormone (3).

The thymus is required for ovaries to develop and function normally. Congenitally athymic nude mice had small ovaries and premature ovarian failure (4) as well as reduced concentrations of circulating luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrone, and testosterone (5). Neonatal thymectomy results in sterility and ovarian dysgenesis in rodents. B6A mice thymectomized at 3 days of age responded with diminished ovarian weight, loss of oocytes and lack of corporea lutea. Circulating antibodies to oocytes were identified and the ovaries were infiltrated with lymphocytes. Neonatal thymectomy also resulted in low levels of FSH, LH, growth hormone, and prolactin (6). A preferential decrease in $CD4^+$ lymphocytes occurred after neonatal thymectomy, and reproductive dysfunction was prevented by injecting $CD4^+$ lymphocytes (7) or implanting a normal thymus (8).

The thymus is sensitive to gonadal hormones in rodents, and high doses of estradiol or testosterone depleted lymphocytes in the thymic cortex (9). In contrast, physiologic doses of estrogen stimulate the thymus. It has been proposed that this stimulation is mediated through influences on factors such as thymulin that are secreted by the thymic epithelium. This contention was supported by finding receptors for both thymic hormones and sex hormones on thymic epithelial cells (10) and by the discovery that estrogen stimulated thymulin secretion (11). Erbach (12) found that replacement doses of estradiol increased antibody formation in ovariectomized female rats. Estradiol was effective as long as the rats had an intact thymus or were treated with thymosin factor 5. Mice were “rescued” from thymectomy-induced ovarian dysgenesis if they were treated with estradiol-17ß before the thymus was removed (13).

Effects of Estrogen in Murine Models of Lupus

Sex hormones play an important role in regulating the severity of disease in F_1 hybrid New Zealand Black (NZB) × New Zealand White (NZW) (NZB/NZW) mice, a model of SLE that spontaneously develops antibodies to antidouble-stranded DNA (anti-dsDNA) and immune complex mediated glomerulonephritis. Reports from colonies in different parts of the world verify that disease in NZB/NZW females starts earlier and progresses more rapidly compared to males. Female NZB/NZW mice die of renal failure at 10 to 12 months of age and males die at the age of 14 to 16 months (14). The females have considerably more IgG anti-DNA antibodies compared to males. At 5 to 6 months of age, females switched from autoantibodies of the IgG and IgM classes to primarily IgG autoantibodies. This switch occurred 3 months later in males (15). Of interest, these mice have concentrations of serum estradiol and testosterone that are comparable to mice that do not develop autoimmune disease (16 ,17). Furthermore, the females do not have abnormalities of estrogen metabolism that would alter 2-hydroxylated or 16-hydroxylated products (18).

Treatment with estrogen accelerates disease in weanling NZB/NZW mice. Mestranol, a synthetic estrogen used in oral contraceptives, induced positive tests for fluorescent antinuclear antibodies in male NZB/NZW mice after 6 weeks

of treatment (19). Roubinian (15 ,20) and Steinberg (21) gave castrated NZB/NZW mice crystalline implants that contained high, nonphysiologic doses (6 to 7 mg) of estradiol-17ß. In mice of both sexes, autoantibody levels were stimulated and recipients of implanted estradiol died prematurely compared with untreated castrates and intact animals (15 ,20 ,21 ,22 ,23).

These early studies linked estrogen with the phenotypic expression of severe autoimmune disease in the NZB/NZW model. It was therefore an unexpected finding that surgical oophorectomy did not reduce the severity of lupus in NZB/NZW females (15). In addition, parturition did not lead to immediate postpartum flares of autoimmune disease. When NZB/NZW mice whelped at 10 and 16 weeks of age and the pups were removed within 24 hours, there was no immediate stimulation of anti-dsDNA, serum immunoglobulins, albuminuria, or blood urea nitrogen (24). In NZB/NZW females that had multiple litters, severity of glomerulonephritis and longevity did not differ from virgin controls (25).

The doses of estrogen used in early experiments (15 ,20 ,21) produced extremely high concentrations of circulating estradiol and fatal estrogen toxicity (26). Toxic wasting, bladder distention, endometriosis and pituitary adenomas were observed in NZB/NZW females that had received the high-dose crystalline implants (23 ,26 ,27 ,28). Newer dosing regimes, however, more closely mimicked naturally occurring levels of estrogen in mice. Brick (16) implanted NZB/NZW mice with 1.5 to 2.0 mg of estradiol-17ß and this dose increased antibody responses. Carlsten (29) used biweekly injections of estradiol benzoate 3.2 µg to reproduce physiologic estrogen concentrations. This treatment accelerated renal disease and shortened longevity in first-generation offspring of NZB/NZW × NZB backcross mice that had inherited the $H2^{d}/H2^{z}$ genotype, which is the same genotype as that of NZB/NZW mice.

The MRL/MpJ-Fos^{lpr} (MRL-lpr) mouse model of autoimmunity has variable responses to estrogenic hormones. High estrogen doses accelerated disease, and male castrates that received continuous long-term treatment with a super-physiologic dose died prematurely with renal disease (30). MRL-lpr females injected with complete Freund's adjuvant, followed by 14 daily injections of high-dose estradiol (0.4 mg/kg/day), responded with proteinuria and early mortality (31).

In contrast, longevity is not influenced strongly by gender in MRL-lpr mice (14). A physiologic dose of estradiol did stimulate anti-DNA, but short-term treatment did not affect longevity in castrated males (16). Unexpectedly, physiologic estradiol suppressed postpartum flares of arthritis (31) whereas raloxifene did not accelerate autoimmunity (32).

Of interest, estrogen stimulates expression of autoimmune disease in mice that are considered “not autoimmune.” C57BL/6 mice do not express autoimmune disease at a young age, but the aged animals spontaneously develop anti-DNA antibodies and inflammatory changes in multiple organs (33 ,34). Estrogen implants in C57BL/6 mice stimulated anti-DNA antibodies (34) and increased numbers of spleen and bone marrow cells that secreted IgG and IgM (35).

Gender influences the SLE models developed by Wakeland (36 ,37) in which specific lupus susceptibility genes were introduced into C57BL/6 mice by congenic matings. The *Sle 1* gene leads to breach of tolerance for chromatin, with development of high titers of antinuclear antibodies. The *Sle 3* gene controls glomerulonephritis and has higher penetrance in females than males (36). When both genes were expressed in B6.NZMc1/c7 mice, severe disease developed that was characterized by splenomegaly, expanded populations of activated B cells and $CD4^{+}$ T cells and glomerulonephritis. Female B6.NZMc1/c7 mice had earlier activation of lymphocytes, higher levels of serum IgG and nephrophilic antibodies with stronger glomerular affinity compared to B6.NZMc1/c7 males. These bicongenic females developed very high levels of anti-dsDNA. It therefore appeared that female gender, and possibly female hormones, promoted the pathogenic spread of autoantibody production from anti-nucleosome antibodies to anti-DNA (37).

Lupus that is induced by manipulating “nonautoimmune” strains is stimulated by treating the mice with estrogen before inducing the autoimmune disease. Female C57Bl/10 × DBA/2 F_1 mice injected with DBA/2 lymphocytes are highly susceptible to chronic graft-vs-host disease. When the lymphocyte recipients were treated with estradiol-17ß before induction of the graft-vs-host reaction, there was increased production of multiple autoantibodies including anti-dsDNA (38). BALB/c mice immunized with the 16/6 Id idiotype of human anti-dsDNA will develop anti-DNA, and this autoantibody response is enhanced by estrogen. BALB/c mice of both sexes were implanted with capsules containing 2 to 3 mg of estradiol-17ß and immunized 3 months later with the idiotype. The mice responded with high titers of antibodies to dsDNA and a number of other autoantibodies (39). Treatment of the idiotype-injected mice with either tamoxifen or anti-estradiol antibody decreased severity of proteinuria and protected against immune complex deposits in renal glomeruli (40).

Effects of Testosterone in Murine Models of Lupus

In contrast to estradiol, androgenic hormones are protective in NZB/NZW mice. In female NZB/NZW mice, subcutaneous implantation of Silastic capsules containing testosterone or 5-dihydrotestosterone suppressed SLE and prolonged life spans (23). It was not necessary for the females to be castrated in order to benefit from exogenous androgens, and androgen treatment was effective even if it was started after the onset of clinical disease (23 ,41 ,42). Autoimmune disease in NZB/NZW females is responsive to relatively low doses of androgen. In fact, the low levels of testosterone that are found normally in female mice afford some protection from autoimmune disease. Long-term treatment with flutamide, a specific blocker of androgen receptors, resulted in accelerated mortality in NZB/NZW females (17). The naturally occurring weakly androgenic steroid, dehydroepiandrosterone sulfate (DHEA), delayed the onset of anti-DNA antibodies and prolonged longevity in NZB/NZW females when treatment was started at 2 months of age. In contrast, there was no response

if treatment began at the age of 6 months (43). Treating NZB/NZW mice with DHEA ameliorated imbalances in several cytokines that are associated with progression of autoimmune disease in this model. Circulating levels of IL-10 and IL-6, which are expected to increase with disease onset and exacerbation, were suppressed by DHEA (43 ,44).

In NZB/NZW males, the course of disease was accelerated by castration. Surgical removal of the testicles before puberty was followed by early production of anti-DNA antibodies, early switch from 19S to 7S autoantibodies and early death (41). Early castration was more effective than late castration, and castration of males at the age of 3 months had little effect on anti-DNA antibodies (45).

The rapidly progressive autoimmune disease in MRL-lpr mice does not display marked differences in severity between genders, and high-dose estradiol therapy is required to accelerate disease progression. High doses of androgens do effectively suppress MRL-lpr autoimmunity. Suppression of systemic autoimmune disease in males has been achieved with implants containing 6 to 8 mg of testosterone or 12 mg of 5-dihydrotestosterone, implanted at 2 to 4 weeks of age (46 ,47).

Lacrimal glands of MRL-lpr mice are infiltrated with periductal and perivascular collections of lymphocytes that disrupt acinar tissue, and lacrimal gland involvement in MRL-lpr females is significantly greater compared to MRL-lpr males. Implantation of high-dose pellets that contained 10 or 25 mg of testosterone resulted in dramatic reversal of the inflammatory lesions (48 ,49).

Immunologic Imprinting of the Fetus

Exposure of the fetal mouse to high-dose exogenous estrogen can stimulate autoimmune responses in a strain that does not ordinarily develop autoimmune disease. In C57BL/6 mice, treating the dam with estrogen at 14 to 16 days of gestation resulted in offspring that had sialoadenitis and increased plaque forming cell responses to bromelain-treated erythrocytes (50 ,51).

Immunologic imprinting by naturally occurring gonadal hormones *in utero* can change the severity of autoimmunity in mice that develop autoimmune disease spontaneously. In the maternal-fetal unit composed of the NZB dam and her NZB/NZW F_1, hybrid fetuses production of steroid hormones was regulated differently compared with “nonautoimmune” mice. Male NZB/NZW fetuses are expected to develop into adults that live longer than NZB/NZW females, The male fetuses were found to develop in utero in the presence of abnormally high levels of serum estradiol. In contrast, testosterone concentrations in the testicles and placentae of fetal NZB/NZW males were abnormally low (52).

Estradiol is considered immunostimulatory in young adult NZB/NZW females, Paradoxically, newborn NZB/NZW females benefited from one-shot treatment with high-dose estradiol. Yamaguchi (53) injected NZB/NZW mice with estradiol-17β 200 ng/g within 2 days of birth. Longevity was increased in the females (50% survival at 18 months of age) so that life spans in the estrogen-exposed females resembled life spans in untreated NZB/NZW males.

Testosterone imprinting of the NZB/NZW fetus ameliorated the course of their autoimmune disease after they grew into adults. However, the beneficial effects were manifested only in males. The prenatal effects of androgen were evaluated by treating NZB dams with Silastic implants containing testosterone on days 13 to 18 of gestation. The male NZB/NZW offspring of these dams had therefore been exposed to increased levels of testosterone *in utero* and in adult life these males had extended life spans compared to male NZB/NZW controls (54).

Responses to Estrogen and Androgen in Murine Lupus

Estrogen has powerful effects on the immune system through mechanisms that are regulated differently through two types of estrogen receptors. Mice from nonautoimmune lineages that were knockout for the estrogen receptor α gene spontaneously developed immune complex glomerulonephritis by 1 year of age with proteinuria, destructive infiltration of B cells in the kidney and serum anti-antibodies DNA, The females had increased serum IgG3. Pro/pre-B lymphocytes ($B220^{low}$/IgM-) and mature B lymphocytes ($B220^{high}$/IgM^+) in bone marrow were decreased (55). In contrast, estrogen receptor ß knockout mice had splenomegaly and myeloproliferative disease resembling chronic myeloid leukemia with lymphoid blast crisis (56). Pro/pre-B lymphocytes were increased in numbers and this inhibition of B cell development resembled the changes noted in mice after oophorectomy. Therefore, the ß estrogen receptor appeared to mediate the effects of estrogen on the immune system (55 ,56).

Exogenous estrogen stimulates $CD4^+$ T cell gene and protein expression of surface receptors that regulate T cell homing (57). Furthermore, estrogen stimulates production of the Th1 cytokine, IFN-γ (58), and has the capacity to enhance production of Th2 cytokines (59). These properties could contribute to the enhancing effects of estrogenic hormones in murine models of SLE. Expression of IFN-γ transcripts was very high in unstimulated T cells from female NZB/NZW mice (60 ,61). Furthermore, NZB/NZW females had high expression of IL-6 genes in unfractionated, unstimulated spleen cells (61). Another survey of NZB/NZW females found limited secretion of Th1 cytokines but unfractionated spleen cells produced high levels of IL-3, IL-4, IL-5, and IL-10 (62). Estrogen is capable of stimulating immune responses in both “nonautoimmune” mice and the New Zealand F_1 hybrid. Treatment with estrogen for periods of either 2 or 4 weeks decreased T suppressor cell activity in C57BL/6 mice as well as autoimmune NZB/NZW mice (63).

Estrogen stimulation is not limited to T cells or to cells that produce antibodies directed against a specific antigen. $CD5^+$ B cells, which spontaneously produce polyreactive IgM autoantibodies (49), were increased in nonautoimmune mice and in young NZB/NZW mice that were treated with estradiol-17β (64 ,65).

Immunostimulatory effects of estrogen are expressed in B cells in lupus-prone mice. Murine B cells have both α and ß estrogen receptors (66). BALB/c mice have a genetic background that predisposes to loss of B cell tolerance. Diamond and associates (67) generated transgenic R4A-γ2b BALB/c mice that express the γ2b heavy chain of a nephritogenic anti-DNA antibody. These mice are normally able to maintain tolerance by deleting DNA-reactive B cells that arise in the immature repertoire. When transgenic BALB/c mice were treated long term with implanted estradiol-17ß that provided serum concentrations equivalent to those found in normal estrus cycles, the mice developed a lupus-like phenotype with elevated levels of circulating anti-DNA antibodies and glomerular immune complex deposits.

Estradiol was thought to have stimulated autoimmunity by increasing the resistance of transitional B cells to apoptosis through up-regulation of the anti-apoptotic protein Bcl-2, and the inhibitory signaling molecules CD22 and SHP-1. Estradiol rescued B cells that were capable of producing high affinity anti-DNA so that these cells were not eliminated from the B cell repertoire. Under the influence of estrogen, the cells matured to a marginal zone phenotype. Both follicular and marginal zone anti-DNA producing B cells were increased in estradiol-treated R4A-IgG2b mice. The greatest increase, however, was in marginal zone cells, which were 10 times more common than follicular cells. Furthermore, the marginal zone B cells were activated by estradiol to secrete high affinity and potentially pathogenic anti-DNA antibodies and were not susceptible to regulation by T cells (68 ,69 ,70).

Testosterone clearly suppresses autoimmune disease in NZB/NZW mice of both sexes. Earlier studies of the mechanisms of androgenic suppression suggested that the immunosuppressive effects of androgens were limited to T cells. Testosterone increased T suppressor cell activity (63) and did not affect $CD5^+$B cells (71) in the NZB/NZW model. Detailed studies of “nonautoimmune” C57BL/6 mice demonstrated that surgical removal of the testes was followed by profound changes in B cells and T cells. This reordering of the immune system could explain some aspects of accelerated autoimmune disease in NZB/NZW male castrates. Surgical castration of male C57BL/6 mice at 6 weeks of age was followed by thymic hypertrophy, splenic enlargement because of expansion of B cells and decreased numbers of splenic T cells. Spleen cells had increased production of IL-2 and IFN-γ, and anti-thyroglobulin antibodies and rheumatoid factor production increased (72). $B220^+$ B cells expanded in the bone marrow following castration of C57BL/6J males, and injections of testosterone cypionate 1 mg or dihydrotestosterone 0.2 or 0.4 mg reversed this effect (73).

Diet Therapy for Murine Lupus

The observation that estrogen can stimulate the immune system led to attempts to manipulate lupus activity with diet. Aromatase-knockout mice are unable to make estrogen. Aromatase-knockout mice raised on a phytoestrogen-free diet developed B cell hyperplasia and destructive leukocyte infiltrates in the salivary glands that resembled Sjögren syndrome. The lesions were completely absent if the mice were fed a diet with normal levels of phytoestrogen. These results suggested that endogenous estrogen paradoxically protects against autoimmune exocrinopathy. Furthermore, phytoestrogens that occur normally in food appeared to play an important role in replacing endogenous estrogenic hormones (74).

Indole-3-carbinol is abundant in cruciferous vegetables and can shift estrogen metabolism away from mitogenic 16α hydroxyestrone, which may fuel disease activity, and toward less estrogenic metabolites. When indole-3-carbinol was fed to female NZB/NZW mice soon after weaning, 80% of the treated mice were alive at 1 year of age compared to 10% of controls. Mice that received indole-3-carbinol from the age of 5 months were all alive at 1 year. Treatment was associated with suppressed anti-dsDNA and suppressed renal disease. The ratio of 2- to 16-α-hydroxyestrone in urine was increased, reflecting a shift in estrogen metabolism that favored production of metabolites with less estrogen activity (75).

Hormone Manipulation to Treat Murine SLE

The aromatase inhibitor 4-hydroxyandrostenedione, which inhibits estrogen biosynthesis, suppressed disease in female NZB/NZW mice. Treatment from 11.5 to 15 weeks of age reduced thymus weights and appeared to retard glomerular inflammation (76).

Tamoxifen, a selective estrogen receptor blocker, was highly effective in NZB/NZW females. A dose of 22 mg/kg body weight was injected at 2-week intervals and the mice were sacrificed after 5 months of treatment. Percentages of B cells and $CD5^+$ B cells were decreased, tumor necrosis factor receptor molecules were fewer in number and renal deposition of immune complexes was suppressed in treated mice (77). More intensive treatment with tamoxifen 800 µg twice a week (about 64 mg/kg/week), resulted in significant reduction of antibodies against nuclear extracts of HeLa cells and calf thymus DNA and significant prolongation of life. Serum anti-DNA antibodies of the IgG3 class a class that appears to be pathogenic in murine SLE, were reduced and glomerular deposits were composed primarily of IgG2a (78).

In the estrogen-sensitive transgenic mouse model, R4A-γ2b BALB/c, tamoxifen blocked the appearance of lupus. When tamoxifen was given in conjunction with estradiol, apoptosis was not affected. Autoreactive B cells expanded but were anergic, and the mice did not develop anti-DNA antibodies or glomerular deposits of IgG. This series of experiments illustrated the ability of tamoxifen to abrogate estrogen-enhanced activation of autoreactive B cells, whereas survival of the cells was not affected (79).

Androgens are beneficial in murine models of lupus, and testosterone and 5-dihydrotestosterone produced favorable responses in NZB/NZW (23 ,41 ,42) and MRL-lpr mice (46 ,47 ,48). Female NZB/NZW mice benefited from long term

intraperitoneal injections of DHEA 100 µg twice a week, if the injections were started at 2 months of age before the expected appearance of overt disease. Anti-dsDNA was suppressed and mean longevity was prolonged so that 71% of treated mice were alive at 41 weeks of age compared to 22% of control mice. DHEA treatment that was started at 6 months of age was not effective (43). NZB/NZW females and castrated males also responded favorably to injections of nandrolone decanoate, an androgen with attenuated virilizing properties. Proteinuria and anti-DNA were suppressed and treatment was effective even if it was begun after disease onset (80 ,81).

The 19-nor-testosterone derivatives norethindrone and norgestrel are progestogens used commonly in oral contraceptive pills. Each compound, in Silastic capsules, was implanted separately in 6-week-old and 24-week-old NZB/NZW females in relatively small doses that were calibrated to suppress reproduction (82). Both progestogens suppressed anti-DNA antibodies, and mice that were implanted with norgestrel at the age of 24 weeks had prolonged life spans (83).

Gender Difference in Occurrence of SLE in Humans

Both hormonal and nonhormonal factors predispose to the increased incidence of SLE in women (84 ,85 ,86). Human SLE clearly has a predilection for females of childbearing age (87) and may appear or exacerbate when serum concentrations of reproductive hormones are elevated and when hormones are undergoing rapid change.

The mean age of menarche was delayed significantly in a small group of young women with SLE (13.5 years) compared to healthy adolescents (12.5) but the majority of the adolescents with lupus had normal gonadal function (88). Disease flares were increased in the 2-week period before onset of the menstrual period (89). Flares were fewer in number following natural menopause (90) or ovarian failure induced by cyclophosphamide therapy (91). In the era before corticosteroids were used to treat SLE, improvement was noted after surgical removal of the uterus and ovaries or treatment with testosterone. Irradiation of the ovaries, however, did not change the course of disease (92).

The theory that SLE activity is influenced by reproductive hormones is supported by the finding that disease flares are increased during pregnancy (93 ,94 ,95 ,96 ,98 ,99). The number of pregnancies and live births that occur during a woman's lifetime did not predict an increased probability of developing lupus *de novo* (97). In patients with pre-existing SLE, the risk of increased lupus activity was elevated during pregnancy. Increased flares were observed at the St. Thomas' Lupus Pregnancy Clinic (98). At the Hopkins Lupus Pregnancy Center, flares (defined by a change of 1.0 on a 0 to 3 visual analog scale) occurred in 60% of lupus pregnancies. The rate of flares in pregnancy was significantly greater compared to the same patients after delivery and to nonpregnant SLE patients (96 ,99).

Th1 cytokines, including IL-2, IFN-γ, and tumor necrosis factor (TNF)-ß, are deleterious during pregnancy and may lead to fetal demise. It has therefore been proposed that a shift away from Th1 responses and towards Th2 responses occurs during normal pregnancy and is important in maintaining the intact fetal-maternal unit (100). Are reproductive hormones involved in this shift? Estrogen has the potential to stimulate both Th1 and Th2 responses (58 ,59). It is of interest to speculate that estrogen in the high concentrations found in pregnancy or estrogen acting in concert with other reproductive hormones can stimulate increased Th2 cytokines. This response would be desirable during a normal pregnancy. In the patient with SLE, excessive Th2 responses could cause increased production of antibodies and lead to activation of disease (101).

This theory was not supported by a recent study of adrenal and gonadal hormone levels in 17 pregnant SLE patients. Surprisingly, estrogen concentrations did not increase during the second and third trimesters. This abnormal response was thought to result from damage to the placenta or an abnormality of steroid metabolism in the placenta (102).

Responses of Peripheral Blood Mononuclear Cells to Estrogen in SLE

Peripheral blood mononuclear cells (PBMC) from patients with SLE have abnormal responses to estrogen stimulation. In vitro apoptosis was decreased when lupus patients' PBMC were cultured with estrogen, a response that could permit abnormal cells to survive and perpetuate autoimmune responses (103). In another series of experiments, culturing PBMC from SLE patients with estradiol 17-ß enhanced production of total IgG and IgG anti-dsDNA antibodies. In contrast, PBMC from normal donors responded to estradiol by producing increased IgG but not anti-dsDNA. Adding IL-10 to estradiol had an additive effect on increasing antibody production (104).

Estrogen Receptors

Variations in estrogen receptors have been identified in human lupus. Normal individuals were found to have both wild-type receptors and an isoform which lacks exon V. In contrast, SLE patients had either the wild type or the truncated form of the receptor. It was suggested that the presence of mutated or differentially spliced forms of estrogen receptor accounted for abnormal responses to estrogen in SLE (105). Calcineurin, an enzyme which participates in T cell signal transduction and has the potential to promote activation of IL-2 and other cytokine genes, may serve as a link between estrogen and the immune system in lupus. Estrogen bound to the estrogen receptor evoked a direct, gender-specific increase in calcineurin mRNA expression in T cells from women with SLE (106 ,107). Estrogen also stimulated the amount of surface CD40 ligand mRNA on T cells from lupus patients compared to normal controls (108).

Oral Contraceptives in SLE

The appearance of autoantibodies and overt SLE has been reported in previously healthy women taking combination oral contraceptives with a high content of potent synthetic estrogen (109 ,110 ,111). In contrast, other investigators noted that rheumatic symptoms did not accompany the use of estrogen-containing contraceptive pills (112 ,113). The current practice of using increasingly smaller doses of estrogen (<50 µg) in combination pills probably accounts for the apparent decrease in lupus-like side effects noted in the past three decades. In metropolitan Philadelphia, oral contraceptives were not associated with new-onset SLE in patients diagnosed between 1985 and 1987 (114).

A recent report by Sanchez-Guerrero (115) did suggest that the use of oral contraceptives was associated with a small risk of developing SLE. A cohort of women in the Nurses' Health Study was followed prospectively every 2 years between 1976 and 1990. When those who had used oral contraceptives were compared with those who never used oral contraceptives and a stringent case definition of SLE was used, the relative risk for developing SLE was found to be 1.9.

In women with pre-existing SLE, oral contraceptives with high dose estrogen were reported to stimulate flares in pre-existing SLE in the 1960s and 1970s. Pimstone (116) and Chapel (117) reported lupus flares in women taking combination pills that contained 50 µg of ethinyl estradiol and 100 µg of mestranol, respectively.

Data from the prospective multicenter Safety of Estrogens in Lupus Erythematosus-National Assessment (SELENA) trial showed that triphasic oral contraceptives did not increase lupus flares in selected subjects over a 1-year period. Premenopausal women (*n* = 145, completed follow-up) with either inactive or stable active SLE took either oral contraceptive pills (35 µg ethinylestradiol/0.5–1.0 mg norethindrone) or placebo for 12 cycles. The participants did not have a history of thrombosis, moderate/high titer antiphospholipid antibodies or lupus anticoagulant. Severe flares occurred in 7.7% of oral contraceptive users and 7.6% of the placebo group. Mild to moderate flares were found in 15% of patients taking oral contraceptives versus 16% of those taking placebo (118).

Postmenopausal Estrogen Replacement in SLE

The routine use of hormone replacement therapy after the menopause is not recommended by the US Preventive Services Task Force (119) because “the harmful effects of combined estrogen and progestin are likely to exceed the chronic disease prevention benefits in most women.” A review of earlier reports of women taking estrogen after the menopause is of interest, however, because the question has arisen that these women could be at increased risk to develop SLE. The Nurses' Health Study showed that women who had taken hormone replacement had an increased risk of *de novo* SLE and the risk had a positive relation to duration of the therapy (120). In a separate case-control study, women who used postmenopausal estrogens for 2 or more years were at risk to develop both discoid lupus and SLE (121).

Postmenopausal estrogen replacement has also been associated with flares of SLE. A case has been reported in which a patient with lupus had remission when she became menopausal at 38 years of age. The disease recurred when she was treated with estrogen for osteoporosis at the age of 64 years (122). In contrast, 2 studies of relatively small groups of SLE patients showed no lupus flares associated with conventional hormone replacement (123 ,124). Transdermal estrogen was given without flares to a group of 15 postmenopausal SLE patients for a period of 1 year (125).

The SELENA trial affirmed that postmenopausal hormone replacement therapy could contribute to flares of SLE, but the flares were of mild or moderate severity. In the hormone replacement arm of the study (*n* = 286, women who completed followup), menopausal women with inactive or stable active SLE were enrolled between March 1996, and June 2002, and randomized to take either 0.625 mg of conjugated estrogen for the entire month plus 5 mg of medroxyprogesterone on days 1 to 12 or placebo. Exclusion factors included high blood pressure, history of venous or arterial thromboses or pulmonary emboli or myocardial infarction, high-titer anticardiolipin antibodies or lupus anticoagulant and migraine headaches with neurologic sequelae. The trial was stopped in 2002, after all the patients were enrolled, based upon the reported results of the Heart and Estrogen/progestin Replacement Study (126) and the Women's Health Initiative trial (127). Severe flares were observed in 7.5% of women receiving hormone replacement and 4.5% of the placebo group, but the difference was not significant. In contrast 59% of hormone-treated SLE patients had mild to moderate flares with an overall incidence rate (taking into account multiple flares of any type) of 1.25 flares/person-year. Flares did occur in 50% of patients taking placebo but the incidence rate of flares was significantly lower (0.93 flare/person-year, $p = 0.006$) (128).

Assisted Reproduction and SLE Flares

Ovulation induction increases circulating estradiol to 10 times the concentration found in nonpregnant women, and an increasing number of reports have documented the association of ovulation induction and in vitro fertilization (IVF) with new-onset SLE and flares of SLE (129). Three women who underwent 6 to 10 cycles of ovulation induction developed very high estradiol levels (1,560–2,850 pm/L) and lupus (130). Another presented with acute lupus (butterfly rash, peritonitis, ascites, anti-dsDNA, hypocomplementemia) following 11 cycles of gonadotropin-human chorionic gonadotropin (HCG) ovulation induction (131). A woman who underwent 27 uncontrolled gonadotropin-HCG-stimulated ovulation induction cycles had three pregnancy losses between the ages of 26 and 32 and developed in sequence, insulin-dependent diabetes mellitus, arthralgia, and thrombocytopenia followed by full blown lupus with a photosensitive rash, cells LE, anti-dsDNA, hypocomplementemia, and anticardiolipin antibodies (132).

In women with established SLE, case reports described moderate flares (133) and a fatal exacerbation of SLE in women undergoing ovulation induction (134). Increased lupus activity was observed in 25% of ovulation induction/IVF fertilization cycles in SLE patients and 13% were complicated by the ovarian hyperstimulation syndrome (129). In another series SLE flares appeared after 13 of 62 cycles. The flare rate was higher after gonadotropin induction (27%) compared to clomiphene induction (6%) (135).

Sex Hormones in SLE Patients

Gonadal hormones are abnormal in both women and men with SLE. In women the metabolism of estradiol-17β and estrone is shifted to favor production of 16-hydroxyestrone and estriol and these metabolites are increased significantly compared with normal women. The 16-metabolites are potent mitogens that are thought to have the potential to stimulate inflammation (136 ,137). Women with lupus also have low plasma androgens. Very low levels of circulating androgens have been described in women with active SLE (138). This abnormality could result from increased testosterone oxidation at C-17, or increased tissue aromatase activity (139 ,140 ,141).

DHEA has shown promise as a treatment for SLE. A study conducted in Taiwan enrolled 120 women with active lupus to receive either DHEA 200 mg/day, or placebo for 24 weeks. In the treatment group, testosterone levels increased significantly but overall disease activity measured by the systemic lupus activity measure (SLAM) score did not differ from the control group. Numbers of patients with flares, those with serious adverse events and patient estimates of disease severity were decreased significantly in the DHEA group (142). A multicenter double-blind United States study of women with SLE resulted in improvement in 58.5% of 147 women treated with DHEA 200 mg/day compared to 44.5% of control women, a difference significant at the 0.017 level. Of interest, levels of both testosterone and estradiol increased in the treatment group (143). A significant decrease of circulating IL-10 occurred in DHEA-treated patients (144).

In men with SLE, imbalances in estrogens and androgens could contribute to increased susceptibility to active disease. Estradiol levels have been reported to be increased (145 ,146), normal (147) or low (148) in male SLE patients. Males with lupus had elevated serum 16-hydroxyestrone (136 ,140) and estrone (148) concentrations. Some men with SLE have a state of functional hypoandrogenism with low levels of plasma testosterone (147 ,148) and elevated LH (149).

Prolactin

Effects of Prolactin on the Immune System

Prolactin, a peptide hormone secreted in the anterior pituitary, has the potential to stimulate the immune system and has been implicated as a factor that can activate SLE (150). There is increasing interest in the role of prolactin in SLE and other autoimmune diseases, and the relationship between prolactin and autoimmunity is addressed in several recent reviews (151 ,152 ,153). Prolactin is produced in sites outside the pituitary, including the brain and lymphocytes (154). Prolactin is a cytokine. It has comparable structural motifs, is synthesized in multiple sites including lymphocytes and has similar receptor structures and signal transduction pathways. Prolactin receptors are distributed throughout the immune system (155) and are included in a novel receptor family that includes receptors for IL-2β, IL-3, IL-4, and IL-6 (156). Prolactin can influence the immune system through the thymus (157), inducing IL-2 receptors on lymphocytes (158) and acting as a growth factor for lymphocytes.

Lymphocytes synthesize and release a biologically active form of prolactin (159), which is employed by the cells as an autocrine and paracrine growth factor. It is possible that treatment with corticosteroids affects lymphocyte production of prolactin. Dexamethasone reduces circulating prolactin concentrations and inhibits gene expression of both pituitary prolactin and lymphocyte prolactin (155).

In rodents, prolactin affects the immune system at almost every level and influences T cells, B cells, macrophages and natural killer cells (160 ,161). Prolactin is important in maintaining normal immune function and sustaining life. Rats that were deprived completely of prolactin by hypophysectomy and injections of antiprolactin antibody became anergic and anemic and died within 8 weeks. Replacement injections of either prolactin or growth hormone stimulated expression of the c-myc growth promoting gene and reversed involution of the spleen and thymus (162).

High levels of circulating PRL stimulate immune responses. Hyperprolactinemia that was created in mice by either implanting syngeneic pituitary glands or injecting exogenous prolactin increased primary humoral antibody responses (163), and low levels of prolactin in cysteamine-treated mice were associated with thymic atrophy and immune suppression (164).

Th-1 cytokines are involved in initiating autoimmunity and Th-2 cytokines contribute to production of antibodies by B cells. The transcription factor gene, interferon regulatory factor-1 (IRF-1), which is exquisitely sensitive to prolactin, is an important regulator of T cell and B cell differentiation and maturation. IRF-1 is required for Th1 immune responses. Prolactin, which stimulates IRF-1, has the potential to regulate expression of Th1 cytokines such as IFN-γ and IL-15 (161). The potential of IRF-1 to promote autoimmunity was demonstrated when type II collagen-induced arthritis was induced in mice that were either IRF-1 deficient (-/-) or IRF-1 positive (+/-). Disease was reduced in the IRF-1 -/- mice compared to the +/- mice (165).

Estrogen-Prolactin Interactions

Estrogen is a potent stimulus for production of pituitary prolactin in rodents and estrogen stimulates autoimmunity in the NZB/NZW lupus model. Female NZB/NZW mice treated with either ethinyl estradiol or estradiol-17β developed pituitary adenomas and serum prolactin levels that were up to 91 times greater than controls (26). This secondary elevation of

prolactin could have contributed to the apparent stimulation of autoimmune disease that was attributed previously to estrogen.

The estrogen-sensitive transgenic R2A-γ2b BALB/c mouse model of Bynoe et al. (70) developed lupus-like serology when it was oophorectomized to remove the major source of estrogen and treated with a prolactin dose that caused a twofold increase in circulating prolactin. The T1/T2 ratio was inverted, with more T2 than T1 cells. Bcl-2, was increased and all B cells increased. The autoimmune stimulating actions of prolactin were genetically determined and did not occur in R4A-γ2b C57Bl/6 mice. A second group of lupus-susceptible transgenic BALB/c mice was treated with both estrogen and bromocriptine in order to determine if estrogen could stimulate lupus in the presence of extremely low concentrations of prolactin. Bromocriptine did not block the appearance of DNA-reactive B cells, but these cells were functionally inactive. It therefore appeared that an adequate amount of circulating prolactin was required in the blood stream in order for estrogen to stimulate the lupus phenotype in a lupus-prone mouse with a permissive genetic background (166 ,167).

Effects of Prolactin in Murine Models of SLE

Hyperprolactinemia In NZB/NZW mice resulted in premature death from autoimmune renal disease. Female NZB/NZW mice were made chronically hyperprolactinemic by grafts of two syngeneic pituitary glands and developed premature glomerulonephritis and early mortality. In contrast, mice treated with the prolactin-lowering drug, bromocriptine, had delayed appearance of antibodies to dsDNA and significantly prolonged life spans (168). Neidhart (169) treated mature NZB/NZW females from the age of 36 weeks with a dose of bromocriptine (5 mg/kg/day) that suppressed serum prolactin to undetectable levels. After 12 weeks of treatment, autoantibodies were not suppressed in mice that received bromocriptine, but bromocriptine did suppress the occurrence of proteinuria. No mice treated with bromocriptine had histologic evidence of glomerulonephritis, but glomerulonephritis was found in 70% of the control animals.

The effects of very high levels of prolactin were examined in a group of NZB/NZW females that had 4 transplanted pituitary glands. Twelve weeks after the pituitary glands were implanted, 80% of recipient mice had antibodies to ds DNA and hypergammaglobulinemia (170). Male NZB/NZW mice, which develop an indolent form of SLE, responded to hyperprolactinemia with accelerated disease (171). Another study determined that naturally occurring hyperprolactinemia was detrimental in parous NZB/NZW mice after whelping and suckling 2 liters or after experiencing prolonged pseudopregnancy (172). Elbourne (173) found that mice with high circulating estrogen and high serum prolactin had accelerated albuminuria and premature appearance of antibodies to DNA (75% positive at 16 weeks of age). In contrast, autoimmune disease was retarded in females with high estrogen levels that were treated with bromocriptine. These mice had delayed appearance of albuminuria and antibodies to DNA (10% positive at 16 weeks of age).

Prolactin and Autoantibodies

Disease-associated autoantibodies have been detected in hyperprolactinemic individuals who did not have clinically apparent autoimmune diseases. Women with prolactinomas had antimicrosomal antibodies and antithyroglobulin, each occurring in 21% of subjects. Antithyroglobulin antibodies were found in 19% of hyperprolactinemic men (174). In another survey of 33 women, 82% of whom had pituitary adenomas, antibodies to at least one autoantigen were detected in 76% and 8 had seven or more different autoantibodies. The most common antibody targets were ssDNA and dsDNA, Sm pyruvate dehydrogenase, and SSA/Ro. The subjects did not have clinical evidence of autoimmune disease (175). Anticardiolipin antibodies were found in 22% of hyperprolactinemic women and men (176).

Hyperprolactinemia in SLE

Many surveys have shown that circulating prolactin is increased in SLE patients. Eight series, six of which included both men and women with SLE, reported serum prolactin concentrations above the norm in 20% to 40% of subjects (150). The analyses of McMurray and May (153) found that prolactin values were above normal in 7 of 10 groups of female lupus patients and 2 of 4 groups of males with lupus. Examination of 19 groups comprising a total of 1,149 patients revealed that 21% were hyperprolactinemic compared to 3% of healthy controls. The association between SLE and hyperprolactinemia was supported by the subsequent publication of three reports of elevated prolactin in lupus patients (177 ,178 ,179).

Prolactin concentrations above the norm were found in 12% of serum samples that were submitted to the Antinuclear Antibody Laboratory at the University of Missouri for routine diagnostic testing. High prolactin was most common in women 50 years of age or younger who were diagnosed with SLE and women older than age 50 years who had antibodies to both SSA/Ro and SSB/La. The expected incidence of hyperprolactinemia in normal populations was 1.3% (180).

Elevated prolactin concentrations did correlate positively with active SLE in 7 reports (179 ,181 ,182 ,183 ,184 ,185 ,186 ,187) and in analyses of five published studies (188), whereas six series showed no correlation (178 ,189 ,190 ,191 ,192 ,193). Jara-Quezada (194) reported five pregnant women with SLE whose hyperprolactinemia exceeded that expected during gestation, and the question was raised that excessive increases of prolactin were associated with disease flares during pregnancy. In a larger series, 68 lupus patients were randomized to receive either bromocriptine 5 mg/day for 14 days starting within 12 hours postpartum or no drug. The bromocriptine-treated subjects did not nurse their infants, but 21 of the 44 controls did nurse. Treated mothers had a significantly lower rate of relapses, a lower cumulative dose of prednisone and a lower cumulative dose of cyclophosphamide in 1 year of follow-up (195).

Causes of Hyperprolactinemia in SLE

Why is serum prolactin elevated in some patients with SLE? Hyperprolactinemia can result from stress (196), certain drugs, hypothyroidism or renal insufficiency (180). Prolactinomas have been reported in at least 43 individuals with lupus (197 ,198 ,199 ,200 ,201 ,202), and it has been proposed that the non cycling secretion of abnormally high concentrations of prolactin stimulates autoimmune responses. In six instances, lupus improved with bromocriptine therapy (197 ,198 ,199 ,200 ,202) and 11 patients had flares when bromocriptine was stopped (197 ,198 ,200 ,201). Lymphocytes from SLE patients actively secrete prolactin (203) and may serve as an extrapituitary source of measurable amounts of bioactive circulating 60 kDA prolactin (204). Antiprolactin antibodies have been identified in SLE patients with idiopathic hyperprolactinemia. It is possible that anti-prolactin activity interferes with attachment of prolactin to receptors so that a “false low” level of prolactin is presented to the pituitary and feedback mechanisms involved in regulation of prolactin secretion are disrupted (205). The association between inactive SLE and low levels of circulating homovanillic acid suggests that impaired dopamine turnover and altered dopaminergic tone results in hyperprolactinemia (206). Circulating cytokines that cross the blood–brain barrier in SLE may also stimulate the anterior pituitary to release excessive prolactin (160).

Prolactin-Suppressing Therapy in SLE

Suppression of circulating prolactin controls symptoms and prevents flares in SLE (207 ,208 ,209 ,210). Seven lupus patients with mild to moderately active disease, 6 of whom had normal prolactin concentrations before treatment, responded to bromocriptine. After 6 months, bromocriptine was stopped and 5 patients became hyperprolactinemic. All 7 patients had increased lupus disease activity in the 5 months after treatment was discontinued (207). The double-blind study of Alvarez-Nemegyei (208) showed the potential for daily treatment with a fixed dose of bromocriptine (2.5 mg/day) to reduce lupus flares. In a more recent study, patients with active but not life threatening SLE were randomized to receive either bromocriptine, in a dose designed to suppress serum prolactin to a concentration <1 ng/mL, or hydroxychloroquine 6 mg/kg. Treatment continued for 1 year. In 11 patients who received bromocriptine, the SLE Activity Measure (SLAM) decreased from (mean ± standard error of the mean) 14.0 ± 1.1 at entry to 7.5 ± 0.8 (p <0.05) after 1 year of treatment. In 13 SLE patients who received hydroxychloroquine, the SLAM score decreased from 13.4 ± 1.3 at entry to 9.0 ± 1.4 (p <0.001) posttreatment. Prednisone doses, the numbers of patients who started and stopped prednisone, and numbers of patients who left the study were similar in both treatment groups (209). Hrycek (210) demonstrated that the benefits of prolactin suppressive therapy were not confined to use of bromocriptine. Low-dose quinagolide was given to 20 SLE patients and 17 healthy individuals were the controls. The SLEDAI measure of disease activity decreased significantly during therapy, as did prolactin and IL-6. It was concluded that quinagolide treatment could have a role in managing SLE.

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Chapter 17

Neuroendocrine Immune Interactions: Principles and Relevance to Systemic Lupus Erythematosus

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A large body of both animal and human studies provides evidence for communication between the immune system and the central nervous system (CNS), and for the importance of this communication in susceptibility to inflammatory disease (1). This chapter first outlines the general principles of this communication, its hormonal and neuronal components, and the physiologic role that it plays in autoimmune/inflammatory disease. Subsequent sections define the afferent and efferent limbs of this communication in more detail, the contribution of specific immune cells and their mechanism of action to initiate disease, and evidence from animal and human studies that these mechanisms are operative in systemic lupus erythematosus (SLE).

The immune system and CNS are the body's initial interface with the constant environmental perturbations that threaten homeostasis. These two systems are designed to recognize environmental perturbations and respond, to re-establish homeostasis. Each system recognizes different kinds of foreign stimuli—whether chemical, antigenic, or infectious in the case of the immune system or psychological and physical in the case of the CNS—but both use many of the same transducing systems to translate perturbing signals into stabilizing responses.

These transducing systems can be either hormonal or neuronal and can lead to regulation at the systemic, regional, and local levels. The cytokines of the immune system not only signal other immune cells but also stimulate the CNS through neuronal and hormonal routes. In turn, the hormones of the stress response and neurotransmitter and neuropeptide release from autonomic and peripheral nerves modulate immune and inflammatory responses (Fig 17-1) (1).

These multiple levels of communication between the immune system and CNS represent an important physiologic mechanism for modulating the intensity of immune and inflammatory responses and for controlling susceptibility and resistance to inflammatory disease. Interruptions of this communication, at any point and through any mechanism, lead to enhanced susceptibility to or severity of autoimmune/inflammatory disease. Conversely, reconstitution of the communication reduces autoimmune/inflammatory disease expression. Thus, the degree of inflammation in response to a foreign stimulus depends not only on the nature of the stimulus, its potency, dose, route, and duration of exposure, but also on the intensity of the modulating neuroendocrine and neuronal responses. This principle has important implications for understanding the effects of pharmacologic agents or stress on autoimmune/inflammatory disease. Drugs that are not primarily designed to affect autoimmune/inflammatory disease severity might alter disease course if they alter the communication between the immune system and the Similarly CNS, these principles provide a rationale and potential mechanism for previously anecdotal evidence that “stress” can affect or precipitate autoimmune/inflammatory disease, because hormones of the stress response that are activated by stress have a profound impact on immune responses.

Expression of Cytokines within the CNS

In addition to acting as signals between immune cells and from peripheral immune cells to the brain, cytokines are also expressed within the CNS (2). Such CNS cytokines are produced by neurons as well as by nonneuronal cells, including cerebral vascular endothelial cells, astrocytes, and glia. Oligodendrocytes, astrocytes, and microglia are the support cells of the CNS; neurons are the functional elements of the CNS, which transmit neuronal impulses and synthesize and secrete neurotransmitters and neuropeptides. Oligodendrocytes and astrocytes are derived from neural ectoderm, while microglia are CNS macrophages that are derived from bone marrow. The glial elements produce growth factors, including cytokines, which are important in neuronal cell growth, differentiation, survival, and cell death. Neuronal peptides, which are synthesized in neuronal cell bodies, are transported to neuronal axon terminals, where both peptides and neurotransmitters are stored in vesicles. Upon electrical stimulation and depolarization, these products are released into the synapse.

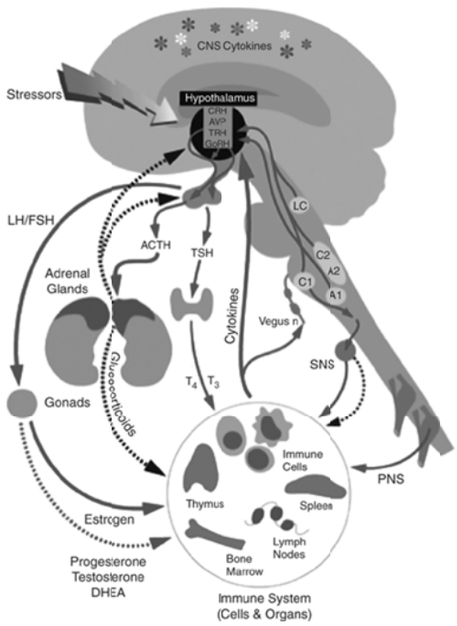

Figure 17-1. Schematic illustration of neural immune connections, including immune signaling of central nervous system via systemic routes and the vagus nerve (Vagus n.) and CNS regulation of immunity via the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system (SNS) and parasympathetic nervous system and peripheral nervous system (PNS). Cytokine expression within the CNS is represented by asterisks within the brain. Dotted lines represent negative regulatory pathways, solid lines represent positive regulatory pathways. CRH, corticotrophin releasing hormone; AVP, arginine vasopressin; ACTH, adrenocorticotropin hormone, SNS, sympathetic nervous system; PNS peripheral nervous system; LC, locus ceruleus; A1, C1, A2, C2, brainstem adrenergic nuclei. (Reprinted with permission from Marques-Deak A, Cizza G, Sternberg E. Brain-immune interactions and disease susceptibility. *Mol Psychiatry* 2005;10(3):239–250.)

Cytokines can be expressed or induced in CNS resident cells (2 ,3 ,4) and can also be expressed in inflammatory cells invading inflamed cerebral tissue. This latter situation might be likely to occur in diseases such as SLE, in which CNS inflammation occurs. In addition to cytokines themselves, the entire molecular machinery that allows these molecules to be functional is also found within the brain. The best defined of these systems is the IL-1, system, in which all components have been identified in the brain. These include both types of IL-1, (i.e, IL-1α and IL-1ß), both types of IL-1, receptors (i.e, types I and II), the enzyme that activates IL-1ß (i.e, IL-1- converting enzyme), and the endogenous IL-1-receptor antagonist (i.e., IL-1Ra), a molecule that blocks the effects of IL-1.

CNS cytokines perform a different physiologic role than stimulation of the brain by cytokines produced by peripheral immune cells. Thus, central cytokines appear to play an integral role in modulating nerve cell death and survival (3 ,5 ,6), while peripheral cytokine signals to the CNS have a primarily neuroendocrine function. In vitro studies show that IL-1, is toxic to mature neurons but that it prevents the naturally occurring neuronal cell death that takes place in immature neurons (7). Both animal and human studies provide evidence that this growth-and-death effect of cytokines on neuronal tissue is relevant to whole organisms. Neurotoxicity results when cytokines are overexpressed in the brains of transgenic mice, in which cytokine genes are targeted to astroglial cells through a promoter that is specific for astroglia, glial fibrillary acidic protein (8). Such mice develop neuropathology and related neurologic manifestations in areas where cytokines are overexpressed. Thus, mice in which IL-6, is targeted to the CNS develop astrogliosis, inflammation, and angiogenesis in areas of the brain, including the cerebellum. Clinical manifestations in these mice include paralysis, seizures, and ataxia.

In human diseases in which inflammatory cells invade cerebral tissue, molecular techniques that allow visualization of gene expression in situ show overexpression of cytokines in regions of neurodegeneration. In brain tissue from patients who died with AIDS dementia, overexpression of certain cytokines, including IL-1, and TNF, has been shown to be concentrated in areas around invading giant cells (9). This suggests that some of the neurologic features of AIDS may result from the neurotoxicity of cytokines. These findings also suggest the possibility that other inflammatory diseases characterized by involvement CNS, such as SLE, some of the neuropathology and neurologic features might be caused by cytokine overexpression from inflammatory cells as well.

IL-1, also may act in concert with other neuropeptides to regulate neuronal cell death and survival. For example, CRH and IL-1, both are induced in neurons during ischemia. Neuronal damage is diminished by the administration of antiserum against CRH and is enhanced by the administration of IL-1Ra antisera (10). These data suggest that IL-1Ra and CRH both play reciprocal roles as neuroprotective and neurodegenerative agents during ischemia, respectively.

More recently it has also been shown that activated T cells contribute to neuronal repair after injury (11), indicating that immune factors are not only deleterious, but also play an important role in neuronal survival and regeneration. These findings have important implications for new avenues for therapy of spinal cord injury and prevention of paralysis.

Definition of the Neuroendocrine Stress Response

The central anatomic component of the neuroendocrine stress response, the hypothalamus, is located at the base of the brain adjacent to the third ventricle. It responds to a variety of incoming stimuli by synthesizing and secreting the neuropeptide corticotropin-releasing hormones (CRH) from

cells of the paraventricular nucleus (PVN) (12). In turn, CRH is secreted into the rich hypophyseal portal blood supply stimulates the anterior pituitary gland to secrete adrenocorticotropin (ACTH), which in turn stimulates the adrenal glands to synthesize and secrete corticosteroids (13).

Hypothalamic CRH secretion is held under tight regulatory control by several positive and negative neurotransmitter systems that result in regulation of glucocorticoid release from the adrenal glands. The noradrenergic, serotonergic, and dopaminergic systems upregulate CRH via α1-adrenergic receptors (14) 5-HT receptors (15), and dopamine (D1) receptors (16) respectively, while the opiates, gamma-aminobutyric acid (GABA)/benzodiazepine, and glucocorticoid feedback suppress hypothalamic CRH via opiate receptors (17), GABAergic receptors (18), and glucocorticoid receptors (19), respectively (20 ,21).

In addition to its neuroendocrine effects via the pituitary, CRH also acts centrally within the brain as a neuropeptide to induce a set of behaviors that are characterized by cautious avoidance, vigilance, enhanced attention, and suppression of vegetative functions such as feeding and reproduction (22 ,23). Together, this constellation of behaviors is known as the classic “fight or flight” response (14 ,24). Many of these effects are mediated through hypothalamic and extrahypothalamic CRH and interactions of these centers with other neurotransmitter systems, such as the brain stem-noradrenergic system and the sympathetic nervous system (25 ,26).

The hypothalamic CRH system communicates with noradrenergic pathways, which are also activated during the stress response, via anatomic connections between the hypothalamus and the noradrenergic centers in the brain stem (27). In turn, the brain stem-noradrenergic system sends signals to the periphery via the sympathetic nerves. Through such connections, the physiologic components of the stress response, such as increased heart rate, muscle tone, and sweating, are coordinated with behavioral responses to form the generalized stress response. Many studies indicate that the modulation of immune responses by both the sympathetic and the neuroendocrine systems are an important physiologic component of the stress response (28 ,29 ,30 ,31 ,32 ,33).

Afferent Limb Stimulation of the CNS by Signals from the Immune System

Hormonal Signaling of the Brain by Immune Signals: Routes of Communication

The first suggestion that immune signals could stimulate the brain came from animal experiments (34), which showed that after intraperitoneal injection of bacterial lipopolysaccharide, IL-1, could be detected in brain tissue. The next series of experiments (35 ,36 ,37 ,38) that further delineated this signaling between the immune system and the brain showed that IL-1, itself could directly activate the hypothalamic-pituitary-adrenal (HPA) axis cascade of hormones by direct stimulation of the hypothalamus and pituitary. IL-1, directly stimulates secretion of ACTH from cultured pituitary cells, induces CRH mRNA expression in the paraventricular nucleus of the hypothalamus, and induces CRH secretions from explanted hypothalamic tissue in culture (39). Subsequent studies showed that many cytokines stimulate the axis HPA, including IL-6, tumor necrosis factor (TNF), IL-2, and interferon (IFN)-α (40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48).

Cytokines from the peripheral immune system can stimulate the brain, through several routes (49 ,50 ,51 ,52). Cytokines may be actively carried from the blood to the brain across the blood–brain barrier, or they also may cross passively at certain anatomically “leaky” points in the blood–brain barrier, such as the organum vasculosum lamina terminalis or the median eminence. This route may be more prevalent during inflammation or illness, when the blood-brain barrier is more permeable (53). Such conditions are particularly likely to occur in an illness such as SLE, in which inflammation may involve the CNS.

It is not necessary, however, for cytokines to cross into the brain to exert their effects. Cytokines can be expressed in the endothelial cells lining cerebral blood vessels (54) and can stimulate second-messenger release (55) in surrounding brain tissue, thus stimulating neurons in these areas. Thus some cytokine effects, such as fever, are indirectly mediated through second messengers, such as prostaglandins (56). The fact that prostaglandin antagonists such as acetyl salicylic acid and other nonsteroidal anti-inflammatory agents block these effects of IL-1, provides evidence for the importance of this mechanism.

Finally, peripheral cytokines can signal the brain via direct neuronal routes. Intraperitoneal cytokines can activate specific CNS regions via the vagus nerve (51). The brain stem nucleus of the tractus solitarius (NTS) is the first brain region that exhibits electrical and biochemical activity after intraperitoneal injection of IL-1. Cutting the vagus nerve prevents this activity. Thus, signaling via the vagus nerve constitutes the most rapid mechanism by which cytokines activate the brain and may be an important mechanism for IL-1-induced increases in plasma corticosteroids and hypothalamic norepinephrine depletion (57). Intravenous IL-1β also has been shown to activate efferent activity in branches of the vagal nerve that innervate the thymus (58). Indeed, activation of the vagus nerve by pathogen products and cytokines and the subsequent release of ACh that suppresses inflammation is an important route of CNS control of inflammation, that has been termed the “vagal inflammatory reflex” (31).

Behavioral Effects of Cytokines on the CNS

One characteristic set of behaviors induced by cytokines is termed sickness behavior (59). This is characterized by loss of appetite, decreased mobility, loss of libido, withdrawal from social interaction, depressed mood, increased somnolence, and fever. Some of these behavioral and functional effects (i.e., fever) are mediated secondarily through prostaglandins, and others are initially activated via the vagus nerve. The frequent occurrence of depression and suicidality in patients treated with cytokines (e.g., IFN or IL-6) for bone marrow suppression has led to the hypothesis that

some forms of depression may be associated with imbalances in cytokines CNS, Indeed, antidepressant pre-treatment of patients who are about to receive cytokine therapy prevents depression and suicidality in these subjects (60). Although not studied in specifically SLE, these findings suggest that increases in CNS cytokines could contribute to depressive symptomatology in SLE.

Modulation of Immune and Inflammatory Responses by the Neuroendocrine System

Glucocorticoid Modulation of the Immune System

If the stimulation of the CNS by peripheral cytokines is viewed as the afferent limb of the neuro-immune communication, the final end point of the efferent limb of this communication is the glucocorticoid effect on the immune response.

Glucocorticoids modulate immune cell function by acting through glucocorticoid receptors present in immune cells. The molecular structure and mechanism of action of glucocorticoid receptors is described below. The overall functional effect of glucocorticoids on the immune response depends on the preparation, dose (whether pharmacologic or physiologic), and temporal sequence of glucocorticoid exposure in relation to antigenic or pro-inflammatory challenge.

Glucocorticoids have profound effects on the immune/inflammatory response at the molecular, cellular, and whole-organ level. Exposure to stress levels of glucocorticoids results in rapid involution of the thymus, as a result of glucocorticoid-induced thymocyte apoptosis (i.e., programmed cell death). Glucocorticoids also regulate the immune response by inducing apoptosis in proliferating lymphocytes (61). There is evidence to suggest that such glucocorticoid-regulated apoptosis could take place within the thymus through induction of an intrathymic glucocorticoid system, because the enzymatic machinery for glucocorticoid synthesis is present within the thymus (62).

Glucocorticoids also induce redistribution of circulating white blood cells, with neutrophilic leukocytosis, eosinopenia, monocytopenia, and altered ratios of T-lymphocyte subtypes, resulting in decreased peripheral blood CD4, and increased CD8, cells. At the same time that this peripheral redistribution occurs, there is decreased infiltration of neutrophils and monocytes into tissues.

Molecular Mechanism of Glucocorticoid Action

Glucocorticoid receptors are members of a hormone receptor superfamily that are structurally related, including the glucocorticoid receptor (binds corticosterone and dexamethasone), the mineralocorticoid receptor (binds corticosterone and aldosterone), androgen receptor (binds testosterone), estrogen receptor (binds estradiol), progesterone receptor (binds progestins), thyroid hormone receptor (binds thyroxine), and retinoic acid receptors (binds all-trans retinoic acid). All members of the superfamily act by binding to a soluble cytosolic receptor made up of three functional regions: (1) a C-terminal hormone-binding region, (2) a DNA-binding region, and (3) an N-terminal immunogenic region that is involved in transactivation (61 ,63). The unbound receptor located in the cytosol, is folded and inactive, bound to a 90kDa heat-shock protein (64) (hsp90) and immunophilins (Fig 17-2). When the hormonal ligand binds to the receptor, hsp90, is displaced, resulting in a conformational change in the receptor that allows the active ligand-receptor complex to displace to the nucleus and bind to hormone receptor-binding elements (HREs) on DNA either as a homodimer or heterodimer. The hormone receptor complex then acts as a transcription factor, either suppressing or stimulating DNA gene transcription. In addition, the glucocorticoid receptor interacts with over 200, other conuclear factors including NF-κB and AP-1, (65 ,66), inhibiting the stimulatory effects of these transcription factors. The recruitment of either coactivators or corepressor complexes is involved in transcriptional regulation and can determine whether or not a gene is transcribed (67). Other accessory proteins, such as HDAC6, also contribute to transcriptional regulation (68).

The glucocorticoid receptor (GR) binds corticosterone with a lower affinity than the mineralocorticoid receptor (MR). Therefore, GR tends to respond to higher levels of glucocorticoids secreted during stress (i.e., stress levels), while the MR responds to basal or nonstress levels (69). While GRs generally bind to glucocorticoid receptor DNA-binding elements (70) as homodimers, it is also possible for GRs and MRs to form heterodimers (71 ,72). These different mechanisms of binding to DNA response elements confer additional specificity of action to the glucocorticoid and mineralocorticoid receptors.

Further specificity of action of these two receptor types is conferred by tissue distribution of the receptors. The primary glucocorticoid receptor in immune cells is GR, which is consistent with the physiologic role of glucocorticoid regulation of the immune system by stress levels of these hormones (1 ,73 ,74). An additional level of specificity is conferred by the tissue distribution of the corticosterone-metabolizing enzyme 11ß-hydroxysteroid, which metabolizes corticosterone but not aldosterone. Thus, where this enzyme is present (e.g., kidney), the primary ligand that is available for binding to the MR is aldosterone rather than corticosterone (70). Where the enzyme is not present (e.g., brain), the primary ligand for the MR is corticosterone. The MR in the brain plays a role in regulation of basal function HPA, such as circadian rhythm.

Effects of Glucocorticoids on Immune Cells

Glucocorticoids have effects on a variety of immune cells involved in both innate and adaptive immunity, B and lymphocytes T, including natural killer (NK) cells, and dendritic cells (DC). Glucocorticoids suppress NK cell function both in vitro and in vivo in animals as well as humans. Mice treated with glucocorticoids show a reduction in the number of splenic cells NK, and these NK cells have reduced cytolytic activity. In a study of children with asthma, glucocorticoids

were shown to decrease the expression of intracellular adhesion molecule-1, (ICAM-1) and L-selectin, suggesting an inhibition in the ability of these cells to migrate to inflammatory sites (75). Glucocorticoids have also been shown to affect development and function of dendritic cells both in vitro and in vivo. Glucocorticoids, reduce DC production of the TH1, cytokine IL-12, downregulate expression of costimulatory molecules, and strongly reduce allostimulatory capacity in vitro. However, the suppressive effect was not observed with dendritic cells activated with lipopolysaccharide (LPS), indicating that stage of dendritic cell maturation influences the effect of glucocorticoids (76).

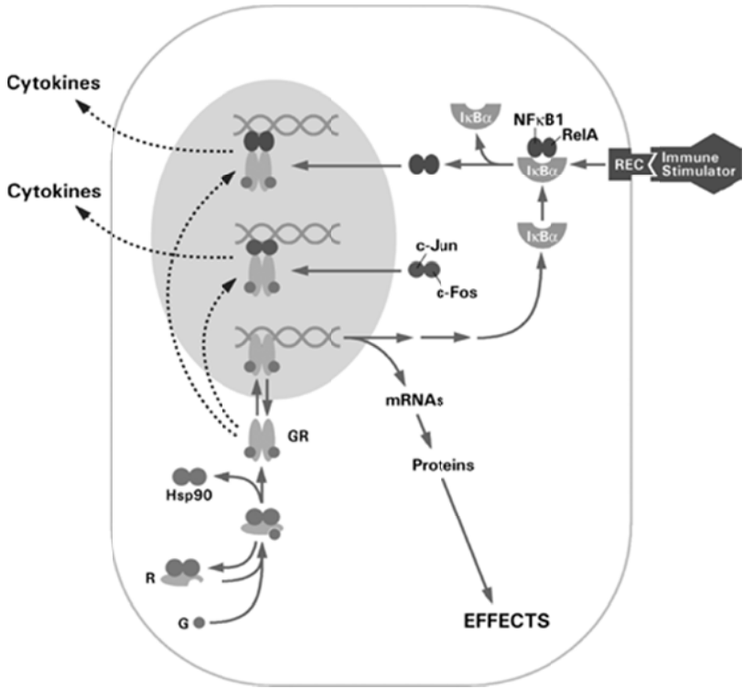

Figure 17-2. Schematic diagram of the molecular mechanism of glucocorticoid receptor regulation of cytokine production. Glucocorticoid hormone (G) binds to the cytosolic glucocorticoid receptor (GR), displacing heat shock protein 90, (HSP90). This allows dimerization, movement into the nucleus and binding of the G-GR complex to DNA, with resultant transcription and translation of proteins, including IkB IkB indirectly suppresses cytokine production by sequestration of NFkB In addition, the G-GR complex can interact with NFkB directly to suppress cytokine production.

Glucocorticoids have been also shown to suppress not only differentiation and maturation of cells T, but also modulate the function of mature effector T cell subtypes, such as cytolytic and helper cells T, In functional assays, a large body of data shows that glucocorticoids suppress mitogen- and antigen-stimulated T- cell proliferation (77). In contrast, some studies indicate that corticosterone in rats can enhance lymphocyte proliferation depending on the conditions of culture (78). While the physiologic relevance of these in vitro studies is not known, this dual suppressing or enhancing effect of glucocorticoids on lymphocyte proliferation underlies their potential physiologic role as immunomodulators rather than pure immunosuppressors.

This modulation is thought to be most critical in T helper cell populations, which are skewed from a TH1, to a TH2, response in the presence of glucocorticoids. This skewing is evidenced by the inhibition of production of TNF-α, IL-2, IL-6, IL-12, and IFN-γ and increase in production of IL-10, IL-4, and IL-13. The effects of glucocorticoids on cytotoxic T cells could be an indirect result of their effect on T helper cells, which do not stimulate cytotoxic T cells in a TH2-type cytokine environment. The suppressive effects of glucocorticoids on B cell proliferation are variable, depending on the stimulus to proliferation and the dose of glucocorticoids used. In general B cell proliferation is suppressed less consistently than T cell proliferation. At the same time, glucocorticoids consistently enhance production of all classes of immunoglobulins.

Thus, while the overall effects of glucocorticoids on immune/inflammatory responses at the cellular level are immunosuppressive, this effect is attained through suppression of many stimulatory components of the immune cascade

and stimulation of some immunosuppressive or anti-inflammatory elements. The relatively greater sensitivity to glucocorticoid suppression of components of cellular versus humoral immunity tends to shift the immune response from a cellular to a humoral pattern (1). Glucocorticoids have also been shown to upregulate IL-6, receptor expression. This would further enhance the action of IL-6, which is a key B cell growth factor (79). In a disease such as SLE, which is characterized by excess or inappropriate antibody production, it is important to delineate whether glucocorticoids may contribute to or exacerbate this shift. Indeed, dexamethasone has been shown to stimulate IL-6, mRNA production in vitro in SLE lymphocytes (80).

Glucocorticoids also downregulate the expression of class II major histocompatibility complex (MHC) antigen expression, thus reducing recognition and binding of antigen. They also inhibit production of pro-inflammatory molecules and mediators (1 ,81), including complement components, arachidonic acid products, histamine, bradykinins, and cytokines. At the same time that many of these immunostimulatory/pro-inflammatory molecules are suppressed, their endogenous suppressors may be enhanced by glucocorticoids. An example of this balance occurs in the complement system, where glucocorticoids suppress C3, while inducing the complement-suppressor factor H Glucocorticoids suppress the production of most cytokines (1 ,82), including IL-1, IL-6, IL-2, IFN-γ, TNF, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Their effects on IL-4, are variable, and in some cases, IL-4, production increases as IL-2, production is suppressed. This pattern of a shift from IL-2, to IL-4, production is consistent with the humoral-, antibody-, and B cell-supportive effects of glucocorticoids and their tendency to shift the immune response from a TH1, to a TH2, pattern (83 ,84). Additionally, while glucocorticoids suppress IL-1, production, they also enhance the expression of IL-1ß receptor (85). Physiologic doses and preparations of glucocorticoids also stimulate delayed-type hypersensitivity (DTH) (74) and induce anti-inflammatory cytokines IL-4, and IL-10.

Taken together these findings indicate that in addition to their suppressive role, glucocorticoids can also stimulate some aspects of the immune or inflammatory response depending on the dose and timing of exposure. Glucocorticoids in this sense may be viewed as physiologic modulators of the immune response (86).

Sympathetic Nervous System Effects on Inflammation and the Immune System

The sympathetic nervous system serves an important role in regional regulation of immunity (87 ,88). Many immune organs, including the spleen, thymus, and lymph nodes, are richly innervated by sympathetic nerves (89). A number of studies, including denervation and ablation studies, indicate that these anatomical connections as well as the neurotransmitters of the sympathetic nervous system play an important physiologic role in inflammatory responses (87). The effects of the sympathetic nervous system are mediated by norepinephrine (NE) released from sympathetic nerve fibers and epinephrine (E) released from the adrenal medulla. Both NE and E exert their actions through adrenergic receptor. The ß2-adrenergic receptor (ß2AR), a G-protein coupled receptor, is the main receptor found on lymphocytes (90). Adrenergic influences on immune cells, including T and B-lymphocytes potently inhibit TH1, cytokines and thereby suppress cell-mediated immune responses. NE and epinephrine also stimulate production of TH2, cytokines, such as IL-10, and could contribute to SLE disease activity (91).

Evidence that the sympathetic nervous system affects the exudation component of peripheral inflammation is provided by sympathetic ablation studies using 6-OH dopamine (6-OHDA) (92) or sympathetic ganglionic blockers such as chlorisondamine (93), or noradrenergic antagonists and agonists (94). Noradrenergic denervation studies have shown differential effects on inflammation depending on the location of denervation. Thus, denervation of the noradrenergic fibers of lymph nodes (95) is associated with exacerbation of inflammation, while systemic sympathectomy or denervation of joints is associated with decreased severity of inflammation (96 ,97). Treatment of neonatal rats with 6-OHDA, which interrupts both central and peripheral systems NA, is associated with exacerbation of experimental allergic encephalomyelitis (EAE) (98). Pharmacologic studies show decreased inflammation in experimental arthritis with beta-blockade (99) and decreased severity of EAE with ß-adrenergic agonists (98).

Peripheral Nervous System Effects on Inflammation and the Immune System

Immune organs and local sites of inflammation are innervated by peripheral nerves that release neuropeptides, such as substance P (SP), that play a role in peripheral inflammation. SP also plays a role in the severity of arthritis (96 ,100) and in the cellular component of inflammation (101). Substance P, which probably is released in retrograde fashion from sensory nerve endings at sites of inflammation, acts as a chemoattractant and stimulator of cellular proliferation and cytokine production. It also plays a role in the early arteriolar changes associated with inflammation (102). Both denervation of SP nerve fivers with local capsaicin denervation of lymph nodes and systemic capsaicin treatment are associated with diminished peripheral inflammation (95 ,96 ,100). Other peripheral neuropeptides that play a role in inflammation include VIP and CGRP, which generally suppress inflammatory responses (103 ,104 ,105 ,106 ,107).

Parasympathetic Nervous System and Immunity

The parasympathetic nervous system both sends immune signals to the CNS through the afferent fibers of the vagus nerve, and also regionally modulates immune responses

through efferent fibers of the vagus nerve. Ganglia outside the spinal cord receive projections from the brainstem, and further innervate visceral organs such as the heart, lungs, gut, liver, and spleen. IL-1, receptors on paraganglia cells located adjacent to parasympathetic ganglia bind IL-1, and activate the vagus nerve, thus signaling the presence of peripheral inflammation to the brain (108). Inflammation in the gut or peritoneum leads to the “inflammatory reflex” which results in the release of acetylcholine from efferent vagus nerve fibers and negative feedback control of inflammation (31). Cutting the vagus nerve prevents immune signaling to the brain and therefore prevents further activation of cholinergic brainstem regions (31 ,108 ,109). Acetylcholine is the primary parasympathetic neurotransmitter, which binds to two receptor subtypes, nicotinic and muscarinic cholinergic receptors, each of which consist of several different subunits which heterodimerize to provide cell and tissue specificity of cholinergic effects. Immune cells contain both of these receptors, but the α7, subunit of the nicotinic receptors specifically mediate cholinergic anti-inflammatory effects in macrophages.

Estrogens and Immunity

Estrogen has a profound influence on immune responses. The two forms of the estrogen receptor, ERα and ERβ (110) are both found in immune cells, including lymphocytes, monocytes, and macrophages (111). Females of all mammalian species have an increased incidence of autoimmune/inflammatory disease compared to males, and the presence of estrogen increases the severity of autoimmune disease. Surgical removal of the ovaries (oophorectomy) in addition to testosterone treatment reduces this tendency in females while estrogen replacement restores it. However, castration (orchidectomy) and estrogen replacement increases autoimmune/inflammatory disease susceptibility in males to levels similar to females. Estrogen has many direct effects in immune cells, which are dose dependent. Estrogen tends to directly enhance immune responses, but may also suppress through indirect effects on the HPA axis (112 ,113).

Prolactin and Immunity

Prolactin is another important endocrine modulator of the immune system. Prolactin, a 200, amino acid peptide hormone, is produced by cells of the anterior pituitary, and other cells including immune cells. Prolactin binds to specific membrane receptors that are members of the cytokine receptor superfamily. Prolactin receptors are expressed on many cells of the immune system such as T cells cells B, monocytes, macrophages, cells NK, and neutrophils. High concentrations of prolactin, similar to levels found during pregnancy, increase the production of pro-inflammatory cytokines IL-12, and TNF in whole blood cultures after LPS exposure in vitro (114).

Physiologic Role of Neural-Immune Communications and Dysregulations in Autoimmune Disease

A multilevel infrastructure exists to allow anatomic, molecular, and functional communications between the immune and nervous systems (115 ,116 ,117). Animal studies in which these communications are interrupted on a genetic, pharmacologic, or surgical basis provide evidence that this interaction plays an important role in regulating susceptibility to and severity of inflammatory and autoimmune diseases. Human studies also provide evidence that dysregulations of such neuroimmune interactions are associated with autoimmune/inflammatory disease, including Sjogren syndrome, SLE, RA, asthma, dermatitis, and irritable bowel syndrome (118 ,119). Some genetic inbred animal strains show a simple association between a relatively blunted HPA axis and autoimmune disease. The obese-strain chicken, which develops spontaneous thyroiditis, and its thyroiditis-resistant counterpart also exhibit relative HPA-axis hypo- and hyper-responsiveness (116). Some, but not all, lupus-prone mouse strains (MRL but not NZB) have a relatively blunted HPA-axis response (120). The concept that an intact HPA axis response protects against inflammatory/autoimmune disease has been shown through intervention studies in disease models including streptococcal cell wall-induced arthritis (121), EAE induced by myelin basic protein (122), and the lethal effects of salmonella (123). Interruptions of the HPA axis in these models surgically, through adrenalectomy or hypophysectomy (i.e., pituitary excision), or pharmacologically, through the glucocorticoid-receptor antagonist RU486, results in enhanced inflammatory disease mortality. Conversely, reconstitution of the axis HPA, surgically or pharmacologically reverses inflammatory disease susceptibility in inflammation-susceptible strains (121 ,122 ,124).

Glucocorticoid Resistance

Impaired glucocorticoid control of inflammation may also result from a lack of responsiveness in cells and tissues that normally respond to circulating glucocorticoids because of impaired receptor function. Glucocorticoid resistance may result from polymorphisms of the receptor or associated cofactors that are necessary for it to function or overexpression of the glucocorticoid receptor-β (GR-β), an inactive form of the GR that binds hormone but does not bind to DNA (1 ,125). Chronic inflammation can itself result in enhanced expression of the GR-β and associated glucocorticoid resistance. Glucocorticoid resistance has been associated with several autoimmune, inflammatory and allergic diseases (see 119).

Together, these studies underline the biologic principle that neuroendocrine responsiveness plays an important modulating role in susceptibility and resistance to autoimmune/inflammatory disease. When the feedback suppression of the immune system by the anti-inflammatory/immunosuppressive effects of the glucocorticoids is interrupted, either by blocking the production of glucocorticoids or by preventing their action

with receptor antagonists, or in the presence of impaired receptor function, enhanced inflammatory susceptibility results. It is likely that in human autoimmune/inflammatory diseases, the HPA axis could be impaired or interrupted at different points in different diseases, or in the same disease in different individuals. Further, neuroendocrine responsiveness varies with time on a circadian basis, in females in relation to the menstrual cycle, and throughout life with aging. Thus, the degree to which neuroendocrine responses modulate inflammatory disease also may vary over time, and this may account for some of the temporal waxing and waning of these illnesses. Understanding the degree to which such hormonal and neuronal inputs control inflammatory disease will provide new insights for future therapeutic approaches in these diseases.

Neuroendocrine Mechanisms in SLE

Neuroendocrine immune interactions could play a role in the pathogenesis of SLE in several ways. As in susceptibility or resistance to other inflammatory illnesses, premorbid neuroendocrine responsiveness might predispose to an increased susceptibility to the development of SLE Once develops SLE, and if the CNS is involved, the local effects of cytokines on neuronal tissue could contribute to some of the specific neuropathologic or neuropsychiatric features of SLE At the effector endpoint of the axis HPA, differences in glucocorticoid receptor number or sensitivity could play a role in the pathogenesis of SLE as well as in clinical response to treatment with steroids. In addition, regardless of the premorbid reactivity of the axis HPA, chronic inflammation itself could alter HPA-axis responses. Studies supporting these possibilities in animal models and in humans, suggest that a variety of neuroimmune mechanisms may be relevant to many features in the pathogenesis of SLE.

CNS Cytokines in Animal Models of SLE

The classic animal models for SLE are (NZB/NZW) F1, mice and mice MRL, These strains of mice develop spontaneous autoimmune disease with many of the characteristic features that are seen in human SLE, including renal disease, vasculitis, and antinuclear antibody production. Neuroendocrine responses and CNS expression of cytokine components have been examined in both these strains. Studies indicate that NZB mice exhibit a profound deficiency of IL-1, receptor expression in the dentate gyrus of the hippocampus (10% of controls) (126), while (NZB/NZW) F1, mice show a 50% deficit and MRL mice show no difference in CNS expression of this receptor as compared with control strains. Although this deficiency is present before the mice develop clinical disease, it is not clear what role it plays in pathogenesis of the syndrome. Differences in expression of the receptor for this cytokine in two strains that both develop lupus-like disease could be interpreted as indicating that the defect is unrelated to the pathogenesis of the disease, or that different molecular mechanisms could lead to the same final disease outcome. Further studies in this area are required to determine which interpretation is correct.

CNS Cytokines in Human SLE

Two clinical aspects of lupus CNS, vasculitis and neuropsychiatric manifestations, could be related in part to the presence of cytokines in the CNS that are released from inflammatory cells. Elevated cytokines have been detected in the cerebrospinal fluid (CSF) of patients with lupus CNS, In one study, IL-6, was increased during clinical exacerbations of CNS lupus and fell to control levels in association with clinical improvements after treatment with methylprednisolone (127). Clinical symptoms in these patients included seizures, organic brain syndrome, nonorganic psychosis, chorea, and focal lesions. In a similar study of lupus psychosis, CSF IFN-α (128) increased and returned to baseline, corresponding with clinical exacerbations and remissions. In both studies, the CSF cytokines were higher than plasma levels, suggesting a CNS source for the cytokines. Further, cytokines were not elevated in patients with seizures from other causes. Although the elevations of CSF cytokines could be viewed as simply a marker for the presence of inflammation, it is noteworthy that in animal models, such as transgenic mice in which IL-6, is specifically overexpressed in brain, clinical features such as focal lesions and seizures are also seen (129 ,130). These transgenic mice also exhibit a reduction in neurogenesis in the dentate gyrus region of the hippocampus, a brain region known to play an important role in learning and memory (131). This suggests that the elevations in IL-6, that are measured in human SLE CSF could play a pathogenic role in development of some of the neuropathologic and clinical features of lupus CNS.

HPA Axis Responsiveness in SLE

Several studies have shown a blunted HPA axis response to a variety of stimuli in human autoimmune/inflammatory and allergic diseases including SLE (Table 17-1). In these

studies, basal cortisol responses do not differ between patients and controls, however patients showed significantly lower cortisol rises in response to stimuli than did controls. Specifically SLE patients showed lower cortisol responses to ovine CRH, compared to controls (132). Studies of HPA axis responsiveness in mouse models of SLE have shown relatively blunted corticosterone responses in MRL, but not NZB/NZW F, mice (133).

Table 17-1: Inflammatory/Autoimmune Diseases Correlated with a Dysfunctional HPA Axis in Humans

Inflammatory/Autoimmune Disease	Reference
Rheumatoid arthritis	(162,163)
SLE	(132)
Sjösgren syndrome	(164)
Dermatitis	(165)
Multiple sclerosis	(152,166)

(Adapted with permission from Marques-Deak A, Cizza G, Sternberg E Brain-immune interactions and disease susceptibility. *Mol Psychiatry* 2005;10(3):239-250.)

Evidence for Glucocorticoid Resistance in SLE

In contrast to patients with other autoimmune diseases such as rheumatoid arthritis, patients with SLE often require large doses of glucocorticoids before a therapeutic effect is seen. Furthermore, even during treatment with large doses of steroids, patients with SLE are less likely to develop Cushingoid features. This anecdotal clinical evidence suggests that patients with SLE may display some degree of glucocorticoid tissue resistance (134). The contribution of the glucocorticoid receptor to potential glucocorticoid resistance has been explored in some studies examining the binding number and affinity characteristics of the GR in lupus. Patients who exhibit hormone resistance have been found to have abnormally high levels of GRß (135) or defective, mutated GR (136 ,137). A decrease in GR number in mononuclear cells was also found in lupus patients (136). Patients also had a higher percentage of lymphocytes with high P-glycoprotein activity, a molecule responsible for transporting steroids outside the cell inhibiting their effects (138).

In a study of patients with SLE who had not received steroid treatment within the previous 6, months, glucocorticoid receptor numbers in peripheral blood mononuclear cells were significantly higher than in controls (139). There was no correlation with disease activity, nor was there a difference in affinity of the GR in these patients. In another study, no difference in GR number was found between patients and controls, however, patients who were on low-dose glucocorticoid treatment were not excluded from this study (140). Because exogenous treatment with glucocorticoids suppresses the responsiveness of the axis HPA, the GR numbers measured in glucocorticoid-treated patients may reflect treatment rather than intrinsic factors. Thus, the discrepancy between these studies could be related to differences in exogenous glucocorticoid exposure in these patients, underscoring the inherent difficulty in studying GR binding and number in such patients.

Prolactin and SLE

Hyperprolactinemia is found in 20% of patients with SLE (141 ,142 ,143). These studies suggest that reducing serum prolactin levels may ameliorate disease activity. Inhibiting prolactin secretion with the drug bromocriptine, a dopamine receptor agonist that inhibits prolactin secretion, results in clinical improvement in lupus patients (144). In vitro prolactin also increased secretion of IFN and IL-2, by peripheral mononuclear cells from SLE patients (145), which suggests that elevated levels of prolactin may be pro-inflammatory. Conventional immunosuppressive therapy decreases prolactin levels, and this decrease correlates with a reduction in SLE activity (146). This suggests that prolactin plays an important role in the pathogenesis of SLE, and that therapeutic interventions targeted at modulating prolactin may provide important new therapeutic approaches for management of SLE (see Section VI).

Effects of Stress in SLE

Until recently, studies of the effects of stress on physical illness, including autoimmune diseases such as SLE, were viewed with skepticism. This related in part to the inherent difficulty in accurately defining and quantifying stressful stimuli and response outcomes. However, recent advances in defining and quantifying not only stressors but also neuro-endocrine transducing signals, disease outcomes, and molecular components of the immune/inflammatory response in animals and humans have allowed more accurate assessment of the effects of stress on autoimmune/inflammatory disease and of the mechanisms by which such effects are transduced. While most such studies have been carried out in models of infectious disease (147 ,148), it is clear from such studies that activation of both the HPA axis and the sympathetic system play an important role in modulating immunity during stress. The evidence from animal models that these systems interact provides direction for the future design of human studies to substantiate old or anecdotal claims that stress is associated with exacerbation or precipitation of disease in SLE.

A number of recent studies have suggested that emotional stress might trigger the onset of SLE or worsen its course, and some studies have shown an association between flares of disease and emotional stress or number and severity of daily stressors (149 ,150 ,151).

Chronic inflammation itself can be viewed as a chronic stressor that can alter HPA-axis responses. The effects of chronic inflammation include hypercortisolism and a shift from primarily CRH control of the stress response to primarily vasopressin (AVP) control (152 ,153). The latter shift results from a switch from CRH to AVP expression in hypothalamic neurons (154) and occurs in response to cytokines such as IL-1(155). Studies in another autoimmune disease, multiple sclerosis (MS), have shown both a shift towards an AVP driven stress response (152) as well as glucocorticoid resistance in PBMC in some subsets of multiple sclerosis (MS) patients (relapsing remitting) (156), indicating that an impaired HPA and glucocorticoid responses and glucocorticoid resistance may contribute to its pathogenesis in different subpopulations of patients.

Sympathetic, Parasympathetic, Peripheral Nervous Systems in SLE

In addition to the changes in endocrine function in SLE, other neuronal regulatory systems such as the sympathetic, parasympathetic, and peripheral nervous systems may also

be dysregulated in SLE NE and epinephrine are dysregulated in SLE and may contribute to disease activity (157). Sympathetic nervous system outflow is increased in patients SLE, as demonstrated by increased levels of serum NPY (158). NPY has also been shown to be increased in a mouse model of SLE (159 ,160). The peripheral nervous system can also contribute to disease development in SLE but is considered clinically to be less frequently affected. Peripheral neuropeptides such as VIP, SP, CGRP are increased in SLE (160 ,161). Further investigation of the connection between these systems and SLE is needed.

Summary

It is apparent that neuro-endocrine-immune interactions play an important role in the pathogenesis of many features of SLE at multiple levels, within and outside the CNS, and at the molecular and whole-organ level. This chapter has mainly focused on two aspects of neuro-endocrine interactions with the immune system, HPA and sympathetic immune interactions, as well as on cytokines within the CNS Many additional hormonal systems, briefly reviewed here, also play an important role in modulating immune function, including the female sex hormones estrogen and progesterone as well as prolactin. All of these hormones play an important role in the pathogenesis of SLE and are reviewed in more depth elsewhere in this text.

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Chapter 18
Animal Models of Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is the most heterogeneous autoimmune disease that affects virtually any organ. This heterogeneity in disease expression has posed difficulty in formulating its mechanistic investigations. Consequently, many investigators have turned toward inbred animal models, which spontaneously develop a homogeneous disease recapitulating some of the serological and histopathological features of SLE. Examples of such models include the New Zealand (NZ) strains, Murphy's Recombinant Large (MRL)/MpJ, C57BL/6 × satin beige [SB] (BXSB), Palmerston North (PN), and C57BL/6 × DBA2 (BXD2) strains.

SLE is characterized by the presence of autoantibodies against a variety of ubiquitous self antigens, deposition of autoantibodies and immune complexes in tissues with development of local inflammation, and finally tissue fibrosis and damage in multiple organs (1). Deliberate attempts or serendipitous discoveries have led to identification of animal models that may recapitulate these various steps or stages and individual disease manifestations. For example, BALB/c mice injected with a DNA surrogate peptide develop extensive immune deposition but have no renal inflammation (2), whereas BALB/c mice injected with hydrocarbon oil pristane generally develop immune deposition and a limited kidney inflammation (focal glomerulonephritis) but no kidney failure (3 ,4). In contrast, genetically lupus-prone mouse strains spontaneously develop immune deposition-mediated renal disease which is lethal, with strain dependent variation in disease patterns and severity (5). Numerous single gene knock-out and transgenic strains have also developed autoimmune features (6 ,7) that recapitulate some aspects or stages of SLE disease.

Consistent disease pattern in individual mouse strains and relative ease in testing renal disease have driven extensive investigations into lupus nephritis. Model investigations into lupus involvement of other organs, however, have been limited (Table 18-1), perhaps because of variability in disease expression or lack of appropriate tools to investigate these manifestations. For example, although some forms of neuropsychiatric lupus develop in many lupus susceptible strains, animal investigations have been limited. Similarly, although a few animal strains develop coronary occlusions and myocardial infarction as a result of immune complex deposition, it is unclear whether a similar mechanism characterizes increased cardiovascular disease in human SLE. Other strains develop dermatitis, hemolytic anemia, arthritis, and vasculitis, but their incidence is generally variable. Further, although the animal investigations have helped in understanding some steps in the pathogenesis of SLE, mouse models have not fully recapitulated the waxing and waning nature and the full spectrum of human SLE, suggesting a need for continuing search for additional model systems. In that regard, like human SLE, SLE in dogs is a chronic disease with alternating periods of remission and relapses and manifests with fever, polyarthritis, glomerulonephritis, mucocutaneous lesions, lymphadenopathy, and splenomegaly.

This chapter provides an overview of animal models of lupus highlighting what has been learnt from animal models about the pathogenesis of human SLE. The chapter will also review studies of efficacy of therapeutic interventions in murine lupus that have formed the basis for similar interventions in human disease. Table 18-1 provides an overview of major characteristics of various animals with lupus-like disease.

Clinical Disease, Autoantibodies, Immunologic Abnormalities, and Genetics in Spontaneous Polygenic Murine SLE

Numerous murine models of spontaneous SLE have been studied. This section reviews the principal characteristics of the most extensively studied strains.

New Zealand Mice

NZB/Bl (NZB) Mice

The New Zealand Bielschowsky black (NZB/Bl) mouse was bred by Bielschowsky, who was mating mice by coat color to derive cancer-susceptible strains. In 1959, she reported that NZB mice died early from autoimmune hemolytic anemia (8). Shortly thereafter, her colleagues described a hybrid between NZB and unrelated strains including the New Zealand white (NZW) that were characterized by early death in females from nephritis associated with lupus erythematosus (LE) cells, thus providing the first animal models of SLE (9 ,10).

Tables 18-1 and 18-2 show characteristics of NZB mice. They also are discussed in several review articles (11 ,12 ,13 ,14 ,15).

Table 18-1: Major Characteristics of Animal Strains Developing Systemic Lupus Erythematosus (SLE)

TABLE 18.1.1: Disease Manifestations in Lupus-Prone Animals

Strain	Nephritis	Dermatitis	Arthritis	Neu.-Psy.	Hematol.	Vascular	Other
NZB/Bl	Mild and delayed				Hemolytic anemia		Peptic ulcer 50%
NZB/NZW F1 (BW)	Proliferative, progressive			Yes			Choroiditis 60%-90%, oophoritis 35%
NZM.2410	Early glomerulosclerosis			Yes			
NZM.2328	Proliferative, progressive						Sialoadenitis, dacryoadenitis
NZB/SWR F1 (SNF1)	Proliferative, progressive						
MRL/Mp-lpr/lpr (MRL-l)	Diffuse proliferative; Severe interstitial	Epidermal hyperplasia, ulceration, chronic	75%, Pannus and infiltrate	Yes		Vasculitis 56%	Sialoadenitis 100%, conjunctivitis 85%, band keratopathy 90%, choroiditis 100%, oophoritis 72%
MRL/Mp-+/+	Mild and delayed	Mild and delayed	75%, Pannus and infiltrate			Vasculitis 8%	Milder/later than in MRL-l; sialoadenitis 95%, conjunctivitis 50%, band keratopathy 90%, choroiditis 100%, oophoritis 72%
BXSB	Diffuse proliferative						Neutrophilic infiltrate in joints
BXD2	Diffuse proliferative		Erosive				Splenomegaly
Flaky skin (fsn/fsn)	Glomerulonephritis	Psoriasiform			Anemia		Splenomegaly, hepatitis, lymphadenitis, hyper-IgE, stomach hyperplasia
NZW/BXSB (WBF1)						Ath.*	*Coronary infarct
Hydrocarbon oil-induced	Focal proliferative		Yes				Hepatitis
Anti-DNA Id induced	Yes				Leukopenia	Thrombosis	Elevated ESR
Dog	65%	60%	90%		Thrombocytopenia,	Clotting	Relapsing/remitting disease, Fever, lymphadenopathy, splenomegaly
Heparan sulfate-induced in dog	100%	100%	40%		Anemia		Interstitial pneumonitis

*Ath, atherosclerosis; BXD2, C57BL/6J _ DBA2J; BXSB, Hematol, hematological; MRL, Murphy's Recombinant Large; lpr lymphoproliferative; Neu-Psy, neuro-psychiatric; NZB, New Zealand black mice; NZM, NZW, New Zealand white mice;.

TABLE 18.1.2: Autoantibodies in Lupus-Prone Animals

Strain	dsDNA	Anti-CL	RF	RBC	snRNP	Cryo	Others
NZB/Bl				+			gp70, NTA
NZB/NZW F1 (BW)	100 (4–5 mo), IgG	+	+ (rare)	20–40	0	+	gp70, RNA poly I, RNA, IL-2, ubiquitin, helicase
NZM.2410	IgG						
NZM.2328	IgG						
NZB/SWR F1 (SNF1)	IgG						
MRL/Mp-+/+	100		+	10	83 (9 mo)	+	gp70, albumin, transferrin, La, Ro, ribosome P, S10 RNA poly I
MRL/Mp-lpr/lpr (MRL-l)	100 (4–5 mo), IgG	+	+	10	37 (5 mo)	+	gp70, albumin, transferrin, La, Ro, Su, ribosome P, S10 RNA poly I, laminin, collagen, ubiquitin, mitochondria, CIC
BXSB	IgG, 100 (4–5 mo)			20–40		+	gp70, albumin, transferrin, anti-nucleolar (ref. 703)
BXD2	+		+				
NZW/BXSB (WBF1)		+					
Hydrocarbon oil-induced	+	+	+		+		Su, ribosomal P, tRNA synthetase, helicase
Anti-DNA Id induced	+						
Dog	<30				<30		ANA (90%), histones (90%), <30% Ro, lymphocytes and platelets
Heparan sulfate-induced in dog	0						ANA >1:128 (100%), heparan sulfate

Numbers indicate frequency of specific autoantibodies (at tested age, if known). CIC, circulating immune complexes; cryo, cryoglobulins; dsDNA, double-stranded DNA; RBC, erythrocyte; RF, rheumatoid factors; RNA poly I, RNA polymerase I; snRNP, small nuclear ribonucleoprotein. All strains have ANA (antinuclear antibody) positivity.

TABLE 18.1.3: Genetic and Other Features of Lupus-Prone Animals

Strain	Coat Color	Sex Dominance	Life Span	Age for 50% Mortality (mo)	H-2 Locus	Mls-1 Locus	Vα	Vβ	IgH-C	IgH-V	Ig_
NZB/NZW	Brown	F	245 (F),	8.5	d/z	a/b					
F1 (BW)	Black	M/F	406 (M)	16	(d/u)	a	c	b	n	d	b
NZB/Bl	White		430(F)		d		d	a	n	d	c
NZW			469(M)		z (u)						
NZM.2410	Agouti	F/M									
NZM.2328	Agouti	F		~9.5							
NZB/SWR	Brown	F		6	d/q	a/a	c	a/b	n/p		
F1 (SNF1)					q		c	a	p		
SWR											
MRL/Mp-+/+ 75%	White		476 (F),		k		a	b	J	J	a
LG/J 12.6%			546 (M)		d/f		a	b	d	j	j
AKR 12.1%					k		a	b	j	d	a
C3H/Di 0.3%					k		a	b	b	k	c
C57BL/6					b		b			b	c
MRL/Mp-lpr/lpr	White	M/F	143 (F),	6	k	b	a	b	J	h	a
(MRL-l)			154 (M)	5							
BXSB* 50%	Brown	M	574 (F),		b	b	b	b	b	b	c
C57BL/6 50%			161 (M)								
SB/Le											
BXD2			14 mo								

F, female; M, male
*The *Yaa* gene represents a gene copy number polymorphism presenting as the duplication of the *TLR7* gene because of a 4-Megabase expansion of the pseudoautosomal region. Increased expression of TLR7 results in an intrinsic bias of Yaa-containing B cells toward nucleolar antigens.

Clinical Characteristics and Autoantibodies

NZB mice are characterized by hyperactive B cells, which are present in fetal life, that produce primarily immunoglobulin M (IgM) antibodies to thymocytes, erythrocytes, single-stranded DNA (ssDNA), and the gp70 glycoprotein of murine leukemia virus (12 ,13 ,14 ,15 ,16 ,17 ,18 ,19). The first antibody to appear in serum is natural thymocytotoxic antibody (NTA) (20 ,21); by 3 months of age, 100% of mice have this antibody. NTAs are cytotoxic for all thymocytes, 50% to 60% of thoracic duct and peripheral blood lymphocytes (both $CD4^+$ and $CD8^+$ populations), 50% of lymph node cells, 33% of spleen cells, and 5% of bone marrow cells. These figures are similar to the reactivity of anti–Thy-l sera that recognize all T cells. The antigens that are recognized by NTA are varied. Some NTAs react with cell surface molecules on B lymphocytes, granulocytes, and bone marrow myeloid cells; others react with a 55-kd molecule on most T cells. Other reported reactivities include an 88-kd glycoprotein, which is thought to be a T-cell differentiation antigen, and surface molecules of 33- and 30-kd sizes (15 ,22 ,23 ,24).

The primary clinical problem in NZB mice is hemolytic anemia, which is fatal in most at between 15 and 18 months of age (8 ,12 ,15). There is mild disease acceleration in females, with death occurring approximately 1 month earlier than in males. IgM and IgG antibodies to erythrocytes cause the hemolysis (25 ,26 ,27) and can be directed against erythrocyte surface antigens that are exposed by treating the red blood cell (RBC) with bromelein, against erythrocyte membrane protein band 3 (28 ,29 ,30), or against spectrin (31). Early in life, the antierythrocyte antibodies are polyreactive; later, they become more specific for band 3 or spectrin, suggesting antigenic stimulation (31). Anti-RBC appears in the serum by 3 months of age and is found in 100% of mice by 12 to 15 months. Clinical hemolysis begins 1 to 5 months after the antibodies appear. Severe anemia occurs in 56% to 87% of females and 77% of males (32). The expected sequelae of hemolysis occur (i.e., erythrocyte sequestration and extramedullary hematopoiesis in liver and spleen, splenomegaly, hepatomegaly, and deposits of hemosiderin in multiple tissues). The ability to make antibodies to erythrocytes probably depends on up to a dozen genes (33); at least two are major—one designated Aia3 on chromosome 7 and the other in the Nba2 region on chromosome 1, a region that plays a major role in several aspects of NZB/Bl lupus (34).

Table 18-2: Characteristics of NZB/Bl Mice

A. Clinical
   1. Females live a mean of 431 days, males 467 days
   2. Death usually is caused by autoimmune hemolytic anemia
   3. Fifty percent mortality by 15 to 17 months of age

B. Histologic
   1. Glomerulonephritis with immunoglobulin and C3 deposits
   2. Marked thymic atrophy
   3. Mild lymphoid hyperplasia

C. Autoantibodies
   1. IgM NTA
   2. IgM and IgG antierythrocyte
   3. IgM anti-ssDNA
   4. Anti-gp70
   5. ANAs by late life
   6. Modest elevations of circulating immune complexes

D. Immune abnormalities
   1. B cells are unusually mature, hyperactivated, and secrete immunoglobulin spontaneously from a very early age (in fetus and in newborn mice); this abnormality is required for autoimmune disease in NZB mice and in hybrids mated with NZB mice
   2. Numbers of B-1 (CD5+) B cells in spleen and peritoneum are increased; these cells make primarily IgM autoantibodies; however, their elimination by introduction of the xid gene protects from SLE
   3. B cells resist tolerance to T-independent antigens
   4. Older mice develop aneuploidy in B-1 B cells
   5. Thymic epithelium is atrophic by 1 month of age; this is a striking abnormality in NZB mice
   6. Antithymocyte antibodies react with immune T cells and may inactivate/delete precursors of suppressor T populations
   7. T cells are required for maximal autoantibodies formation
   8. A unique form of retroviral gp70 antigen is secreted, and high quantities are found in serum
   9. Clearance of immune complexes by Fc-mediated mechanisms is defective

E. Genetics
   1. Multiple dominant, codominant, and recessive genes participate in the immune abnormalities
   2. One set of genes controls the constellation of polyclonal B cell activation, expression of gp70, and antithymocyte antibodies; another set of genes controls B cell tolerance defects, antibodies to gp70, anti-ssDNA, and anti-RBCs; the gene sets segregate independently; neither of these sets is dependent on H-2
   3. The disease is linked to MHC
   4. Analysis of the NZ genome by microsatellites suggests that NZB donates two to five genes located on different chromosomes, some transmitted in a dominant and others in a recessive fashion, to lupus in mice with NZ backgrounds

ANA, antinuclear antibody; IgM, immunoglobulin M; MHC, major histocompatibility complex; ssDNA, single-stranded DNA.

Clinical glomerulonephritis (GN) may be observed in some NZB mice, but it is mild compared with the nephritis of other lupus murine models, probably because, in contrast to the IgG anti–double-stranded DNA (anti-dsDNA) that arise in the other strains, the IgM anti-ssDNA that dominates the NZB response does not contain many nephritogenic subsets of anti-DNA. However, histologic changes of glomerulonephritis, nephrotic syndrome, and renal insufficiency occur in some mice late in their life span, especially in virgin females (35 ,36). The incidence of antinuclear antibodies (ANAs) in

NZB mice is variable. ANAs are not regularly present in high titers as they are in other lupus-prone strains, but approximately 80% of mice are positive by 9 months of age (12). Some NZB mice exhibit learning disabilities (37), which probably relate both to the cortical ectopias that occur in approximately 40% and to the autoimmune process. Autoantibodies to Purkinje cells of the cerebellum have been found (38), and the numbers of interleukin-1 (IL-1) receptors that are expressed in the dentate gyrus are much lower than in normal mice (39).

Abnormalities of Stem Cells and B Cells

NZB mice are remarkable for inherent abnormalities in their B cells that probably originate in bone marrow stem cells. In comparison to normal mice, there are increased numbers of IgM-secreting cells and increased synthesis of IgM by individual B cells, which are characteristics that may be controlled by different genes (11 ,40 ,41 ,42). This hyperactivation of B cells begins quite early and is detectable in fetal liver. The IgM hypergammaglobulinemia of NZB mice may depend on gene(s) in a region on chromosome 4 that is 70 to 90 centimorgan (cM) distal to the centromere (43). Putative bone marrow pre–B cells exhibit increased growth both in vitro (44) and in vivo (45); this property is lost after 10 months of age (46). The mature B cells are committed to secretion of Ig, particularly IgM. They are resistant to normal control mechanisms involving engagement of the B cell receptor (BCR). Normally, if the BCR is bound, B cells cannot respond to lipopolysaccharide (LPS) stimulation by secreting Ig; NZB B cells in this situation secrete IgM, probably because of abnormal downstream signaling events. Regulation of B cell activation is not completely askew, however; binding of major histocompatibility complex (MHC) class II interrupts signaling by LPS in a normal fashion (47).

Another B cell abnormality that is highly characteristic of NZB mice is the appearance of aneuploidy in B cells, primarily in $CD5^+$ (also designated Ly-1 or B-1) B cells, as the mice age. Hyperdiploid B-1 B cells with additional chromosomes 10, 15, 17, and × are common (48 ,49). Lymphoid malignancies are more common in NZB than in other murine lupus strains, prevalence varying in different colonies between 1% and 20% (20 ,49 ,50 ,51); they may be a model of B cell chronic lymphocytic leukemia (49). Malignant B-1 B cells secrete large quantities of IL-10, which can skew T-cell repertoires away from T-helper-1 (Th1) and toward Th2 phenotypes (52). In young NZB mice, numbers of nonmalignant B-1 B cells are increased in the spleen and peritoneum (11 ,53 ,54); these cells make IgM autoantibodies to RBCs, thymocytes, and ssDNA(11 ,53 ,54 ,55). B-1 cells are also present among marginal zone (MZ) B cells in lymphoid tissues: MZ B cells respond more rapidly to antigen than do other B cells (56). B-2 (CD^5) B cells are more likely to be the source for IgG autoantibodies (57 ,58). However, elimination of B-1 B cells by introducing the X-linked recessive gene, Xid (41), or by lysing the cells with water in the peritoneal cavity (where these cells are renewed) reduces antibodies to RBC and hemolytic anemia (59), thus demonstrating the importance of B-1 cells to NZB disease. Finally, splenic B cells in NZB mice are probably resistant to apoptosis because of the influence of the Ifi202 gene, which is upregulated in this strain and plays a major role in sustained autoantibodies production in hybrids with NZB, and probably in the parent strain as well (60).

Abnormalities of Dendritic Cells

Since the recent recognition of the connections between innate and acquired immunity, there has been great interest in the role of dendritic cells (DC) as mediators of immune tolerance, and as a source of APC that activate T cells. Notably, DC can respond to CpG oligonucleotides (ODNs) derived from bacteria or from nucleosomal DNA in immune complexes, thus becoming activated APC that probably enhance autoimmune responses to DNA. In fact, NZB mice, compared to normal strains, respond to injections of CpG ODN with increased release of IFN-α; the source of that cytokine is DC. Furthermore, cell numbers of DC and mRNA for toll-like receptor 9 (TLR9), which binds ODN in DC, are increased in NZB mice. On the other hand, other features of DC that promote inflammation are abnormally low in NZB DC, including IL-12 production, expression of the homing chemokine CCR7 and the activation surface marker CD62L (61). Whether these abnormalities are primary defects contributing to autoimmunity, or whether they represent an activation stage in DC responding to abnormalities in apoptosis, phagocytosis of apoptotic materials and immune complexes, or intrinsic abnormalities in B and T cells, remains to be determined.

Abnormalities of Thymus and T Cells

Abnormalities of the thymus also are characteristic of NZB mice, and T cells interact with hyperactive B cells to further increase autoimmune responses. NZB mice exhibit dramatic involution of thymic tissue; thymic epithelium is atrophied and immunologically defective by 1 month of age (before the appearance of NTA), with epithelial cell degeneration, accumulation of TdT^+ large immature T cells in the subcapsular region of the cortex, cortical atrophy, and increased lymphoid and plasma cell infiltrates in the medulla (12 ,24 ,62 ,63 ,64 ,65). NZB thymic epithelial cells are functionally defective compared with cells from normal mice, having low expression of surface Ia molecules, low secretion of IL-1, high secretion of prostaglandin E2 (PGE2) and PGE3, and diminished ability to educate nonthymic cells to express Thy-l (63 ,64 ,65 ,66 ,67).

As in the B cell compartment, NZB bone marrow contains increased prothymocyte activity, and these prothymoctes have an increased growth advantage when they are transferred to histocompatible recipients (68). T cells probably play a major role in disease, because MHC class II is an important predisposing factor for autoimmunity. The hybrid combination of NZB d/d and NZW z/z or SWR q/q to make d/z or d/q MHC molecules predisposes hybrids to GN that is mediated by IgG anti-dsDNA (69 ,70 ,71 ,72 ,73 ,74 ,75 ,76), which are antibodies that NZB mice do not make. NZB mice that are congenic for H-2b (NZB.H-2b) have less disease than the wild-type NZB.H-2d. However, introduction of a mutated I-A chain (bm12) converts this

animal (i.e., H-2Bbm12) to a phenotype that is similar to the BW hybrid, with high-titer IgG anti-dsDNA and severe clinical GN (77 ,78 ,79 ,80). MHC class II likely plays a role in disease by shaping the repertoires of $CD4^+$ T cells. In fact, $CD4^+$ cells that proliferate in response to the RBC membrane protein band 3 and to spectrin (i.e., the major RBC antigens that are recognized by antierythrocyte Ab) have been isolated from NZB spleens (28). The importance of T cells to autoantibodies formation also is indicated by experiments in which anti-CD4 nondepleting antibody was administered to NZB mice: anti-erythrocyte antibodies were significantly decreased, although anemia was not prevented (81 ,82).

Genetics

NZB mice have a gene(s) located on chromosome 1 (Nba2) linked with elevated serum levels of antibodies to dsDNA, chromatin, histone, and gp70 as well as hypergammaglobulinemia. One gene within the Nba2 region that is particularly important is Ifi202, a interferon type 1-regulated gene which when increased in expression probably renders splenic B cells and non-T non-B cells relatively resistant to apoptosis, thus contributing to sustained autoantibodies production (60). Recent studies have shown Nba2, Nba4 (chromosome 5), Lbw4 (chromosome 6), and Nba5 (chromosome 7) increase antibody responses to chromatin in hybrids with SWR (83). Anti-gp70 responses are increased by genes in the regions Nba2 and Nba5, and gp70 antigen levels are increased by Sgp3 on chromosome 13 in crosses of NZB with C57Bl6 bearing the Yaa lupus-acclerating gene on the Y chromosome (84). There are contributions from the MHC region (H-2d/d) that enhance Nba2 and other gene region influences on autoantibody formation and nephritis (85,86), and from a region on chromosome 4 that predispose NZB/NZW F1 mice to nephritis (GN) (43,85,86). Studies of backcrosses between (NZB × SM/J) F1 and NZW mice, and of (NZB × NZW) F2 intercross mice, have suggested that NZB genes on chromosomes 1, 4, 7, 10, 13, and 19, in various combinations, contribute to GN (69,86). In a preceding paragraph we have noted that a recently discovered locus Aia3 on chromosome 7 along with a locus within Nba2 on chromosome 4, are key in development of anti-RBC and hemolytic anemia (84).

Summary

In NZB mice, the combination of inherent B cell hyperactivity and thymic loss, in addition to expansion and activation of DC in bone marrow, probably results in the abnormal shaping of T- and B cell repertoires. NZB mice are characterized by a fatal hemolytic anemia that is induced by antierythrocyte antibodies. Other autoantibodies in their repertoire include predominantly IgM NTA, anti-ssDNA, and anti-gp70. Their dominant immunologic abnormalities are hyperactivated B cells from fetal life onward, early degeneration of thymic epithelium, and increased numbers of $CD5^+$ (B-1) B cells that develop aneuploidy with age. These manifestations are controlled by multiple different genes. Sex differences are not marked, but disease is slightly accelerated in females.

New Zealand White Mice (NZW)

This strain is of great interest because it is clinically healthy but provides many genes that predispose to SLE in hybrids with other lupus-prone strains (15 ,43 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97). Therefore, the NZW genome must contain controlling or repressor or epistatic genes that protect from SLE, and those controlling genes must be powerful enough to allow the animal to appear normal.

Clinical Characteristics and Autoantibodies

The NZW mouse has a slightly shortened life span and develops largely nonpathogenic autoantibodies, some of which are only intermittently detectable. The autoantibodies pattern is characterized primarily by IgG antibodies to ssDNA and histones (89 ,98).

Genetics

Two groups of genes are clearly important in predisposing hybrid mice of an NZW parent to lupus and nephritis: (a) the MHC class II gene z (probably identical to u), and (b) several non-MHC genes.

Inheritance of the MHC class II z genes from NZW (I-Ez, I-Az, also referred to as I-Eu and I-Au) predisposes hybrid mice to nephritis (89 ,99 ,100 ,101). H-2d/z mice, compared to H-2 d/d mice, have a higher incidence of nephritis; the d/z haplotype increases the risk for nephritis 30-fold (100). These MHC II genes are important in class switching of various autoantibodies from IgM to IgG (89). H-2d/z predisposes to antibodies to ssDNA, dsDNA, chromatin, and histones, but not to gp-70 (99 ,100). Because the MHC region is closely linked to the gene encoding tumor necrosis factor-α (TNF-α) in the same gene region, and low production of that cytokine is a dominant genetic feature of NZW mice (102), it has been somewhat difficult to sort out the role of each gene. This has assumed great importance with the interest in considering TNF-α blockade in the treatment of human SLE. Fujimura et al. (101) made three different H-2 congenic mice of BW bearing distinct haplotypes at class II and TNF-α regions (H-2d/z:A(d/u)E(d/u)TNF-α (d/z), H2(d/u):A(d/u)E(d/u)TNF-α(d/d), and H-2(d/d):A(d/d)E(d/d)TNF-α(d/d); studies of nephritis in each group showed that both the NZW MHC class II and the unique TNF-α allele are important predisposing genes.

Genome scanning has shown regions in several NZW chromosomes outside MHC that increase susceptibility to SLE. These include regions in chromosomes 1, 11, 16, and 19 (which are linked to IgG autoantibody production), with a selective linkage of a region on chromosome 14 with IgG antihistone Ab (89). Additionally, chromosomal regions from NZW parents labeled SLE1, SLE2, and SLE3, have been linked with disease susceptibility in NZM2410 mice (products of matings of NZB/NZW F1 brother/sisters with categorization and further mating of various offspring according to severity of nephritis) and congenics of these mice and the normal strain C57B1/6J (87); see also (88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96)). SLE1 is located in the distal portion of chromosome 1 and promotes breaking of

tolerance to chromatin, with mice developing IgM antichromatin (92 ,94), which sets the stage for production of IgG anti-dsDNA if other permissive genes are present. The presence of the *z* allele of *SLE1* impairs B cell anergy, receptor revision, and deletion, which have been attributed to the lupus susceptibility gene *Ly108* (705). SLE2 is located on chromosome 4 and promotes B cell hyperactivity (91). Identified in NZM2410 mice, SLE2 on a C57Bl/6 background has been divided into SLE2a and 2b from NZW and SLE2c from NZB. The NZW-derived loci increased lymphocyte expansion and renal disease, whereas the NZB-derived locus did not, although it was associated with expansion in B-1 B cells (97). SLE3 is located on chromosome 7 and promotes T-cell hyperactivity (95). If normal mice are made homozygous for SLE1 or SLE3, few develop clinical nephritis. However, if they are made to express either one or two copies of both SLE1 and SLE2, some 85% develop severe nephritis (87 ,103). This illustrates the point that more than one susceptibility gene is required to develop clinical SLE. Another gene, probably located in or near the NZW MHC region, labeled SLE1-s (for "SLE1-suppressor"), when expressed in a normal strain along with SLE1 and SLE3, reduces the incidence of severe nephritis approximately 50% (87 ,103). Therefore, NZW mice appear healthy because their genome contains not only SLE susceptibility genes but also genes that protect from SLE, and the latter dominate in this strain. However, hybrids of NZW in which the other parent also contributes powerful susceptibility genes develop severe SLE. At the time of this writing, the exact gene(s) within these chromosomal regions have not been identified.

Identifiable single gene regions other than MHC/TNF-α, SLE1, SLE2, SLE3, and SLE1-s have been proposed as important to SLE susceptibility in NZW mice (104). For example, a locus designated lbw3, identifed on NZW chromosome 5, is associated with renal disease (69). Recent work (105) suggested that the P2RX7 gene within the lbw3 region contributes to cell death by aponecrosis, which may provide abundant autoantigens such as nucleosomes to the immune systems of mice predisposed to SLE, such as the NZB/NZW F1 hybrid.

The p8.6 gene, encoded upstream of the mouse TCRVa1 gene, contains regions that are consensus motifs for SH2 and SH3 binding motifs known to activate phosphorylation of some molecules. A gene mutation present in NZW (and BXSB) mice is associated with dysregulation in signaling through the BCR or T-cell receptor (TCR), which could play a role in the hyperactive responses or these cells in hybrid mice (106). Additionally, one of two murine Rt6 genes is deleted in NZW mice (107). Rt6 is a T-cell–restricted GPI-anchored membrane protein, a member of the family of mono[adenosine diphosphate(ADP)-ribosyl]transferases, also known as PARP. These enzymes are activated by apoptosis and play a role in DNA repair.

A portion of the TCR, encompassing Db2-Jb2, is deleted in NZW mice (108). Although one genetic backcross study showed lupus-like disease segregating with the abnormal TCRs (74), studies by other groups have not confirmed the importance of this deletion (75).

(NZB/NZW) F1 Mice (BW)

The BW hybrid cross between NZB and New Zealand white (NZW) mice is considered by many to be the murine model that most closely resembles human SLE. The disease is more severe and earlier in females, with high titers of IgG anti-dsDNA, antichromatin, ANA, and LE cells occurring in virtually all females; death results from immune glomerulonephritis (12 ,36 ,109) (Tables 18-1 and 18-3).

Both NZB and NZW parents contribute genetically to the immune abnormalities that cause disease, as discussed in the preceding sections. The B cell hyperactivity that is characteristic of the NZB is inherited by the BW, with abnormally high secretion of immunoglobulin being detectable by 1 month of age (13 ,14 ,15 ,19). However, the T-cell dependence of the response is more striking than in the NZB parent and probably is responsible for the isotype shift from IgM anti-DNA to IgG anti-DNA that precedes clinical disease (110 ,111). The ability to make this shift depends in part on genes located on chromosome 1 from the NZB (Nba2) parent and gene(s) on chromosomes 1 (SLE1), 4 (SLE2) and possibly 7 (SLE3) from the NZW parent.

Clinical Characteristics and Autoantibodies

The large quantities of IgG antibodies that bind both dsDNA and ssDNA, and are widely designated as anti-dsDNA, are striking and can be abrogated by removal of $CD4^+$ (formerly called $L3T4^+$) T cells (70 ,112). IgG antibodies to dsDNA clearly contain subsets that cause nephritis. Transfer of certain monoclonal BW IgG2 anti-dsDNA antibodies to normal BALB/c mice induces nephritis in the recipients (113 ,114). Infusion of anti-DNA into rodent kidneys induces proteinuria (115), and normal mice secreting BW IgG anti-dsDNA encoded by transgenes develop GN (116 ,117). (Chapter 21 discusses in detail characteristics of pathogenic subsets of anti-DNA.)

Anti-DNA and immune complexes containing gp70 and anti-gp70s are the most important autoantibodies made by BW mice that contribute to nephritis (118 ,119). ANAs are detectable in most females by 2 to 3 months of age; they include antibodies that bind subnucleosomes, nucleosomes, chromatin, dsDNA, ssDNA, dsRNA, transfer RNA (tRNA), polynucleotides, and histones (111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119). IgM anti-DNAs arise in females between 3 and 5 months of age; by 5 to 7 months, IgG anti-DNAs appear (12 ,110 ,111). The IgG2a and 2b subclasses are most frequent, which is important because these subclasses fix complement well. The IgM to IgG switch and the dominance of IgG2a and 2b thereafter occur in BW females responding not only to DNA but also to other thymic-independent and -dependent antigens (120). Shortly after the switch to IgG, IgG and complement deposit in the mesangia of BW glomeruli, spreading later to capillary loops and interstitial tubular regions (12). Proteinuria appears between 5 and 7 months; azotemia followed by death occurs at 6 to 12 months of age. Approximately half of the females are dead by 8 months and 90% at 12 months (12 ,14 ,114). With regard to immune complexes containing gp70, that antigen is an endogenous

retroviral glycoprotein produced by hepatic cells that is found in all mouse strains. However, lupus-prone mice (MRL-Fas(lpr), BXSB, NZB, NZW, NZB/NZW F1) all have high serum levels. It is likely that past integrations of murine leukemia viruses into the mouse genome account for production of gp70. DNA/anti-DNA complexes are difficult to detect in mouse sera, whereas gp70/anti-gp70 complexes are found more frequently than free antibody to gp70. It may be that many antibodies to DNA cause nephritis by direct attachment to planted or cross-reactive glomerular and tubular antigens, whereas passive trapping of gp70/anti-gp70 immune complexes is important in inducing nephritis. Both anti-gp70 and anti-nucleosome bind to endogenous xenotropic virions; immmunization of BWF1 mice with such virions induced stronger antibody responses than did immunization with chromatin. Therefore, the gp70 response may be very important as an indicator that pathogenic antinucleosomal antibodies can be made (121). NZB chromosome regions Nba2 (on chromosome 1, contains the Ifi202 gene) and H2 (on chromosome 17) are linked to high levels of gp70/anti-gp70 immune complex production, as they are linked to high titers of IgG anti-DNA. In contrast, the ability to make high levels of gp70 antigen is linked to regions on chromosomes 4 and 13 (119).

Table 18-3: Characteristics OF NZB/NZW F1 Mice

A. Clinical
   1. Females live a mean of 280 days, males 439 days
   2. Death usually is caused by immune glomerulonephritis
   3. Fifty percent mortality by 8 months in females and 15 months in males

B. Histologic
   1. Glomerulonephritis with proliferative changes in mesangial and endothelial cells of glomeruli, capillary basement membrane thickening, and chronic obliterative changes; mononuclear cell infiltrates in interstitium
   2. Glomerular immune deposits of IgG (predominantly IgG2a) and C3; similar deposits in tubular basement membrane and interstitium
   3. Thymic cortical atrophy by 6 months of age
   4. Myocardial infarcts with hyaline thickening of small arteries
   5. Mild lymph node hyperplasia and splenomegaly

C. Autoantibodies
   1. IgG anti-dsDNA (also binds ssDNA), enriched in IgG2a and 2b
   2. ANA and LE cells in all
   3. IgG antibodies bind chromatin, nucleosomes, and phospholipids
   4. Antithymocyte in most females and some males
   5. Renal eluates contain IgG anti-dsDNA concentrated 25 to 30 times greater than in serum; IgG2a isotype usually is dominant
   6. Modest elevations of circulating immune complexes; these include gp70–anti-gp70
   7. Low serum complement levels by 6 months of age in females

D. Immune abnormalities
   1. Polyclonal B cell activation
   2. B cells are resistant to tolerance to some antigens
   3. Strict dependence on T cell help for formation of pathogenic IgG anti-DNA, $CD4^+CD8^-$ and $CD4^-CD8^-$ α/ß TCR cells, as well as $CD4^-CD8^-$ γδ TCR cells, can provide help
   4. IgG repertoire becomes restricted with age to certain public Ids; there is some restriction of B cell clonality in the IgG anti-DNA response
   5. Thymic epithelial atrophy by 6 months of age; medullary hyperplasia; effect of thymectomy on disease varies
   6. Clearance of immune complexes by Fc- and complement-mediated mechanisms is defective
   7. Disease and autoantibody production is sensitive to sex hormone influences

E. Genetics
   1. The expression of high-titer IgG anti-dsDNA requires heterozygosity at MHC, namely $H\text{-}2^{d/z}$
   2. Additional complementary non–H-2-linked genes are required from both NZB and NZW parents to permit full expression of the IgG anti-DNA response; by microsatellite analysis of DNA, there are approximately 10 genes on as many chromosomes, with multiple genes required for early mortality, glomerulonephritis, antichromatin, and splenomegaly; this suggests a multigenic inheritance, with certain groupings predisposing more strongly than others to disease, rather than a simple additive model
   3. The large deletion in the ß chain of the TCR of the NZW parent probably does not predispose to disease

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dsDNA, double-stranded DNA; TCR, T cell receptor.

Antibodies eluted from glomeruli are composed predominantly of IgG anti-DNA; 50% of the total IgG is anti-DNA according to some reports (122 ,123). In colonies in our laboratory, anti-DNA accounts for as much as 85% of the total glomerular IgG (124). IgG2a is the dominant isotype in glomerular deposits, suggesting a role for Th1 cells, because production of IgG2a is dependent on interferon-γ (IFN-γ). Other antigens and antibodies have been reported in glomerular eluates, including gp70, antihistones, anti-C1q, and anti-RNA polymerase (125 ,126 ,127). The high serum levels of IgG anti-DNA occur at about the same time as hypocomplementemia, and levels of circulating immune complexes are elevated (12).

Histologic changes in kidneys include chronic obliterative changes in glomeruli, mesangial, and peripheral proliferative changes, capillary membrane thickening, glomerular sclerosis, tubular atrophy, infiltration by mononuclear lymphocytes and monocyte/macrophages, and vasculopathy

(primarily degenerative, occasionally inflammatory) (12 ,36) (Fig. 18-1). Additionally, messenger RNA (mRNA) encoding several molecules that contribute to proliferation, inflammation, and sclerosis is increased in the kidneys of BW mice with GN; those molecules include platelet-derived growth factor (PDGF), MHC class II, insulin-like growth factor-1 (IGF-1), IFN-γ, and basic fibroblast growth factor (bFGF) (128 ,129 ,130 ,131). Other autoantibodies also occur in BW mice. Antibodies to erythrocytes are found in 35% to 78% of BW females, but they rarely cause hemolytic anemia (14). Approximately 50% develop NTAs by 6 months of age. Because the genes governing NTA, anti-DNA, and antierythrocyte antibodies probably segregate separately (13 ,15 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,102 ,103 ,104 ,106 ,107 ,132 ,133 ,134 ,135), New Zealand mouse strains have been bred that have high-titer NTAs but no autoimmune disease. However, NTAs may serve as an accelerator of the disease process that occurs in mice with IgG anti-DNA, because NTAs may alter T-cell function. Both IgM and IgG antiphospholipid antibodies have been detected and obtained as monoclonal antibodies from BW mice (136). Some have anticardiolipin activity and others lupus anticoagulant properties.

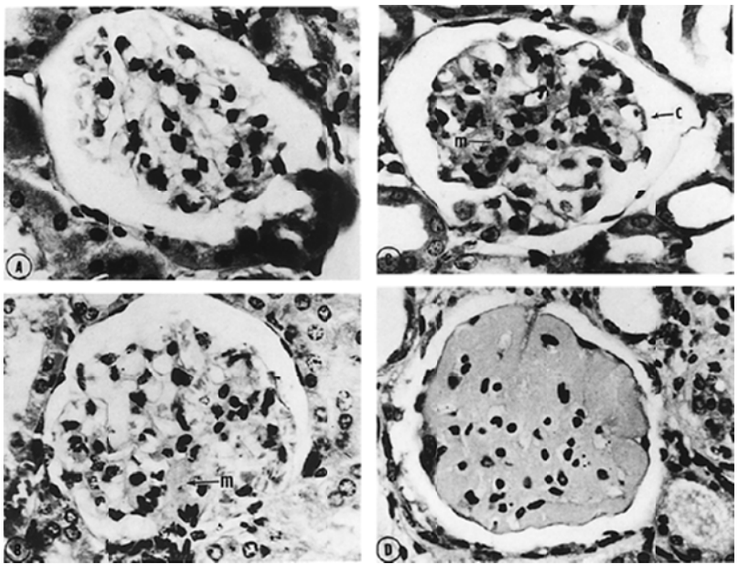

Figure 18-1. Glomerulonephritis in New Zealand mice. Each sample is from a kidney section of NZW mice. **A,** Normal mouse glomerulus. **B,** Mesangial proliferation and thickening (m). **C,** Proliferative glomerulonephritis with thickened glomerular capillaries (c). **D,** End-stage glomerulopathy; the glomerulus is obliterated.

IgG1 antibodies have been eluted from the neurons of BW mice (137); it is not known whether they cross-react extensively with lymphocytes, as do some human lupus antineuronal antibodies. Antibodies to histones, ubiquitin, chromatin, and fibrillarin have been reported, as have cryoglobulins (138 ,139 ,140). Some antibodies to DNA, chromatin, and nucleosomes can bind to and/or penetrate living cells; some of these subsets make glomerular cells proliferate and impair cellular production of protein (141 ,142 ,143 ,144 ,145).

The lymphoproliferative features of NZB mice occur in BW hybrids, which exhibit mild lymphadenopathy and splenomegaly (12 ,14). Lymphoid neoplasia is far less common in BW than in NZB mice. Some investigators have reported a relatively high incidence of thymoma, from 1% to 5% (146), but that has been rare in our colonies unless mice are treated with cytotoxic agents (147). Extrarenal lesions occur in BW mice, including lymphocytic infiltration of salivary glands, mild inflammation around bile ducts in the liver, pancarditis, vasculitis (less common than in MRL-Fas(lpr) and BXSB mice), myocardial infarcts, and deposits of DNA and anti-DNA in the dermoepidermal junction of skin and in the choroid plexus (12 ,148 ,149).

Sex Hormone Influences on Lupus in BW Mice

The femaleness of spontaneous BW disease has been studied extensively. Most BW males develop ANAs, including antibodies to DNA, but they are predominantly IgM. The IgM to IgG switch occurs relatively late in life, usually after 12 months. Histologic evidence of nephritis can be found in males, and most die of slowly progressive chronic nephritis by 15 to 20 months of age (12 ,14).

The BW mouse is particularly sensitive to the effect of sex hormones on disease. Generally, androgens are protective and suppress the expression of autoantibodies and disease, and estrogens are permissive. Castrated male BW mice (and other hybrids of NZB) develop high titers of IgG autoantibodies as early in life as do females (13). Males that are castrated and/or treated with estrogens or testosterone antagonists assume a female pattern: early IgM-to-IgG switch of anti-DNA antibodies and early, fatal nephritis (150 ,151 ,152). Females that are treated with castration and androgens, or with antiestrogens, have prolonged survival, with suppression of IgG anti-DNA and nephritis (153 ,154 ,155 ,156). In old females, androgens can suppress disease without altering the elevated levels of IgG anti-DNA. Addition of exogenous estrogens cause early death in females, but this may result largely from toxic effects rather than from enhancement of immune responses (152). Hyperprolactinemia occurs in some women with SLE. In BW mice, SLE is accelerated in pseudopregnant females in which cyclical increases of prolactin occur, and administration of prolactin to BW mice accelerates disease, while bromocriptin suppresses it (157 ,158 ,159). Manipulations of BW mice that produce both high levels of estrogen and high levels of prolactin shorten survival about 8 weeks (159).

The effects of sex hormones on immune responses are complex and poorly understood. There are receptors for estrogens, progestogens, and prolactin on lymphocytes (157). The administration of estradiol in vivo dramatically suppresses NK cell function, and NK cells downregulate activated B cells (160). Additionally, normal mice transgenic for a murine IgG antibody to DNA show defective B cell tolerance if they are treated with exogenous estradiol (161). Such mice fail to delete B cells producing anti-DNA from unmutated germline genes. In addition to their effects on sex tissues and lymphocytes, sex hormones may regulate the expression of certain genes.

Abnormalities of Stem Cells and B Cells

BW mice exhibit the hyperactivated B cell phenotype of their NZB parent, except that defects appear later in life. BW mice have abnormally elevated secretion of IgM by 1 month of age. Stem cells of the pre-B lineage can partially transfer disease: severe combined immunodeficiency disease (SCID) mice (a mutant strain that lacks most T cells) that are inoculated with BW bone marrow pre–B cells develop autoantibodies (including IgG anti-dsDNA), and approximately 25% develop clinical nephritis (162). These studies suggest that both B and T cells are required for the full expression of BW disease. The B cell repertoire that expresses anti-DNA is somewhat restricted. Public idiotypes (Ids) that are expressed on total serum IgG become increasingly restricted as the mice age (163). Although many different V genes can be used to assemble antibodies that bind DNA (164), most BW monoclonal antibody anti-DNA belong to one of approximately 12 families (165 ,166 ,167 ,168). This type of restriction is seen in normal, antigen-driven antibody responses. B-1 B cells and marginal zone B cells are increased in number. Depleting some of these cells by administering a B cell superantigen (protein A from *Staphylococcus aureus*) delays appearance of serum IgG anti-DNA and reduces proteinuria, confirming participation of these cells in autoimmunity in this mouse strain (169).

Abnormalities of Thymus and T Cells

The degeneration of thymic epithelial cells that is characteristic of NZB mice occurs in BW mice, but at 6 months of age in contrast to 1 month of age in the NZB parent (15). Responses to thymectomy have been variable; there are reports of thymectomy failing to alter disease or even accelerating it (63). Full-blown BW lupus depends on the presence of $CD4^+$ helper T cells; T-cell lines from nephritic mice can accelerate disease in naive young syngeneic mice (170 ,171). Elimination or inactivation of $CD4^+$ T cells prevents the onset of disease and can even partially reverse established nephritis (112 ,172). As BW mice age, the numbers of $CD4^+$ T cells increase fivefold, and these cells are polyclonal (173 ,174). T cells from nephritic BW mice can drive B cells from young normal mice to make pathogenic autoantibodies (170 ,171), whereas B cells from old mice will not secrete anti-DNA when they are cocultured with T cells from premorbid young normal mice (170 ,175).

Pathogenic T cells must receive second signals after TCRs are engaged to develop into activated effectors of disease. Interruption of second signals with blockade of the CD28/CTLA4 T-cell surface molecule's interactions with CD80/CD86 (B7–1/B7–2) on antigen-presenting cells (APCs) prevents disease. Experiments showing this include the administration of cytotoxic T-lymphocyte antigen-4 (CTLA-4)–Ig, which binds B7.1 and B7.2, thus preventing interaction with CD28 (176), and the administration of antibodies to CD80 and CD86 (177). Additionally, blocking second signals that activate B cells (CD40 interacting with CD40 ligand) by administration of antibody to CD40L prolongs survival in BW and other New Zealand-background lupus mice (178 ,179). Blocking both CD28/B7 and CD40/CD40L interactions is probably more effective than blocking either one alone (180).

Because $CD4^+$ T cells of the Th1 phenotype (secreting IL-2 and IFN-γ) generally support cell-mediated reactions, whereas $CD4^+$ cells of the Th2 phenotype (secreting IL-4, IL-5, and IL-6) give help to B cells for antibody production, there has been great interest in the possibility that a skewing toward Th2 plays a major role in the SLE of BW mice. Such skewing has been suggested by the well-known fact that T cells from BW mice secrete less and less IL-2 as the mice age, with a diminution in IL-2 receptors on T-cell surfaces (14 ,175 ,181). One study (181) showed a concomitant decline in IL-2 and IFN-γ, with an increase in IL-4 secretion by 6 to 8 months of age that would support a shift to Th2. Another study, however, showed a decrease in IL-2 and IL-4

and an increase in IFN-γ as mice aged (175). In our colonies, dramatic increases in plasma levels of IFN-γ and IL-4 occur at the time high quantities of IgG anti-DNA appear. Because the dominant isotype that is eluted from glomerular lesions is IgG2a, which depends on IFN-γ for its synthesis, Th1 cells (or Th0 that secrete IFN-γ) are important in inducing disease. Additionally, our laboratory has accelerated disease in young BW mice by the transfer of either Th1 or Th2 cell lines, implying that both subsets play important pathogenic roles. IL-6, which is secreted by Th2 cells and B cells, potentiates autoantibodies formation (182). Administration of IL-6 accelerates disease, and antibodies to IL-6 delay it (183). IL-10, which is made predominantly by monocyte/macrophages, also is important, is increased in SLE, and shifts repertoires from Th1 toward Th2, probably by suppressing IL-6. Thus, administration of anti–IL-10 delays disease in BW mice (184).

Transforming growth factor-ß (TGF-ß) can mediate suppression, which is critical to controlling SLE-like immune responses (185). TGF-ß a is essential for the suppression by some $CD4^+CD25^+$ regulatory T cells (186) and by $CD8^+$ T cells (187), which delay autoautoimmunity in BW mice tolerized with histone or Ig peptides. However, late in disease TGF-ß contributes to glomerular scarring and thus to shortened survival (1). IL-1 and TNF-α are both pro-inflammatory and may be abnormally elevated in BW lupus (188 ,189). The role of TNF-α in murine and human lupus has been debated for several years. NZW mice have an unusual gene that may encode abnormally low levels of TNF-α, and short-term administration of TNF-α to BW mice delays disease (190). However, mRNA for TNF-α is increased in the glomerular tissue of BW mice (189), and chronic administration of the cytokine worsens disease (191 ,192).

Abnormalities of Monocytes/Macrophages

Monocyte/macrophages are primary sources of IL-1; production of that cytokine is reduced in BW and other murine lupus strains (188 ,193). Macrophages also produce IL-12, the major cytokine stimulating Th1 responses. The ability of CRP treatment to delay disease onset in BW mice may relate to the fact that CRP reduces IL-12 production by macrophages following ingestion of apoptotic materials; those macrophages have reduced ability to activate T cells (194 ,195 ,196 ,197).

The Role of Defective Regulatory Cells in BW Lupus (CD4+CD25+, CD8+, NKT cells, B-1 B cells)

Finally, the possibility that regulatory cells that ordinarily suppress activated T and/or B cells are defective or missing from BW repertoires should be considered. $CD8^+$ T cells, which usually are cytotoxic or suppressive, behave abnormally in BW mice. As the mice age, $CD8^+$ T cells fail to expand while $CD4^+$ T and B cells are increasing greatly in numbers, and very few $CD8^+$ cells express surface markers of activation and memory. Furthermore, activating $CD8^+$ T cells from old BW mice results in apoptosis rather than the activation characteristic of normal mice and young BWs (174). Infusing $CD8^+$ T cells from young BW mice suppresses murine lupus (180). Recent studies showed that tolerizing regimens with autoantibodies– or histone-derived peptides induce both suppressive $CD8^+$ T cells and classical $CD4^+CD25^+$ regulatory T cells, each of which can prolong survival in BW or NZB/SWR Fl mice, indicating that regulatory T-cell defects can be “repaired” in vivo (186 ,187 ,198). However, the systemic autoimmunity in NZM2328 mice is not associated with a global deficiency in functional $CD25^+$ Treg cells (199). CD1-restricted NKT cells prevent the development of autoimmune manifestations if activated in early stages of disease in BW (nephritis), pristane-injected BALB/c (nephritis) and MRL-lpr (dermatitis) mice (1 ,3 ,200), but not in late stages of BW disease and in pristane-injected SJL mice (201 ,202). As BW mice age, the numbers of NKT expand and become hyperactive; they can actually increase production of IFN-γ—a major cytokine that enhances SLE in this strain (203).

Regulation provided by B-1 (CD5+) B cells also may be abnormal in BW mice. B-1 B cells can downregulate autoantibody production under normal circumstances; BW mice have large numbers of these cells. B-1 B cells derived from normal CBA/J bone marrow and transferred with BW marrow into chimeric mice, suppressed disease (204). In contrast, mixtures of BW and CBA/N bone marrow (CBA/N carries the xid gene, which eliminates B-1 B cells) transferred BW lupus to recipients (204). Thus, BW B-1 cells increase autoimmunity instead of suppressing it. Monocytes/macrophages, which are defective in IL-1 production in BW mice, also may serve as downregulators in normal circumstances; it is not clear that they serve this function in the BW model.

Abnormalities of DC in BW Mice

DC, which connect innate and acquired immunity, and can be activated by oligonucleotides produced by bacteria and by SLE patients, are abnormal in BW mice. As mice age, DC expand in numbers and acquire ability to attract B cells and to present antigen (205). This activity is particularly brisk in the spleen, where DC stimulate nucleosome-reactive T cells to a much greater extent than normal (206). In BW mice, splenic DC are potent adjuvants for induction of autoantibodies to apoptotic materials (207). Furthermore, DC are a major source of type 1 interferons. High production of these interferons is characteristic of BW mice and of humans with SLE (6). Increased expression of the Ifi202 gene (interferon-inducible) from the Nba2 gene region of NZB mice is important in predisposing to disease (60). Deficiency of the type-I interferon receptor protects NZB mice from disease (208), and administration of IFN-α accelerates disease in BW mice (209). Therefore, abnormal DC play a critical role in promoting lupus-like disease in BW mice.

Genetic Predisposition

Genetic predisposition is discussed fully in Chapter 7 and was reviewed briefly in the preceding sections on NZB and NZW mice. In BW mice, genetic contributions to disease are provided by both NZB and NZW parents. The most important contributors are probably MHC genes (heterozygosity for H-2 d/z) and the Ifi202 gene from NZB encoding

a transcription factor that inhibits lymphocyte apoptosis (6 ,60). Additionally, multiple non-MHC genes on at least eight different chromosomes contribute to disease susceptibility (43 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,95 ,96 ,99 ,100 ,101 ,102 ,103 ,104 ,106 ,107 ,108).

Summary

BW mice develop fatal glomerulonephritis, mediated primarily by IgG antibodies to dsDNA and immune complexes of gp70 and anti-gp70, that occurs earlier and is more severe in females and can be modulated by sex hormones. Multiple genes inherited from both NZB and NZW parents, both MHC and non-MHC, are required for the development of high-titer IgG anti-dsDNA and clinical nephritis. Abnormalities in B-1 and marginal zone B cells, in $CD4^+$ helper T cells and $CD4^+CD25^+$ regulatory T cells, in $CD8^+$ and NK-T suppressor cells, and in DC are all required for the disease to be fully manifest.

(SWR × NZB) F1 (SNF1) Mice

The SNF1 mouse is a model of lupus nephritis that is produced by mating the normal SWR mouse with the autoimmune NZB mouse (210 ,211 ,212 ,213 ,214 ,215); it does not matter which parent is female and which is male (Table 18-4). In contrast to NZW mice, SWR mice are completely healthy, with normal life spans, low levels of serum gp70, and no evidence of autoimmune disease (210 ,211). Their B cells can produce Igs bearing the same public Ids that dominate serum Ig in MRL-Fas(lpr) mice (215 ,216 ,217).

Table 18-4: Characteristics of NZB × SWR F1 (SNF1) Mice

A. Clinical
1. Mean survival in females is 297 days; mean survival in males is 531 days
2. Females die from immune glomerulonephritis between 5 and 13 months of age

B. Histologic
1. Glomerulonephritis with proliferative and obliterative lesions

C. Autoantibodies
1. IgG anti-dsDNA is made by all females
2. Anti-dsDNA is dominated by IgG2b cationic populations with restricted idiotypes
3. ANAs in all females

D. Immune abnormalities
1. B cells are hyperactivated
2. The development of nephritis depends on the presence of T cell help for production of IgG anti-DNA
3. Cationic IgG anti-dsDNA may use the allotype of either the NZB or healthy SWR parent
4. Anti-dsDNA deposited in glomeruli cluster into two main groups defined by their Ids
5. $CD4^+CD8^-$ and $CD4^-CD8^-$ T cells can provide help for the synthesis of cationic IgG anti-dsDNA

E. Genetics
1. Probably similar to BW mice

Clinical Characteristics and Autoantibodies

SNF1 mice are similar to BW mice. Females are dead by 10 to 12 months of age (50% mortality at 6 months) from an immune glomerulonephritis that is mediated primarily by IgG2b antibodies to dsDNA (212 ,214). This model has been of particular interest because of the oligoclonality of the IgG anti-DNA that is deposited in glomeruli (213 ,215). Activated B cells of NZB mice make anti-DNAs that are predominantly IgM, bind ssDNA rather than dsDNA, and are anionic in charge (214). In contrast, B cells of SNF1 mice make predominantly IgG2b anti-dsDNA that is cationic (214 ,215). Cationic charge probably is important in initiating nephritis, because cationic antibodies (or antigens or immune complexes) can bind to polyanions in glomerular basement membranes. IgG in glomerular eluates from BW mice also is enriched in cationic subpopulations (118 ,124), and it is those populations that bind directly to glomeruli when they are infused into old BW mice (218).

The presumed pathogens, IgG2b cationic anti-dsDNA, also are restricted in Id expression. The IgG in the glomeruli of SNF1 mice can be grouped into two families of Ids (213 ,215). The first, Id564, is composed entirely of cationic IgG, and most members bear the Igh allotype of the SWR parent. The second Id cluster, Id512, contains immunoglobulin of anionic, neutral, and cationic charge; the allotypes expressed are both SWR and NZB derived. Id564 is unique to SNF1 mice and is not found in either parent. This Id restriction is similar to that reported by our group in BW mice, where only two public Ids (IdGN1 and IdGN2) dominate the glomerular immunoglobulin deposits (163).

Sequence data show that the expression of Id564 depends on the VH region of the immunoglobulin molecule; $Id564^+$ monoclonal antibodies are closely related structurally and probably derive from a germline gene that is unique to the SNF1 mouse (216). One family of Ids, designated as $IdLNF^+$, has been used to track reciprocal T- and B cell functions that are connected by idiotypy. SNF1 mice also make antihistone antibodies, which are characterized by some clonal restriction and by somatic mutations, as are most autoantibodies in the mouse models (219).

Abnormalities of Stem Cells and B Cells

It is assumed that SNF1 mice inherit hyperactivated B cells from their NZB parent, but there are few data on the subject. The interesting features of this model include the demonstration that a nephritogenic anti-DNA subset can be constructed from the allotype of a normal parent given the appropriate additional genetic background. Idiotypic connectivity between B and T cells also has been particularly well described in this model (220 ,221 ,222). $IdLNF^+$ immunoglobulin does not contain much antibody to DNA, but nephritis and early death correlate with high serum levels of $IdLNF^+$ immunoglobulin and glomerular deposits of the Id, thus illustrating the role of non–DNA-binding immunoglobulin in the glomerular disease. Suppression of $IdLNF^+$ immunoglobulin by the administration of a specific anti-Id does not

downregulate serum levels of IgG anti-DNA, but nephritis is delayed and survival prolonged (222).

Abnormalities of T Cells

Studies suggest that the T-cell abnormalities of BW mice are reiterated in the SNF1 model. B cells from SNF1 spleens (or BW spleens) secrete IgG anti-dsDNA (including cationic subsets) only when they are stimulated by T cells in culture (223 ,224). Those T cells may bear the classic $CD4^+CD8^-$ phenotype of helper T cells, or they may be $CD4^-CD8^-$ (223).

As mice age, their $CD4^+$, $IdLNF^+$-specific repertoire expands greatly. There is little TCR restriction in the expanding $CD4^+$ cells. Transfer of a few T-cell clones that are specific for the Id increased the Id^+ immunoglobulin production in young SNF1 mice (220 ,221). Some of the T cells that help anti-DNA production recognize a small number of peptides in the histones found in nucleosomes (225); autoantibodies production and disease can be dramatically delayed by administration of some of those peptides in very small quantities (186).

Genetics

As in the BW mouse, genes contributed from both parents are necessary for disease in the SNF1. Some genes clearly are linked to H-2. However, one study suggests that nephritis also is influenced by the TCR-ß chain (which contains a large deletion similar to the NZW) as well as by the I-A-chain genes of the SWR parent (224).

Summary

The SNF1 mouse is another example of female-dominant, T-cell–dependent lupus nephritis in a hybrid mouse with an NZB background. The nature of the antibodies that deposit in glomeruli has been particularly well studied and is somewhat oligoclonal, thus providing important information about the characteristics and genetic control of pathogenic subsets of autoantibodies.

(NZB × SJL) F1 (NS) Mice

The female predominance of lupus-like disease was also demonstrated in another NZB-based strain, where the female, but not male, offspring of NZB crossed with SJL mice developed proteinuria and Ig deposits in the renal glomeruli and at the dermo-epidermal junction of the skin at 12-months of age (226). Female NS mice died faster and exhibited ANA, anti-dsDNA antibody and circulating immune complexes earlier in life and in greater amounts than male NS mice. The disease development in female NS mice is associated with thymic pathology characterized by the intrathymic accumulation of mature T and B cells (227). Ovariectomy of NS females reduced but did not prevent the accumulation of T and B cells in the thymus indicating that estrogen plays a minimal role in the thymic pathology of this strain. In contrast, chronic dihydrotestosterone (DHT) treatment of female mice, started at 4 weeks of age, inhibited the development of thymic lesions. Consistently, orchidectomy of NS males induced the appearance of “female-like” thymic lesions that could be prevented by DHT treatment. This demonstrates that the absence of thymic disease in NS males probably reflects an inhibitory action of androgens (228).

New Zealand Mixed (NZM) Mice

In 1993, Rudofsky et al. (73) reported a new strain of mice with SLE. They performed selective inbreeding of the progeny of one cross between NZB and NZW mice, selecting for severity of nephritis and coat colors. They derived 27 strains and studied 12, determining homozygosity for NZB and NZW polymorphic gene markers at H-2, Hc (i.e., a polymorphism for C4 on chromosome 2), and coat-color loci on chromosomes 2, 4, and 7. Most NZM strains have IgG anti-dsDNA antibodies. Some strains develop early onset nephritis in males and others in females similar to the BW F1, and some strains have little nephritis. These initial studies showed that there is not a strict requirement for H-2d/z heterozygosity to develop nephritis, but such heterozygosity increases susceptibility.

Subsequently, selected NZM strains were used to study the segregation of genes with manifestations of lupus. For example, the NZM/Aeg2410 line (i.e., more rapid and severe GN than in BW) was backcrossed to normal C57B1 mice for interval mapping of susceptibility loci (229). Three chromosomal intervals containing strong recessive alleles predisposing to GN were found on chromosomes 1, 4, and 7. All of these are contributed by the NZW parent: they have been designated SLE1, SLE2, and SLE3. Studies in single and double congenic mice have shown that SLE1 allows a mouse to break tolerance to chromatin, SLE2 permits B cell hyperactivity, and SLE3 permits T-cell (and some B cell) hyperactivity. Normal C57Bl/6J mice expressing SLE1 or SLE3 do not develop nephritis, but C57 mice expressing both chromosome regions develop nephritis. See the discussion of genetics of NZW mice above, and Chapter 7 for a more detailed discussion. In SNF1 mice, heterozygosity at H-2 correlates strongly with GN; the MHC alleles seemed to confer susceptibility independently and were additive (229). Subsequent studies in other strains have identified similar regions of chromosomes that contribute to GN, but as more and smaller segments of the mouse genome have been analyzed, the inheritance pattern has looked more like polygenic gene combinations without a strictly additive pattern of inheritance (see Chapter 7). Chapter 7 summarizes the genetics in the NZM and other strains, which are also available in many excellent reviews (230 ,231 ,232 ,233 ,234).

B cells in this strain are abnormal (like the NZB and BW) in that ligation of the BCR does not modulate LPS signals in a normal way (47).

NZM2328 mice, another New Zealand Mixed strain, develop autoantibodies and acute and severe chronic glomerulonephritis with female predominance similar to NZB/NZW F1 and humans with SLE (235). Chronic GN with glomerular sclerosis and tubular atrophy but not acute GN was correlated with severe proteinuria. Using a backcross analysis of (NZM2328 × C57L/J) F1 × NZM2328, Fu et al.

identified loci that could be linked to either autoantibodies production or to acute or chronic glomerulonephritis. Further, in this model adoptive transfer of $CD4^+CD25^+$ cells can suppress anti-DNA antibody production, but do not influence the development of glomerulonephritis (199). These authors further showed that the male NZM2328 mice that normally do not develop glomerulonephritis experience an accelerated onset of acute glomerulonephritis after day 3 thymectomy, but this acute glomerulonephritis does not progress to chronic glomerulonephritis.

When compared to MRL-lpr and BW mice, NZM2410 mice develop an accelerated onset of chronic glomerulosclerosis that can be suppressed by in vivo blockade of IL-4 by monoclonal antibody treatment or by genetic deletion of transcription factor Stat6 that inhibits production of and responsiveness to type 2 cytokines such as IL-4 (236). In fact, levels of IL-4 are markedly elevated in NZM.2410 mice, as determined using an in vivo cytokine assay. Germline deletion of Stat6 in NZM2328 mice has a similar ameliorating effect on glomerulosclerosis (237). Strikingly, antibody blockade or Stat6 deletion has no effect on IgG anti-dsDNA antibody levels and on renal IgG deposition in NZM2410 and NZM2328 strains. Thus, IL-4 effects on lupus nephritis in NZM2410 and NZM2328 models appear to be independent of IL-4 effects on autoantibody production. On the other hand, the germline deletion of Stat4, a transcription factor for type 1 cytokines, suppresses anti-dsDNA antibody production, but does not suppress the incidence of glomerulonephritis in NZM2410 and NZM2328 models (236 ,237).

These observations suggest that anti-DNA autoantibodies production and development of acute and chronic glomerulonephritis can be uncoupled in some models, raising the question of the direct cause-effect relationships between the presence of autoantibodies and lupus nephritis in some of the NZM strains.

MRL/Mp (MRL+/+) and MRL-Fas(lpr) Mice

The MRL-Fas(lpr) strain and the congenic MRL/Mp (MRL/+/+) (also called MRL/n) were developed by Murphy and Roths in 1976 (238). They were derived from LG/J mice crossed with AKR/J, C3HDi, and C57B1/6. By the 12th generation of inbreeding, the MRL-Fas(lpr) which is characterized by marked lymphadenopathy and splenomegaly, large quantities of antibodies to DNA, antibodies to Sm, and lethal immune nephritis, was derived. Lacking the lpr gene, MRL/+/+ mice share over 95% of the genetic material of the MRL-Fas(lpr) (14). The lpr (i.e., lymphoproliferation) trait occurred as a spontaneous mutation in a single autosomal recessive gene; the mutation results in a defective Fas molecule (239 ,240 ,241 ,242 ,243). Interactions of Fas and Fas ligand (FasL) are required for the initiation of apoptosis in activated B and T lymphocytes under normal immunoregulatory conditions (244). Therefore, mice that are homozygous for the lpr mutation (i.e., Fas (lpr), formerly designated lpr/lpr) develop massive lymphoproliferation, large quantities of IgG autoantibodies, and autoimmune disease (245 ,246 ,247). Table 18-5 lists features of this strain.

Table 18-5: Characteristics of MRL/lpr Mice

A. Clinical
1. Massive lymphadenopathy with expansion of $CD4^+$ and Thy1 + B220 + $CD4^-CD8^-$ TCR αß + (double negative or DNT) cells
2. Early death in males and females (50% mortality at 6 months)
3. Congenic strain MRL/++ lacks lpr; 50% mortality at 17 months
4. Deaths usually result from immune glomerulonephritis
5. Approximately one half develop acute necrotizing polyarteritis
6. In some colonies, approximately 25% develop destructive polyarthritis

B. Histologic
1. Subacute proliferation of mesangial and endothelial cells, occasional glomerular crescents, basement membrane thickening; deposits of immunoglobulin and C3 in glomeruli, especially in capillary walls; marked mononuclear cell infiltrate in interstitium
2. Acute polyarteritis of coronary and renal arteries
3. Proliferative synovitis, pannus formation, and destruction of articular cartilage—usually detected microscopically, not grossly
4. Thymic atrophy
5. Massive hyperplasia of all lymphoid organs, sometimes with hemorrhage and cystic necrosis

C. Autoantibodies
1. Monoclonal paraproteins in approximately 40%; IgG3 cryoglobulins are common
2. Most marked elevations of serum IgG, IgM, and immune complexes of all murine SLE models
3. ANAs at highest levels of all murine SLE models
4. IgG and IgM anti-dsDNA and anti-ssDNA
5. Anti-Sm in 10% of females and 35% of males
6. IgM and IgG rheumatoid factors in 65%; some IgG–IgG complexes
7. gp70–anti-gp70 complexes
8. IgM and IgG antibodies to DNA, snRNP particles, and phospholipid often are cross-reactive, suggesting that any of the antigens can activate the entire repertoire
9. Hypocomplementemia

D. Immune abnormalities
1. Lymphoid hyperplasia primarily results from expansion of unusual $CD3^+CD4^-CD8^-$ B220 + α/ß$^+$ T cells; they probably derive from activated $CD8^+$ cells that fail to undergo apoptosis
2. Appearance of these T cells and of early disease is strictly dependent on the lpr gene and also is thymus dependent; thymectomy prevents disease
3. High numbers of hyperactivated B cells appear just before onset of clinical disease
4. Autoantibodies, nephritis, arthritis, and CNS disease are prevented by elimination of $CD4^+$ cells; lymphoproliferation is not
5. Lymphoproliferation is prevented by elimination of $CD8^+$ cells; autoantibodies, nephritis, and arthritis are not affected
6. Defective Fc-mediated phagocytosis and clearance of immune complexes
7. Monocytes/macrophages are abnormal, with low expression of IL-1ß; and defective function

E. Genetics
1. Accelerated disease is produced by a single autosomal recessive gene, lpr; this mutation encodes a defective Fas molecule, so that very low levels of Fas are expressed on cell surfaces; engagement between Fas and FasL is infrequent, making Fas-mediated apoptosis defective; Fas/FasL interaction delivers a major signal for deleting activated T cells by apoptosis; mice homozygous for lpr develop lymphoproliferation on most backgrounds, but clinical autoimmune disease primarily appears in permissive backgrounds, such as MRL/++ and NZB
2. The congenic MRL/++ has a B cell repertoire that makes anti-DNA, anti-Sm, and rheumatoid factors; these autoantibodies probably are controlled by multiple genes, as in the NZB

Clinical Characteristics and Autoantibodies

MRL/+/+ mice are abnormal and develop late-life lupus. They make anti-DNA, anti-Sm, and rheumatoid factors, but serum levels are lower than those of MRL-Fas(lpr) mice. Male and female MRL/+/+ are similarly affected; most develop clinical nephritis with advancing age and are dead by 24 months (12 ,14 ,238).

In MRL-Fas(lpr) mice, the quantities of antibodies that are provided by the MRL/+/+ background are greatly amplified by T-cell help delivered by the $CD4^+$ cells expanded by lymphoproliferation (248 ,249 ,250 ,251 ,252 ,253 ,254 ,255 ,256 ,257 ,258), probably resulting from delayed/defective apoptosis because of the abnormal Fas gene. The most numerous cells that pack lymph nodes and spleen are not $CD4^+$; they bear the surface phenotype $CD3^+CD4^-CD8^-$ $B220^+$. They bear α/ß TCRs and therefore are part of the T-cell lineage. Presumably in normal mice the double negative cells are rapidly eliminated by apoptosis, and they accumulate in MRL-Fas(lpr) mice because of defects in this process. These mice die at 3 to 7 months of age.

Both male and female MRL-Fas(lpr) mice develop high serum levels of immunoglobulins, monoclonal paraproteins, ANAs, and immune complexes (the highest of all murine lupus strains) (12 ,14). They make IgM and IgG anti-ssDNA and anti-dsDNA, and they die from immune nephritis at a young age (90% dead by 9 months of age). Other autoantibodies in their repertoire include IgG antibodies that bind chromatin, histone, nucleosomes, nucleobindin (i.e., a DNA-binding protein), cardiolipin, erythrocyte surfaces, thyroglobulin, lymphocyte surfaces, Sm, Ul snRNP, Ro, La, Ku, Su, proteoglycans on endothelial cell membranes, neurons, ribosomal P, RNA polymerase I, C1q, and heat shock proteins (256 ,257 ,258 ,259 ,260 ,261 ,262 ,263 ,264 ,265 ,266 ,267 ,268 ,269 ,270 ,271 ,272 ,273 ,274 ,275 ,276 ,277). Additionally, they have gp70/anti-gp7O immune complexes (119). A substantial portion of MRL-Fas(lpr) mice develop IgG3 cryoglobulins, some containing rheumatoid factor activity (140 ,278 ,279). Many of these antibodies are closely related; that is, many antibodies to Sm, La, C1q, and nucleobindin also bind DNA. Anti-DNA, anti-Sm, and anti-La frequently use highly similar VH genes. The following features are found in MRL-Fas(lpr) and never, or rarely, in NZB mice and their hybrids: (a) massive lymphoproliferation, (b) inflammatory erosive polyarthritis (usually detected microscopically rather than grossly), (c) IgM rheumatoid factors, (d) severe necrotizing arteritis, and (e) antibodies to snRNP particles (12 ,14 ,238 ,257 ,269 ,270 ,271 ,272 ,273 ,280 ,281 ,282). In addition to the development of fatal nephritis, most MRL-Fas(lpr) mice develop lymphocytic infiltration of salivary glands, pancreas, peripheral muscles and nerves, uvea, and thyroid (283 ,284 ,285 ,286 ,287). In fact, they develop clinical thyroiditis with hypothyroidism, abnormal electrical transmission in muscles and nerves (suggesting clinical polymyositis and polyneuritis), learning disabilities, sensorineural hearing loss, and band keratopathy (285 ,286 ,287 ,288 ,289).

In females, anti-DNA is detectable in the circulation by 6 to 8 weeks of age, proteinuria begins at 1 to 3 months, and death associated with azotemia occurs at 3 to 6 months (12 ,14). Males lag behind females by approximately 1 month. IgG2a antibodies to DNA deposit in glomeruli, as do IgG1 and IgG3. The IgG3 cryoglobulins may be associated with either wire-loop, membranous-type lesions or with focal proliferative glomerular disease (140 ,278 ,279). The IgG anti-DNA repertoire is dominated by a public Id.H130 (217). Such dominance is reminiscent of the nephritis of BW and SNF1 mice. As for BW mice, there is some evidence that the first stimulating autoantigen is DNA linked to protein, such as chromatin or nucleosomes (274 ,275). After these antibodies mutate, specificities for other autoantigens could develop (e.g., ssDNA, dsDNA, phospholipid, Sm, La, and so on) (266 ,269 ,270). Antibodies to small nuclear ribonucleoprotein (snRNP) antigens, such as Sm, Ro, and La, occur in the MRL-Fas(lpr) and MRL/+/+ lupus-prone strains (12 ,14 ,267 ,268 ,269 ,270 ,276), and not in New Zealand strains. However, antibodies to snRNP have been found in the Palmerston North lupus-prone strain, discussed below (290). In MRL mice, antibodies to snRNP antigens are found in approximately 25% of animals. The reasons why some MRL-Fas(lpr) mice express anti-Sm and others do not is unclear; there are no demonstrable genetic or environmental factors that account for these differences (291). There may be a role for antibody specificities, however. The D epitope of Sm may contain helper epitopes that permit antibody expression, and the B epitope may contain suppressor epitopes (267). Antigen specificity for components of the polypeptides/nRNP complex is similar to the specificities of human anti-Sm. The anti-Sm response is dominated by public Ids (e.g., Y2), which can be found on human anti-Sm and on other human and murine autoantibodies (292 ,293). The ability to make anti-Sm does not correlate with clinical nephritis.

Histologic examination of the kidneys shows proliferation of mesangial and endothelial cells in glomeruli, occasional crescent formation, and basement membrane thickening, as well as interstitial infiltration by lymphocytes. IgG, C3, and anti-DNA are deposited in glomeruli; the presence of gp70 is variable and less constant than in NZB and related strains (294). Antibodies to RNA polymerase I also may contribute to nephritis (259). Renal failure is the primary cause of death.

Polyarthritis occurs in some MRL-Fas(lpr) mice with a prevalence between 15% and 25% (12 ,14 ,280 ,281). Years ago, these mice were reported to develop swelling in the hind feet and lower legs; today, most studies are done examining histology rather than observing gross evidence of inflammation (281). By 14 weeks of age, there is synovial cell proliferation with early subchondral bone destruction and marginal erosions. Cartilage is intact in this early lesion, and the synovial stroma is devoid of inflammatory cells. By 19 weeks of age, there is destruction of cartilage and subchondral bone that is associated with proliferating synovial lining cells and pannus formation. Mild inflammation occurs in synovial stroma but is remote from areas of cartilage damage. Focal arteriolitis can occur. By 25 weeks of age, the inflammatory response in synovium is more marked, but proliferating synovial lining cells continue to be present. Additionally, joint destruction has progressed to the development of periarticular fibrous scar tissue and new bone formation. The animals have rheumatoid factors and antibodies to collagen type II (12 ,14 ,280). There also is a correlation between the presence of IgM rheumatoid factor and arthritis. The rheumatoid factors in MRL-Fas(lpr) mice differ from those in MRL/+/+ and C57Bl6-lpr/lpr in that they are more likely to bind IgG2a than to bind other IgG isotypes (279 ,282). All of these features raise the possibility that MRL-Fas(lpr) mice are a model of spontaneous, genetically controlled arthritis, albeit arthritis that is relatively subtle. It is particularly fascinating that the initial destructive lesions are formed by proliferating synovium without inflammatory cells.

Acute necrotizing arteritis, primarily of coronary and renal arteries, is found in over half of MRL-Fas(lpr) males and females (12 ,14). Many have myocardial infarctions, but these seem to be more related histologically to small vessel vasculopathy than to inflammation of medium-sized arteries. The degenerative vascular disease consists of periodic acid-Schiff–positive eosinophilic deposits in the intima and media of small vessels without inflammation. Ig, C3, and occasionally gp70 can be found in the walls of medium and small arteries, venules, and arterioles.

T Cells, B Cells, Stem Cells, and the Thymus

Lymphoproliferation is the hallmark of MRL-Fas(lpr) mice. In both males and females, lymphadenopathy begins by 3 months of age (12 ,14). Nodes can reach 100 times their normal size and may develop hemorrhage and necrosis. Lymphoid malignancies are rare. Normal mouse strains onto which the Fas (lpr) gene is engrafted yield homozygotes with lymphoproliferation. Most of these develop anti-DNA, and varying proportions develop nephritis (not as universal or severe as in MRL-Fas(lpr) mice) (14 ,249). Therefore, the lpr gene encoding a defective Fas molecule with resultant diminished apoptosis creates a T-lymphocyte population in which highly autoreactive cells are not eliminated in a normal fashion. Other T cells, and probably B cells as well, also proliferate in the absence of some of the usual control mechanisms.

The development of lymphoproliferation may depend on $CD8^+$ cells, which are precursors of the double negative (DN) cells, or some DN cells may be a separate autoreactive lineage that is usually deleted in mice with normal tolerance mechanisms. MRL-Fas(lpr) mice that are treated with antibodies to CD8 or genetically engineered to fail to express CD8 or MHC class I molecules do not develop lymphoproliferation (295 ,296 ,297). The unusual $CD3^+$, $B220^+$, $CD4^-CD8^-$TCR- α/β cell that is so greatly expanded may derive from activated $CD8^+$ T cells that do not undergo the usual apoptosis following activation (244 ,247 ,298). On the other hand, the DN cell may be an independent lineage capable of certain functions. On in vivo transfer to chimeric mice, MRL DN cells did not develop into single positive cells (although the experiment did not strictly rule out the possibility) and their survival was short-lived (299). In other experiments (primarily in vitro), activation of DN cells caused them to express perforin and become cytolytic (300). However, the high levels of TGF-ß in serum of MRL-Fas(lpr) mice after immunization might suppress the cytolytic capacity of the DN cells in vivo (301). The DN T cells also may play a role in nephritis; this is the best evidence that they are active in vivo. Some T cells cloned from kidney infiltrates have the DN surface phenotype and are autoreactive and kidney-specific, proliferating to renal tubular epithelial and mesangial cells (302 ,303). When activated in vitro, they induce MHC class II and intracellular adhesion molecules (ICAM-1) on cultured tubular epithelial cells; the cytokines encoded by mRNA in the T-cell clones include IL-4, TNF-α, and IFN-γ. Tubular epithelial cells may play an important role in MRL nephritis, because they can process antigen and act as APCs (304).

The autoantibodies, vasculitis, arthritis, and Ig-induced nephritis of MRL-Fas(lpr) mice depend largely on $CD4^+$ cells. Studies of mice (a) after the administration of antibodies to CD4, (b) in which MHC class II is knocked out (thus preventing development of $CD4^+$ T cells), or (c) that lack CD4 molecules show that these disease features do not develop (251 ,295 ,296 ,305 ,306). The presence of the lpr gene causes marked expansion of $CD4^+$ cells at the same time that the DN population is increasing. In fact, T-cell help for syngeneic B cells is more marked in MRL-Fas(lpr) than in NZB or BXSB mice (14). T cells probably are not entirely incapable of undergoing apoptosis; the protein kinase C–dependent pathway for apoptosis is intact (307). The genes that are used to assemble the TCRs on MRL-Fas(lpr) cells are diverse (252). There may be some restriction in clonality at the onset of disease; TCR-Vß8 families were abundant in lymphoid or salivary glands in some studies (308). As disease progresses, however, multiple different clones are involved (252 ,309). T cells in the periphery have other abnormal features. The ability of MRL-Fas(lpr) T cells to cap, proliferate, and express IL-2 surface receptors and to

secrete IL-2 after antigenic or mitogenic stimulation is impaired (254 ,310). This may result from deficient signaling via the phosphoinositide pathway (311). There is increased tyrosine phosphorylation of p561ck in splenic T cells of MRL-Fas(lpr) mice with increased levels of intracellular polyamines (312). In lymph nodes, quantities of mRNA encoding IL-6, IL-10, and IFN-γ are increased (313), suggesting the participation of both Th1 and Th2 cells in disease. Cytokine gene therapy has been studied (314), and monthly intramuscular injection of cDNA expression vectors encoding for TGF-ß or IL-2 altered MRL-Fas(lpr) disease. TGF-ß prolonged survival, decreased autoantibodies and total IgG, and suppressed histologic damage to kidneys. IL-2 decreased survival and increased autoantibodies and IgG production.

The Fas-defective T cells of MRL-Fas(lpr) mice can be destructive in non-lpr backgrounds. When T cells are transferred to MRL/+/+ or SCID mice, graft-versus-host wasting disease occurs, probably because there is little Fas on the donor T cells to engage FasL on hepatocytes and other cells in the recipients. Therefore, the donor T cells, when activated, do not enter apoptosis but survive and mediate perforin-induced cytotoxicity of the target recipient organs (315 ,316 ,317).

There is debate regarding the role of B cells in the pathogenesis of MRL-Fas(lpr) lupus. The hyperactivation of B cells and abnormalities of pre-B stem cells that clearly are present in NZB mice, their hybrids, and BXSB mice are far less dramatic in the MRL background. However, MRL-Fas(lpr) B cells that are isolated from T cells are hyperactivated (318). They hyperrespond to stimulation with LPS or IL-1 (319 ,320 ,321), display increased quantities of IL-6 receptors on their surfaces (322), and do not undergo anergy or receptor editing (two mechanisms of B cell tolerance) as efficiently as B cells in normal mice (323). Perhaps all of these qualities reflect the importance of normal Fas/FasL interactions in B cells, or the influence of the large populations of helper T cells to which the B cells are exposed. There is restricted B cell clonality to several autoantigens, such as rheumatoid factor that binds IgG2a, but this is similar to the situation in both BW and normal mice making antibody responses after stimulation by specific antigens (324). In MRL-Fas(lpr) mice the contribution of the MRL background apparently provides B cells with appropriate antibody repertoires to cause autoimmunity.

Stem cells in these mice may be less abnormal than stem cells in other SLE mouse models. One group has reported significant delay in disease onset after syngeneic bone marrow transplantation (325). MRL-Fas(lpr) mice underwent immunoablation with high-dose cyclophosphamide and then received syngeneic bone marrow that was depleted of Thy1.2 cells. Mean survival was 350 days, compared with 197 days in untreated controls, and lymphadenopathy did not develop. This is a curious finding, because all background genes, as well as the lpr gene, would be transferred with the marrow. It suggests that removing T cells can reset the thermostat for autoimmunity, and many weeks are required for disease to begin again.

The thymus is structurally abnormal in MRL-Fas(lpr) mice, as it is in all strains that develop spontaneous SLE (326). Thymic cortical atrophy is severe and medullary hyperplasia common, as in NZB and BW mice (62 ,63). The numbers of epithelial cells in the subcapsular and medullary regions are decreased, and there are cortical holes in which no epithelial cells can be seen. Total cortical thymocytes are decreased in number. Levels of DN cells are high, while levels of single-positive cells are low, thus suggesting the inability of activated DN cells to undergo apoptosis. Studies with superantigens have suggested that early intrathymic deletion of autoreactive T cells is normal in MRL-Fas(lpr) mice (327 ,328), but that this may be impaired at older ages (329 ,330). Both thymic and peripheral deletion mechanisms for T cells likely are affected profoundly by the defect in apoptosis, which eliminates highly autoreactive activated T cells from the repertoire (244 ,245 ,246 ,247 ,329 ,330). In fact, SLE in MRL-Fas(lpr) mice may be more thymus dependent than in other strains. Thymectomy of newborn MRL-Fas(lpr) mice prevents development of lymphoproliferation and autoimmune disease (13 ,14 ,331), and MRL-Fas(lpr) thymus engrafted into MRL/+/+ mice causes lymphoproliferation and early death from autoimmune nephritis (14).

Abnormal cell functions also extend to populations other than lymphocytes. Neutrophils from MRL-Fas(lpr) (but not MRL/+/+) mice have a marked defect in Fc-receptor–mediated phagocytosis, which develops at the time of onset of autoimmune disease; this may result from elevated levels of TGF-ß in the serum. Their ability to access areas of inflammation also may be impaired (332). Macrophages make abnormally small quantities of IL-1 (188 ,333), and immune complexes are not cleared as efficiently as in normal mice (334).

Genetics

The role of the lpr gene (and of the defective Fas molecule it encodes) as a disease accelerator is fairly well understood. The lpr allele on chromosome 19 is a mutation in the Fas gene resulting from an early retroviral transposon insertion in the intron between exons 2 and 3, which results in abnormal RNA splicing with a frame shift and premature termination of the mRNA (239 ,240 ,241 ,242 ,243). Mice that are homozygous for lpr express very small amounts of Fas on their cell surfaces. Normal mice express high levels of Fas on activated T and B lymphocytes and on $CD4^+CD8^+$ thymocytes, and lower levels on proliferating cells in the thymus, gut, skin, heart, liver, and ovary. Fas in concert with the FasL transduces signals, which usually results in stimulation via activation of protein tyrosine kinase, which phosphorylates a nuclear RNA-binding protein TIA-1, a ceramide-mediated apoptosis pathway, IL-1–converting enzyme (ICE), cysteine proteases, and protein tyrosine phosphatase 1C gene (PTP1c) to promote apoptosis. On the other hand, Fas/FasL interactions can activate the Abl kinase to inhibit apoptosis (335 ,336 ,337 ,338 ,339 ,340 ,341 ,342 ,343 ,344 ,345).

Presumably, mice that are homozygous for lpr are unable to delete highly autoreactive T (and possibly B) cells in the periphery, which accounts in part for their high production of multiple autoantibodies. Activation-induced cell death (AICD) has been shown to depend in part on Fas/FasL interactions in

$CD8^+$, Th0, and Th1 $CD4^+$ T cells, and B cells (346 ,347 ,348). Lpr B cells resist apoptosis (349). Fas/FasL also is essential for killing by $CD4^+$ Th1 cells and is one of two pathways that are used by $CD8^+$ cytotoxic lymphocytes (350 ,351). Anergic autoreactive B cells are normally eliminated by $CD4^+$ T cells; in the absence of normal Fas/FasL interactions, the B cells are activated rather than killed (352). The genetic defect in Fas also accounts for the accumulation of $B220^+$, $CD4^-CD8^-$, and TCR^+ T cells that cause the massive lymphadenopathy associated with lpr/lpr. MHC class II knockout MRL-Fas(lpr) mice that lack $CD4^+$ cells do not develop SLE despite massive lymphadenopathy (305). MRL-Fas(lpr) mice that are transgenic for the gene encoding normal Fas molecules do not exhibit the acceleration of disease characteristic of the wild strain (353). The introduction of Fas(lpr) into any mouse strain results in lymphadenopathy of various degrees and production of autoantibodies; only strains that are genetically susceptible to SLE develop high-titer autoantibodies and full-blown clinical autoimmune disease.

MRL+/+ background genes are essential for the development of full-blown SLE. As in NZB and NZB hybrid mice, backcross studies have shown that the abilities to secrete large quantities of immunoglobulin and to make several different autoantibodies segregate independently of each other. More recent analysis of the MRL-Fas(lpr) mouse genome by microsatellite methods has identified two regions that associate with nephritis, one on chromosome 7, and one on chromosome 12. Interestingly, no linkage was found with the region on chromosome 17 that encodes the MHC (240). This is different from other murine models of spontaneous SLE. Genome scanning of MRL-Fas(lpr) and C57B1/6-Fas (lpr) mice (354) showed that lymphadenopathy and splenomegaly are linked to regions on chromosomes 4, 5, 7, and 10, designated Lmb l–4. Lmb l, 2, and 3 were also linked to anti-DNA but not nephritis; in contrast, Lmb4 was linked to nephritis. Lmb 1 was derived from the C57B1 background; Lmb 2, 3, and 4 were from MRL. These loci appeared to be additive. At the time of this writing, the single gene(s) within these chromosomal regions have not been identified.

Summary

MRL-Fas(lpr) mice are particularly interesting as a model of the accelerating factor for autoimmunity that can be provided by a single gene being added to a susceptible host. The massive lymphoproliferation that is associated with the autosomal-recessive lpr gene almost surely results from defective apoptosis. The resultant expansion in $CD4^+$ T cells drives predisposed MRL B cells to make the largest array of autoantibodies that occurs in murine lupus. The production of pathogenic autoantibodies and the presence of cytolytic DN cells and of $CD4^+$ T cells in target organs such as kidneys and salivary glands result in accelerated autoimmunity and early death from lupus-like nephritis. Some MRL-Fas(lpr) mice develop destructive polyarthritis, which often is associated with IgM rheumatoid factors. MRL mice are the only strains that spontaneously make anti-Sm. They also develop vasculitis, which can be severe.

BXSB Mice

The BXSB strain was developed by Murphy and Roths (355 ,356). BXSB is a recombinant inbred (RI) strain; RI mice are derived by brother/sister matings within each generation, usually extending for 12 to 20 generations. The RI technique is used to produce strains with high frequencies of homozygosity at many loci to see the expression of recessive genes. The initial mating was between a C57BI/6 (B6) female and a satin beige (SB/Le male), hence the designation BXSB.

The unique features of BXSB mice are that disease is much worse in males than in females, and the disease-accelerating gene that is responsible for this difference is located on the Y chromosome. The gene is called *Yaa,* for Y chromosome-linked autoimmunity accelerator. The female BXSB mice develop late-life lupus; therefore, additional genes contribute to disease, as in all other models of spontaneous lupus studied to date (Table 18-6).

Clinical Manifestations and Autoantibodies

BXSB mice make an autoantibodies repertoire that includes IgG antibodies to ssDNA and dsDNA, chromatin, C1q, ANA, and antibodies that are directed against brain cells (12 ,14 ,357 ,358). Additionally, a small proportion make antierythrocyte, NTA, monoclonal paraproteins, and gp70anti-gp70 immune complexes (12 ,14). By an early age (3 months), they have elevated levels of circulating immune complexes and hypocomplementemia (12). Serum levels of C4 diminish as clinical disease appears (359).

Death is caused by immune glomerulonephritis (12 ,14 ,358). Histologically, the disease is more exudative than in other mouse models. That is, there are neutrophils invading glomeruli along with IgG and C3 deposition, proliferative changes in mesangia and endothelial cells, and basement membrane thickening (12). The progression from nephritis to death is rapid, with 50% of males dead by 5 months of age (12 ,14 ,358 ,359).

T Cells, B Cells, Stem Cells, and the Thymus

Lymphoproliferation occurs in BXSB mice; it is more marked than in BW but less dramatic than in MRL-Fas(lpr) (12 ,14). In contrast to MRL-Fas(lpr) mice, the hyperplastic nodes contain predominantly B cells (13 ,14), and for some time it was thought that B cell defects were the primary abnormality in BXSB mice. As in the other models, B cells are hyperactivated, higher portions are mature (expressing IgD and IgM on their surfaces), higher proportions display CD40L on their surface, and secretion of IgG and IgM is increased (13 ,14 ,360 ,361). The B cells are resistant to tolerance with human gamma globulin; the resistance is a property of the B cell itself and does not reflect abnormalities in APCs or T cells (362). Studies in Yaa^+Yaa-double bone marrow chimeric mice show that Yaa^- T cells can activate Yaa^+ B cells to make autoantibodies (or increased antibodies to foreign antigens), but Yaa^+ T cells cannot drive Yaa- B cells to make autoantibodies (363 ,364).

This probably indicates that Yaa+ B cells present antigen to T cells and the two cells cross-activate each other. However, BXSB T cells play an important role in disease (365 ,366) by providing help for autoantibodies formation. As mice age, they develop the typical T-cell defects of SLE mice (i.e., abnormally low proliferative responses to antigens/mitogens, reduced production of IL-2). Elimination of CD4+ T cells (but not CD8+) suppresses autoantibodies, monocytosis, and nephritis (366). Production of mixed chimerics in BXSB mice created by lethal irradiation followed by transfer of bone marrow from nonautoimmune BALB/c mice plus congenic marrow depleted of T cells prolongs survival, prevents nephritis, and restores normal primary immune responses (which are abnormal in mice receiving only allogeneic cells). Depletion of BXSB T cells is essential to the success of this approach (367). In sum, it is clear that T cells are required for development of full-blown disease. Disease is delayed by the prevention of second signal-mediated T-cell activation after administration of CTLA41g (368). As BXSB males age, their T cells acquire a memory phenotype and secrete lymphokines that are characteristic of both Th1 and Th2 cells (369 ,370). Recent experiments showed that BXSB disease is not altered in mice deficient in IL-4 (371).

Table 18-6: Characteristics of BXSB Mice

A. Clinical
1. Males die early of lupus (50% mortality at 5 months; 90% at 8 months)
2. Females have late-onset lupus (50% mortality at 15 months; 90% at 24 months)
3. Major cause of death is immune glomerulonephritis

B. Histologic
1. Males show severe acute to subacute glomerulonephritis, with proliferation and exudation of neutrophils into glomeruli
2. In males, IgG and C3 deposit in mesangium and glomerular capillary walls by 3 months of age; deposits in tubular basement membranes and interstitium also occur
3. Lymph node hyperplasia (10–20 times normal size) in males
4. Myocardial infarcts in 25%, without arteritis
5. Thymic cortical atrophy with medullary hyperplasia; thymic epithelial cells contain crystalline inclusions

C. Autoantibodies
1. All males develop ANAs and IgG anti-dsDNA and anti-ssDNA
2. Less than one-half of males develop monoclonal paraproteins, antierythrocyte antibodies, gp70–anti-gp70, and thymocytotoxic antibodies
3. Hypocomplementemia in males by 3 months of age; low C4 levels
4. Elevated levels of circulating immune complexes
5. Defective monocyte/macrophages

D. Immune abnormalities
1. B cell is the most frequent cell in hyperplastic lymph nodes
2. B cell hyperactivation and advanced maturity
3. B cells are resistant to tolerance with some antigens
4. Male bone marrow transferred to female BXSB mice produces accelerated disease; female marrow confers late lupus when transferred to males; mature male B cells do not accelerate disease; abnormality is contained in marrow stem cells
5. Monocytosis occurs
6. Elimination of CD4+ T cells diminishes anti-DNA, monocytosis, nephritis, and mortality
7. Disease is not influenced substantially by thymectomy
8. Disease is not influenced substantially by sex hormone therapies and/or castration
9. Defective Fc-mediated immune complex clearance

E. Genetics
1. A single gene that accelerates disease, Yaa, is present on the Y chromosome; Yaa is probably overexpression or duplication of the Tlr 7 gene
2. Additional genes that behave as X-linked recessives confer susceptibility to disease; they may account for late-life SLE in females

The thymus shows cortical atrophy and defects similar to those in other SLE strains (326 ,372). Crystalline structures have been described in the thymic epithelial cells of BXSB males; they are thought to represent abnormal storage of thymic hormones (373). Apoptosis of thymic cells is delayed in all SLE strains studied, including BXSB. Thymectomy has accelerated disease in some studies and has not altered it in others (13 ,374). The effects are not as consistent and dramatic as the protection from disease that is conferred by thymectomy in MRL-Fas(lpr) mice(13 ,14 ,331).

An additional feature of BXSB mice is monocytosis. By 2 weeks of age, BXSB males have increased numbers of monocyte colony-forming units in spleen and lymph nodes (375). Further, the monocytes/macrophages are abnormal; they make unusually large quantities of procoagulants, which might contribute to the rapid damage of glomeruli that characterizes lupus in this strain (376).

Studies of lymph nodes show dramatic increases in mRNA for IL-1, with some increase in IL-10 and TGF-ß, all of which probably come from monocytes. IFN-γ also is increased, suggesting simultaneous increase in monocyte/macrophage and T-cell activity (313).

There is good evidence that a stem cell abnormality is crucial to the development of disease in BXSB mice (14 ,377), because male BXSB bone marrow can transfer disease and normal marrow grafted into male BXSB mice can prevent disease (14 ,377 ,378 ,379). This stem cell defect may lead to a single abnormality that affects both B and T cells, or there may be multiple genes influencing multiple responses leading to hyperactivity in each type of lymphocyte and in monocytes.

Manipulations such as castration and androgen therapy do not dramatically alter outcome (13 ,14 ,380), in contrast to mice with New Zealand backgrounds.

Genetics

Multiple genes predispose to SLE in BXSB mice, as in the other models. There is an inherent tendency toward autoimmune disease in BXSB mice of both sexes; that tendency is dramatically accelerated by the introduction of the *Yaa* gene on the Y chromosome. This accounts for the earlier, more severe disease in males. If normal mice are generated that bear the *SLE1* gene

from NZW mice (a gene associated with ability to break tolerance to nucleosomes) and the *Yaa* gene, fatal autoimmune nephritis occurs (87). *Yaa* has recently been shown to be overexpression (or duplication) of the *TLR7* gene (703 ,706). The *Yaa* gene alone is not sufficient to permit the development of autoimmunity: MHC and other genes play important roles (381 ,382 ,383 ,706). The *Yaa* gene represents a duplication of the *TLR7* gene because of a 4-megabase expansion of the pseudoautosomal region (703). This results in increased expression of TLR7, a single-stranded RNA-binding innate immune receptor, which appears to cause an intrinsic bias of Yaa-containing B cells toward nucleolar antigens. Thus, the Yaa locus represents an example in which qualitative phenotypic differences in disease pathology are derived from a copy number polymorphism, a genetic event that has been shown to be common both in mice and humans (704). Mice of the H-2b haplotype (BXSB is H-2b) do not express MHC class III-E molecules; introduction of the I-Eα chain into BXSB males permits the mice to display I-E on cell surfaces and prevents disease (384 ,385 ,386). This effect is controlled by MHC and occurs only in mouse strains with “permissive” MHC such as H-2b (387). The I-A molecule in the transgenic mice contains peptides from I-E, and it is possible that those peptides prevent the presentation of other peptides that induce and sustain pathogenic autoantibodies production (387). Susceptibility to autoimmunity is transmitted as an autosomal dominant trait in some F1 hybrids that are derived from BXSB (381 ,382 ,383), and in others susceptibility behaves as if it were controlled by autosomal-recessive genes (14).

Results of genome scans of backcrosses between BXSB and B10 mice have been published (388 ,389). Three to four regions on chromosome 1 and a region on chromosome 3 are linked to nephritis. One publication reports linkage with a region on chromosome 13, the other with regions on chromosomes 4 and 10. The regions on 1 that are telomeric have been linked to SLE in other strains; the other regions may be unique to BXSB.

Summary

In summary, BXSB mice are unique in that lupus nephritis is more severe and occurs earlier in males than in females; this is from the accelerating effect of a single gene, Yaa, which excludes enhanced expression of TLR7 which is located on the Y chromosome. Disease develops rapidly in BXSB males, with 50% dead of immune glomerulonephritis by 5 months of age.

B cells, T cells, and monocytes all have abnormal functions, most of which suggest hyperactivation. The autoantibodies repertoire is directed primarily against nucleosomal and DNA antigens. Multiple genes participate, some of which are shared in other lupus mouse strains and some of which are probably unique to the BXSB background.

The BXD2 (C57BL/6J × DBA2J) Model of Spontaneous Erosive Arthritis and Glomerulonephritis (“Rhupus”)

The BXD2 strain of mice is one of approximately 80 BXD recombinant inbred (RI) mouse strains that were generated originally by Dr. Benjamin A. Taylor at the Jackson Laboratory (Bar Harbor, Me, USA) by inbreeding the intercross progeny of a cross between C57BL/6J and DBA/2J strains for more than 20 generations (390). During the course of a survey to discover genetic loci that influence T-cell senescence by using a set of 20 of the BXD RI strains, Mountz and colleagues observed the development of spontaneous arthritis in the BXD2 strain in specific pathogen-free conditions (391). The adult mice spontaneously develop generalized autoimmune disease, including glomerulonephritis (GN), increased serum titres of rheumatoid factor (RF) and anti-DNA antibody, and a spontaneous erosive arthritis characterized by mononuclear cell infiltration, synovial hyperplasia, and bone and cartilage erosion. The arthritis affects 50% of female mice by 8 months and 90% at ages >12 months. There is a female predominance, as the incidence of arthritis in male mice is lower than that in female mice. The mice also exhibit splenomegaly, but lymphadenopathy is not a pronounced feature.

The features of lupus and arthritis developed by the BXD2 mice segregate in F2 mice generated by crossing BXD2 mice with the parental B6 and D2 strains. Genetic linkage analysis of the serum levels of anti-DNA and RF by using the BXD RI strains shows that the serum titers of anti-DNA and RF were influenced by a genetic locus on mouse chromosome (Chr) 2 near the marker D2Mit412 (78 cm, 163 Mb) and on Chr 4 near D4Mit146 (53.6 cm, 109 Mb), respectively. Both loci are close to the B cell hyperactivity, lupus or GN susceptibility loci that have been identified previously. Thus, the BXD2 strain of mice is a novel polygenic model for complex autoimmune disease that spontaneously develops both generalized autoimmune disease, including renal disease, and chronic erosive arthritis (391).

The (NZW × BXSB)F1 Model of Antiphospholipid Syndrome and Coronary Artery Disease

Disease Characteristics and Autoantibodies

Male hybrid (NZW × BXSB)F1 mice have been particularly interesting as models of autoimmunity linked to accelerated degenerative coronary artery disease, a combination seen in some patients with SLE. In these mice, 50% of the males are dead by 24 weeks of age, usually with extensive MI, with occlusive disease and intimal thickening in small coronary arteries but not extramyocardial coronary arteries. These mice also develop high serum levels of anti-DNA and immune complexes, with antibodies against both platelets and phospholipids. Some monoclonal anticardiolipin antibodies also bind platelets and DNA, similar to some antibodies from BW mice. Most of the antiphospholipids bind β_2-glycoprotein I; such subsets may be more likely to be associated with clotting than subsets without that characteristic (392). The males also develop glomerulonephritis, hypertension, leukocytosis, gastrointestinal vasculitis, and thrombocytopenia (392 ,393).

Abnormalities in Stem Cells, T Cells, and B Cells

All these cells are abnormal, as in BXSB parents. Serum levels of IFN-γ and IL-10 increase as mice age. Treatment with antibodies to CD4 delays disease, whereas treatment with antibodies to CD8 accelerates it (394). Lethal irradiation of (NZW × BXSB)F1 mice followed by transfer of bone marrow from normal C57B1 mice prevents nephritis, coronary artery disease, and thrombocytopenia, suggesting that all of these manifestations result from immune and inflammatory processes (394). On the other hand, treatment with the calcium channel blocker ticlodipine prolongs survival and lowers the prevalence of myocardial infarction without affecting nephritis (395). Similarly, treatment with nifedipine lowers blood pressure and prolongs survival, protects partially from coronary artery stenosis and myocardial infarction, and reduces the amount of histologic nephritis (396). Therefore, the final expression of disease has immune, inflammatory, and degenerative components.

Genetics

One genome scan has shown linkage between various disease features and different chromosomal regions. Antibodies to cardiolipin, platelet-binding antibodies, thrombocytopenia, and myocardial infarction were each controlled by independently segregating dominant alleles. Regions on chromosomes 4 and 17 are linked to anticardiolipin, on chromosomes 8 and 17 to antiplatelet antibodies and thrombocytopenia, and on chromosomes 7 and 14 to MI (397). This suggests that there is not a simple direct association between antiphospholipid and myocardial infarct or thrombocytopenia; the antibodies and disease expression have complex genetic requirements.

Gld/Gld Mice with Absence of functional Fas Ligand (FasL)

In 1984, Roths et al. (398) reported a spontaneous autosomal-recessive mutation that occurred in the inbred mouse strain C3H/HeJ, which they called gld (for generalized lymphoproliferative disorder). It now is known that the mutation is a single base change in the C-terminal extracellular domain of the FasL molecule, which is encoded on mouse chromosome 6 (399 ,400 ,401); functional FasL molecules are not generated. FasL is expressed on cell surfaces, but the mutation interferes with its ability to bind Fas. Therefore, apoptosis does not proceed normally, highly autoreactive T and B cells persist instead of dying, and SLE results.

FasL plays a major role in apoptosis. Clinically, C3H/gld/gld mice of both sexes develop lymphadenopathy and splenomegaly by 13 weeks of age. Lymphoid organs contain increased numbers of B, T, and DN lymphocytes. The $B220^{+}CD4^{-}CD8^{-}TCR^{+}$ T cell that expands so dramatically in MRL-Fas(lpr) mice probably is identical to the major expanded population in gld/gld mice, since both strains have major defects in apoptosis mediated by Fas/FasL interactions. Recent evidence suggests that these DN cells require MHC class I expression for expansion, and contain populations with high avidity for self antigens such as endogenous retroviral superantigens; such a dangerous population is deleted in normal mice (402). C3H/Gld/gld mice have a shortened life span compared with wild-type C3H/HeJ, with male C3H/gld/gld mice living a mean of 396 days and females 368 days, compared with 688 days in females that are not homozygous for gld. Lymphoid cells and macrophages infiltrate the interstitium of lungs extensively, but other organs rarely are involved. Vasculitis does not occur. Most of these mice do not develop histologic lupus nephritis, although all mice older than 22 weeks have immunoglobulin deposits in glomeruli (primarily confined to the mesangium). By that age, serum levels of gamma globulin are approximately five times normal; this increase occurs in all isotypes but is most dramatic in IgA and IgG2b. ANAs begin to appear at 8 weeks of age, and all C3H/gld/gld mice are positive by 16 weeks. By 20 weeks, all have antibodies to thymocytes and antibodies to dsDNA (398 ,403). The primary cause of early mortality probably is the pulmonary disease. In C3H/gld/gld and BALB/gld/gld mice that live to 1 year of age, B cell malignancies are common (usually $CD5^{+}$ malignant plasmacytoid lymphomas) (404). As in other lupus models, genetic backgrounds in addition to the single point mutation determine the extent of disease: B6/gld/gld mice have milder disease than C3H/gld/gld.

The gld mutation has greatly increased our understanding of the importance of Fas/FasL interactions and of apoptosis in maintaining normal immune homeostasis. For example, lethally irradiated mice reconstituted with stem cells from Fas-deficient MRL/lpr mice develop chronic graft-versus-host disease (GVHD), but stem cells deficient in both Fas and FasL do not produce GVHD, showing that FasL is an important effector in this syndrome. Interestingly, these double-deficient T cells can induce normal B cells to produce autoantibodies (405). In pristane-induced murine lupus, lpr and gld mutations affect some autoantibody production but not others, suggesting that autoantibodies differ in their dependence on Fas and FasL expression by T cells (406). B6/gld/gld mice can clear cytomegalovirus after infection, but they cannot downregulate the resultant inflammatory responses (407). Nonobese diabetic (NOD) mice spontaneously develop autoimmune diabetes resulting from immune destruction of pancreatic ß cells. NOD/gld/gld mice are protected from disease, showing the dependence of the process on FasL-mediated apoptosis (408). Interestingly, lupus-like disease in C3H/gld/gld mice also requires TNF-α; mice deficient in that cytokine or treated with antibodies to TNF-α have milder disease (409). C3H/gld/gld disease can be prevented by lethal irradiation followed by reconstitution with a mixture of normal and gld bone marrow, as long as the normal marrow is not depleted of $Thy1^{+}$ cells, suggesting that T cells expressing FasL can correct the gld defect; $CD8^{+}$ $FasL^{+}$ cells are primarily responsible for suppression of lymphoproliferation (410).

In summary, autoiminune–permissive strains with defective production of FasL develop lymphoproliferation,

autoantibodies, and infiltration of organs with lymphocytes that cannot be deleted normally. Their disease has similarities to human SLE, as does disease associated with production of a defective Fas molecule in lpr-bearing strains.

Palmerston North Mice (PN)

PN mice are descendants of albino mice that were purchased from a pet shop in 1948 and raised at the Palmerston North Hospital in New Zealand. Inbreeding began in 1964, with animals being selected for ANA positivity. Autoantibodies and nephritis were characterized by Walker et al. (411) in 1978.

Clinical Characteristics and Autoantibodies

Fifty-percent survivals are 11 months for females and 15 months for males. The mice develop two main pathologic lesions: (a) necrotizing vasculitis of small and medium arteries; and (b) proliferative glomerulonephritis with fibrinoid necrosis, crescent formation, and glomerular deposits of IgG and C3. Arteritis occurs in spleen, thymus, kidneys, ovaries, and lungs, with sparing of the aorta. Lymph nodes are hyperplastic in some mice, and malignant lymphoma occurs. Thymic cortical atrophy occurs late (at approximately 11 months).

Anti-DNA and ANAs may be present at birth in some PN mice, and both increase with age until most animals are positive. By 1 month of age, all female PN mice have IgM anti-dsDNA and ssDNA, and the majority also produce IgM antibodies to cardiolipin. By 3 months of age, approximately 90% have IgG anti-ssDNa and -dsDNA. By 6 to 12 months, all females have IgG antibodies to cardiolipin and other phospholipids and the majority have IgG antibodies to erythrocytes (411). LE cells have been reported. As the mice age, the proliferative responses of their T cells tends to diminish, as in other lupus strains (412).

The most remarkable finding in PN mice is vasculitis. A recent report detailed the types of cells and cytokines in perivascular and vascular infiltrates. Perivasculitis dominated in arteries and veins in kidney, liver, brain, and lung; vasculitis dominated in veins and venules. The infiltrates were composed mainly of an unusual cell type with T-cell and B cell markers, in addition to $CD4^+$ expression. The predominant cytokines in lesions were IL-4, IL-6, and IL-10 with little to no IL-2, IFN-γ, TGF-β, or TNF-α. Therefore, these T-cell populations are mostly Th2 with little participation of monocytes/macrophages at the time the lesions are full-blown (413).

In summary, the PN mouse is a model of spontaneous autoantibodies, including antibodies to phospholipid, with impressive vasculitis and glomerulonephritis. The genetics are not yet well understood.

“Flaky Skin” (fsn) Mutant Mice

The autosomal recessive mutation “flaky skin” (fsn) causes pleiotropic abnormalities in the immune and hematopoietic systems accompanied by psoriasiform dermatitis, anemia, anti-dsDNA autoantibodies, glomerulonephritis accompanied by immune complex deposition in the kidneys, elevated IL-4 production by spleen cells, increased serum IL-4, and hyper-IgE (414 ,415). Additional systemic lesions include splenomegaly, granulomatous lymphadenitis, mixed inflammatory cell infiltrates and fibrosis around portal triads in the liver, progressive and massive papillomatosis of the stratified squamous epithelium of the forestomach, hyperplasia and dysplasia of the glandular stomach, increased apoptosis of cecal enterocytes and testicular degeneration (415).

The fsn mutation that was mapped to chromosome 17 (416) has recently been identified as a mutation in the tetratricopeptide repeat domain 7 (Ttc7) gene (417). This mutation is due to the insertion of an endogenous retrovirus (early transposon class) into intron 14 of the Ttc7 gene. The insertion leads to the reduced levels of wild-type Ttc7 transcripts in fsn mice and the insertion of an additional exon derived from the retrovirus into the majority of Ttc7 mRNAs. The Ttc7 is expressed in multiple types of tissue including skin, kidney, spleen, and thymus, but is most abundant in germinal center B cells and hematopoietic stem cells, suggesting an important role in the development of immune system cells.

Induction of Lupus in Normal Mouse Strains

In the previously discussed models of spontaneous SLE, multiple genetic factors likely provide the major if not the only important risk factors. Mutations in Fas (Fas-lpr) and FasL (gld) accelerate autoimmunity in these susceptible strains. However, there are several examples of the induction of SLE-like disease in mice that are otherwise healthy, with genetic backgrounds that do not predispose to autoimmunity. These include (a) induction of chronic GVHD; (b) alteration of expression of single molecules (either upregulation via transgene insertion or deletion in knockout mice); (c) transfer of pathogenic autoantibodies or the B cells that secrete them; (d) forced expression of pathogenic autoantibodies via the introduction of transgenes; (e) activation of idiotypic networks that result in the production of pathogenic autoantibodies; (f) inoculations of DNA, DNA/protein, other autoreactive proteins or oligopeptides, and (g) injections of hydrocarbon oils such as pristane. In most of these models, some strains of mice are more susceptible than others, again suggesting that most if not all murine genetic backgrounds contain genes that permit autoimmunity.

Chronic GVHD

GVHD is produced in mice by injecting lymphocytes from a parent into an F1 hybrid differing at one MHC locus from that parent. It is caused by T cells recognizing foreign MHC antigens (418). Acute GVHD is runting disease with failure to thrive, diarrhea, wasting, and early death (419). T cells that are defective in Fas expression (caused by the lpr gene) can cause acute GVHD in recipients with identical MHC class II

molecules, because they do not undergo apoptosis after activation (420). Acute GVHD is not lupus-like. If lymphocytes are injected after the recipient F1 animal has reached at least 6 weeks of age and if certain H-2 gene interactions occur between parent and F1, chronic GVHD results. Chronic GVHD resembles SLE (418 ,419 ,421 ,422 ,423 ,424). Several IgG autoantibodies are made, including anti-dsDNA, anti-ssDNA, and antihistone (418 ,421 ,424). In some combinations, fatal lupus-like nephritis mediated by the IgG anti-DNA occurs.

$CD4^+$ effector cells provided by the donor are required for induction of chronic GVHD (418 ,419); they must be activated by appropriate MHC class II gene products on the surface of the recipient cells (422 ,423). One combination that results in fatal nephritis of chronic GVHD is H-2d donor lymphocytes into an H-2b recipient (423 ,424). In contrast, most recipient H2k haplotypes are resistant. The development of clinical nephritis and of autoantibodies can be separated. Many parental hybrid combinations result in the ability of the recipient to make high-titer IgG anti-DNA, but class II genes I-A and I-E (equivalent to human HLA class II DR and DQ) must contain a susceptible haplotype, such as b, for severe nephritis to result (424). Animals without nephritis confine renal deposits of IgG to mesangial regions of glomeruli; animals with nephritis have IgG deposits along the capillary loops (424).

This model provides an excellent example of lupus nephritis resulting from interactions between $CD4^+$ T-helper cells, $CD8^+$ cytotoxic/suppressor cells, and APCs of a host that differs from the donor at MHC. Disease is initiated by donor $CD4^+$ cells activated by host APC to secrete IL-4, and B cell stimulation with autoantibodies production begins. Ability of the host to mount $CD8^+$ cells that kill the B cells determines whether acute or chronic GVHD will occur. Both acute and chronic GVHD may begin as a Th2 cytokine-mediated B cell stimulation; transition to acute GVHD depends on the education in the host thymus of donor-derived pro-T and pre-T cells to develop into double-positive cells and ultimately $CD8^+$ T cells that terminate the B cell hyperactivity by eliminating activated B cells (both perforin-mediated cytotoxicity and Fas/FasL killing occur). Activation of $CD8^+$ T is promoted by IFN-γ secretion by donor $CD4^+$ T cells. If thymic education does not occur, IL-4 secretion continues and B cells are not downregulated; sustained autoantibody production and lupus-like chronic GVHD result (425 ,426 ,427 ,428). Mice with severe chronic GVHD usually have high levels of IgE and IgG1 in their serum, confirming the important role of Th2 cells in disease. Antibodies to IL-4, or infusion of soluble IL-4 receptor, prevents or suppresses disease (429).

Genetic Alteration of Expression of Single Molecules: Increased Expression in Transgenic Mice

See Chapter 7 for a detailed discussion of genes in murine lupus. In Table 18-7 , various gene-targeted strains are grouped according to potential mechanisms by which over-expression or deficiency of certain genes causes humoral autoimmunity and lupus-like disease. Studies in which single genes are overexpressed in transgenic mice, or single genes are deleted in knockout mice, have all suggested that strategies that permit extended lifetimes for autoreactive lymphocytes or for autoantigens promote the development of SLE-like disease in normal mice. For example, overexpression of bcl-2 (which protects cells from apoptotic death) in normal mice transgenic for that molecule causes them to develop mild autoimmunity (430). Bcl-2 transgenic C57B1/6-lpr mice have lymphadenopathy but no abnormal autoantibodies (431). In C57B1/6-lpr mice transgenic for Pim-l (a cytoplasmic serine/threonine protein kinase that also inhibits apoptosis), lymphoproliferation resulting from the accumulation of $B220^+$ T cells also occurs (432). A molecule that stimulates the growth of B lymphocytes, which is variously named BlyS (B lymphocyte stimulator), BAFF (B cell activating factor belonging to the TNF-α family), THANK (TNF-α homologue that activates apoptosis, nuclear factor [NFI]-xB, c-Jun NH2-terminal kinase), TALL-1 (TNF-α and apoptosis ligand-related leukocyte-expressed ligand 1), and zTNF4, can induce autoimmunity when overexpressed. BLyS is a monocyte-specific TNF-α family cytokine that is a potent costimulator with anti-immunoglobulin M in vitro and with CD40L in vivo for B cell proliferation. BLyS enhances the humoral responses to T-cell–independent and T-cell–dependent antigens by protecting antigen-activated B cells from apoptosis (433 ,434 ,435).

Genetic Alteration of Expression of Single Molecules: Deleted Molecules in Knockout Mice

Table 18-7 summarizes various gene knockout strains that develop lupus-like disease. Two general categories of single gene deletion have led to generation of lupus-like disease in otherwise healthy mice: (a) removal of genes that downregulate accumulation and/or activation of B or T lymphocytes; and (b) deletion of genes that regulate normal degradation and clearing of DNA, immune complexes, or apoptotic cells and bodies. In the first category, normal mice with deletion of Lyn have a marked increase in IgM-secreting B cells and develop high levels of immune complexes and anti-DNA, along with a glomerulonephritis similar to SLE (436 ,437). Lyn is a Src protein tyrosine kinase associated with the BCR that participates in an inhibitory signal after BCR activation; Lyn phosphorylates the BCR coreceptor CD22, a process that recruits the tyrosine phosphatase SHP-1 to the BCR/CD22 complex and controls B cell activation. In the absence of Lyn, B cells exhibit spontaneous hyperreactivity, which doubtless contributes to their lupus-like phenotype (438). Motheaten mice (so called because of patchy alopecia) have spontaneous deletion of a single residue in the N-terminal SH2 domain of the protein tyrosine phosphatase 1C gene (PTP1c). PTP1c activity is absent, which may remove an inhibitory signal for the activation of Lyn and Syk, with resultant B cell

hyperactivation. IgM levels are high, B-1 B cells are abnormally activated, and high-titer ANAs develop, with immune complex deposition in many tissues (439 ,440). On the T-cell side, deletion of PD-1, an immunoglobulin superfamily member bearing an immunoreceptor tyrosine-based inhibitory motif (ITIM) that affects primarily $CD4^-CD8^-$ thymocytes also results in lupus-like disease (441). Similarly, expression of the cell-cycle regulator p21 prevents accumulation of $CD4^+$ memory cells; deletion of that molecule in normal mice results in loss of tolerance for nuclear antigens. Interestingly, female mice with a p21 deletion are particularly prone to develop SLE; they develop IgG antibodies to dsDNA, lymphadenopathy, Ig-mediated glomerulonephritis, and shortened survival (442).

Table 18-7: Gene Targeted Strains with Lupus-Like Phenotypes

Potential Mechanism	Targeted Genes*	References
Impaired apoptosis and cell cycle	Bcl-2Tg, Bim, CD95DIT, CDK, E2F2, Fas, Fas ligand, GADD45, IEX-1Tg, PtenHet, PtenT cell-CKO	(6,7,694,695)
Defective clearance of DNA, apoptotic cells, and immune complexes	C1q, C4, DNase, IgM (secreted), Mer, MFG-E8, SAP	(696,697)
Dysregulated lymphocyte activation caused by mutations in cell receptors and their ligands	BAFFTg, CD22, CD21/CD35, CD45PMt, CD152, FcγRIIB, G2A, IL-2Rß;, PD-1, TACI, TCR-α, TGFßRIIDNT or T cell-CKO	(5,6,698,699)
Dysregulated lymphocyte activation caused by intracellular signaling molecule mutations	Aiolos, Cbl-b/Vav-1, E2F2, Fli-1Tg, Gadd45a, LIGHTTg, Lyn, PKC-d, P21, Rasgrp1, SHP-1, SOCS-1, Stra13, TSAd	(5,6,699)
Cytokine production abnormalities	IFN-γTg, IL-4Tg, IL-10, TGFß	(6,700,701)
Defective hormone signaling	ERα	(702)

*Gene-targeted mice are conventional knockout mutations unless marked as Tg (transgenic overexpression), DIT (dominant interfering transgene that cause deficiency of the targeted molecule), Het (heterozygous for the targeted gene), CKO (conditional knockout), PMt (A point mutation in CD45 prevents dimerization and negative regulation of phosphatase activity), and DNT (dominant negative transgene).

In the second category—gene deletions that influence clearing of DNA, nucleosomes, apoptotic cells, and apoptotic bodies—several single gene deletions have produced lupus-like phenotypes in normal mice. Humans with homozygous deletions of C1q have a very high prevalence of SLE. Similarly, among mice in which the C1q gene was deleted, approximately half developed high-titer ANAs and 25% had clinical nephritis by the age of 8 months; glomeruli showed unusually abundant deposits of apoptotic bodies (443). C1q probably plays a role in clearance of immune complexes, of apoptotic cells, and of apoptotic bodies (444). Serum amyloid P component (SAP) binds to DNA and chromatin, displaces H-1 histones, and solublizes native long chromatin. SAP also binds to apoptotic cells (surface blebs contain chromatin) and to nuclear debris following cell necrosis. It is probably important in the disposal of these materials. Mice with deletions in the SAP gene developed autoantibodies, including anti-DNA, and severe glomerulonephritis (445). Mice deficient in DNAse1 also developed ANA, Ig deposition in glomeruli, and clinical nephritis (446).

In summary, these single gene knockout mice show that one alteration permitting B- or T-cell hyperactivation, or interfering with the elimination of DNA/nucleosomes or apoptotic and necrotic cells that provide stimulatory nucleosomes and other self antigens, is powerful enough to produce lupus-like phenotypes in mice that otherwise are resistant to clinical autoimmunity.

Lupus Induced by Direct Transfer of Pathogenic Autoantibodies or B Cells That Secrete Those Antibodies

See Chapter 21 for a detailed discussion of this topic. Briefly, our laboratory demonstrated that transfer of B cell hybridomas secreting pathogenic IgG anti-dsDNA to normal BALB/c mice resulted in the development of SLE, with circulating IgG anti-dsDNA and immune complexes and severe Ig-mediated glomerulonephritis (113 ,114). In some cases, mice were injected repeatedly with purified IgG rather than with hybridoma cells, with the same results. Injections of the immunoglobulin into C57B1/6 mice did not produce any disease, suggesting that background susceptibility genes, perhaps influencing the composition of the kidney, must be present for this approach to induce disease. SCID mice that were populated with BW pre–B cells developed SLE with the expected secretion of autoantibodies by their adopted B cells (162). Similarly, some human monoclonal antibody anti-DNA inoculated into SCID mice deposited in glomeruli and induced proteinuria (447).

Lupus in Mice Transgenic for Pathogenic Autoantibodies

Transient lupus nephritis developed in normal mice that were transgenic for an IgG2b anti-dsDNA derived from a nephritic BW female (116). The gene construct permitted only small

quantities of the transgenic IgG2b to be expressed on B cell surfaces, thus bypassing early tolerance mechanisms. Therefore, the transgenic mice secreted IgG2b anti-dsDNA for several weeks and, during that time, developed proteinuria. Later, B cell receptor editing occurred, with resultant elimination of the ability of the immunoglobulin to bind DNA; the proteinuria disappeared and the mice lived a normal life span. Mice carrying transgenes encoding anti-DNA from MRL-Fas(lpr) mice have also been generated and studied for B cell tolerance. In MRL-Fas(lpr) mice, anti-dsDNA B cells undergo receptor editing, while anti-ssDNA B cells are functionally silenced (448). In the lupus mice compared to normal BALB/c mice, developmental arrest of autoreactive B cells does not occur; in the presence of the Fas/lpr mutation anti-dsDNA, B cells find their way to lymphoid follicles, along with CD4 T cells, so that T-B interactions continue to drive clinical autoimmunity (323 ,449). To summarize, if normal mice express the transgene-encoded immunoglobulin on B cell surfaces, the cells are developmentally arrested, deleted, anergized, or receptor edited; cells do not reach T-B interaction sites in lymphoid organs, and secretion of the anti-DNA is short-lived if it occurs at all. If the transgenic mouse has an lpr background, these mechanisms of tolerance degrade over time, pathogenic B cells reach follicles where they can interact with T cells, and ANAs encoded by the transgene are secreted with steadily increasing titers. In another model using mice transgenic for the R4A-γ 2b heavy chain of an anti-DNA monoclonal antibody (mAb) (which can combine with multiple light chains to made a DNA-binding Ig), nonautoimmune hosts display a high-affinity population that is anergic, another high-affinity population that is deleted, and a third population that produced germline-encoded antibodies with low affinity for dsDNA that escaped normal regulation (450). Perhaps these low-affinity cells that normally escape regulation undergo activation and receive T-cell help in mice predisposed to SLE and thus become pathogenic anti-DNA–secreting B cells.

Lupus Following Activation of Id/Anti-Id Networks

Chapter 15 discusses the role of Id/anti-Id networks in SLE. Immunization with an Id induces anti-Id; immunization with an anti-Id induces anti-anti-Id and/or Id. This principle has been used to study murine models of SLE and of antiphospholipid syndrome. After immunization of BALB/c or other susceptible strains (again, C57B1/6 is resistant) with Id 16/6 (i.e., a frequently occurring Id in patients with SLE) or anti-Id 16/6, a full Id/anti-Id network appeared in the mice along with autoantibodies to DNA, to phospholipids, and to snRNP particles. The mice developed leukopenia, elevated sedimentation rates, and immunoglobulin deposits in glomeruli (451 ,452 ,453). Normal mice that were immunized with a monoclonal antiphospholipid IgM with lupus anticoagulant activity also developed an Id/anti-Id network, along with thrombocytopenia, lupus anticoagulant, and fetal loss (454 ,455). These models also have been used to test multiple therapeutic interventions (456 ,457 ,458 ,459 ,460 ,461). Both $CD4^+$ and $CD8^+$ cells may be necessary for the development of full-blown disease (460 ,462). It should be noted that C57B1/6 mice are not completely protected from SLE; when mated with a substrain of NZM/Aeg, some hybrids develop severe immune complex GN (73).

Lupus Induced by Immunization with DNA, DNA/Proteins, RNA/Proteins, or Oligopeptides

There has been great debate regarding the nature of the inciting antigens in SLE. Most investigators agree that DNA/protein and RNA/protein molecules and particles likely are the true immunogens in mice or humans who are predisposed to SLE. In general, naked mammalian DNA is a weak immunogen and does not induce SLE in normal mice unless it is bound to a protein (78 ,463 ,464). In contrast, bacterial DNA used to immunize normal mice can induce IgG antibodies to DNA (almost exclusively to ssDNA rather than dsDNA), and some animals develop immune complex nephritis (465). Whether this DNA acquires protein after immunization is unknown. Mammalian DNA used as an immunogen also can induce IgG anti-DNA and nephritis in normal mice if it is bound to protein. Thus, immunization with nucleobindin, which probably combines with nucleosomes that are released from the thymus and other tissues, can induce anti-DNA in normals (261), as can DNA that is combined with a fusion protein (463). Nucleosomes are particles in which DNA is wrapped around histones; they likely are direct immunogens that induce many of the autoantibodies characteristics of SLE.

Immunization of rabbits, mice, and baboons with protein or oligopeptide autoantigens (from Sm B/51, Ro 60-kd peptides, and La/SS-B) have induced epitope spreading, antinuclear antibodies, and proteinuria in a proportion of animals (466 ,467 ,468 ,469). However, one group of investigators found more limited epitope spreading in rabbits and mice after immunization with a peptide of Sm B/B8, less ANA production, and no clinical disease (470). This may reflect differences in environmental stimuli to which animals are exposed in different laboratories.

Lupus Induced by Injection of Hydrocarbon Oil

Chronic inflammation may induce autoantibodies in susceptible mice. Satoh et al. (471) injected pristane into the peritoneal cavities of BALB/c mice. Approximately half of these mice developed IgM anti-ssDNA, IgM antihistone, IgG anti-Sm, and IgG anti-Su. IgM, IgG, and C3 were found in glomeruli, predominantly in mesangial areas. This is excellent evidence that inflammatory stimuli can provoke autoantibodies production; whether that leads to disease, however, probably depends on concurrent immune responses and genetic susceptibility.

A single intraperitoneal injection of 2,6,10,14-tetramethylpentadecane (pristane) induces lupus-like autoantibodies in several inbred strains of mice (472). Pristane-injected BALB/c mice develop autoantibodies to nRNP/Sm, Su, and dsDNA, whereas similarly treated SJL/J mice produce anti-ribosomal P autoantibodies. Associated with

these autoimmune responses, mice of both strains experience an immune-complex-mediated glomerulonephritis (3 ,471). IgM, IgG, and C3 are found in glomeruli, predominantly in mesangial areas. The finding that BALB/c, CBA, and DBA mice develop an inflammatory joint disease after two intraperitoneal injections of pristane further increases interest in this animal model. The pristane-induced arthritis occurs between 100 and 200 days after the initial pristane injection (473). It preferentially involves ankle and wrist joints and presents several similarities to rheumatoid arthritis. A variety of autoantibodies, including rheumatoid factor, autoantibodies to collagen type II, and antibodies to stress proteins, are detected in the serum of affected mice. Contact with environmental microorganisms is necessary for the induction of pristane-induced arthritis, which reportedly does not develop in specific pathogen-free mice maintained in isolator cages. Interestingly, pristane-induced murine lupus is associated with marked hypergammaglobulinemia that is also influenced by microbial stimulation.

Because pristane selectively induces lupus-specific autoantibodies in virtually any strain of mouse regardless of its genetic background (474), this model is increasingly being used in testing the role of various genes on lupus manifestations using transgenic and knockout mice that are generally generated in normal mouse backgrounds. The use of hydrocarbon model in these experiments saves time and resources that would be required to backcross the null mutation from the stock (usually C57BL/6-Sv129) onto genetically lupus-prone strains. Further, pristane injection broadens the spectrum of lupus-like autoantibodies produced in genetically lupus-prone mice. For example, it induces anti-nRNP/Sm and Su antibodies that are not generally detected in genetically lupus-prone NZB/NZW F1 mice. Pristane injection also enhances anti-chromatin/DNA antibodies in these mice and dramatically accelerates lupus nephritis (475). Thus, environmental influences can trigger and exacerbate autoimmunity in genetically lupus-prone animals. Furthermore, injection with adjuvant mineral oils, such as Bayol F (Incomplete Freund's adjuvant: IFA) and squalene (MF59), also induces lupus-like autoantibodies production in otherwise normal BALB/c mice (476).

A Brief Overview of the Pathogenesis of Murine Lupus

Chapter 5 summarizes current concepts regarding the pathogenesis of SLE. Here, the information from murine lupus is briefly synthesized (Table 18-8).

Spontaneous murine lupus results primarily from genetic predisposition (almost always involving multiple genes in certain pathogenic combinations), from environmental stimuli, or both. It is likely that a susceptible genetic background is always required, because no single environmental trigger that accounts for disease induction or flare in all patients has been identified. However, the relative importance of genes and environmental triggers may vary.

Table 18-8: Pathogenesis of Autoimmunity in Murine Models of SLE

A. Genetic susceptibility
1. MHC genes (NZ and BXSB)
2. Multiple genes on different chromosomes, not linked to MHC
3. Single accelerating genes: lpr, Yaa, me

B. Immune abnormalities
1. Excessive T cell help by $CD4^+$ and $CD4^-$ $CD8^-$cells
2. Excessive B cell activation, partially independent of T cells, including B-1 and marginal zone B cells.
3. Defective generation of regulatory/inhibitory T cells, including $CD8^+$
4. Defects in bone marrow stem cells (NZ and BXSB mice)
5. Abnormal architecture and function of the thymus, with marked cortical atrophy
6. Defective clearance of apoptotic materials and immune complexes

C. Production of autoantibodies
1. Antibodies against DNA/protein and RNA/protein antigens
2. Antibodies against cell surface molecules including erythrocytes, lymphocytes and neurons
3. Antibodies against phospholipids

D. Infiltration of target organs by T cells capable of damaging the organ

E. Activation of dendritic cells by oligonucleotides from bacteria, viruses and DNA/anti-DNA complexes: DC then activate T cells

F. Environmental factors influencing disease
1. Diet
2. Sex hormone status
3. Infections
4. Neuroendocrine system

Examples of Spontaneous and Induced Mouse Models of SLE Illustrate These Points

In mice, multiple genes are required for the development of spontaneous SLE. Addition of accelerator genes to these backgrounds makes the disease appear earlier, such as Fas/1pr, gld and Yaa, each of which is discussed above. In most if not all strains MHC class II genes are critical, probably because they shape the $CD4^+$ T-cell responses that drive abnormal B cells, which already are prone to secrete large quantities of IgM and autoantibodies. Microsatellite analysis of DNA in the mouse genome has shown that multiple genes on different chromosomes are required for development of all SLE manifestations that are known to develop in NZ hybrids. There may be at least two or three genes that influence each manifestation (e.g., anti-DNA, early mortality, glomerulonephritis, and so on). In most strains, there is a region on the telomeric portion of chromosome 1 that contains at least one gene that significantly increases susceptibility; a syntenic region on human chromosome 1 has been

associated with SLE in several human populations. In total, there may be ten or more genes in each strain that in combination cause all of the manifestations that are associated with murine lupus, with some providing a higher proportion of susceptibility than others. (These concepts are discussed in detail in Chapter 7 .)

Additionally, there are several single genes that can be knocked in or knocked out of the genome of normal mice that result in lupus-like clinical disease. Most of them alter B- or T-cell survival and/or affect apoptosis.

The Role of Stem Cells, Thymus Cells, B Cells, and T Cells in Murine Lupus

Most investigators think that abnormalities occur in B and T cells of NZB, NZ hybrids, and BXSB mice. Therefore, a defect (or, more likely, multiple defects) in stem cells may underlie the disease. Transfer of bone marrow cells likely to be stem cells from NZ and BXSB mice have transferred the ability to make autoantibodies and develop disease to otherwise normal recipients (45 ,136 ,367 ,378 ,379). There is evidence that activation of both T and B cells is abnormal in that cells are too sensitive to stimulation, not eliminated properly after activation, or both. If there are one or a few abnormalities in cell activation that characterize any one strain, however, they have eluded detection thus far.

Thymic architecture is abnormal in all mice with spontaneous SLE (10 ,62 ,63 ,64 ,326 ,372). Basically, cortical atrophy occurs in all, and medullary hyperplasia may be seen. The function of thymic epithelial cells is abnormal. There must be profound effects on positive and negative selection of T-cell repertoires in these thymuses, but again, a basic functional defect that is common to all lupus mice remains to be identified. Thymectomy prevents development of disease in MRL-Fas(lpr) mice, but probably not in NZ strains or BXSB mice.

B cells are abnormal in NZ and BSXB mice. They are easily activated and increase in numbers over time (15 ,18 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,52 ,53 ,54 ,55 ,57 ,58 ,59 ,162 ,204 ,212 ,318 ,320 ,321 ,323 ,364). The IgM antibodies they make spontaneously probably are the origin of the pathogenic IgG autoantibodies that mediate tissue damage. All that it takes is the addition of T-cell help, and the disease becomes florid. B cells from MRL mice also may be abnormal, although the evidence for this is weaker than in the other strains. After all, the MRL-Fas(lpr) mouse makes far more autoantibodies than any other strain, both in terms of antigens that are recognized and total quantities of each antibody. Does this simply mean that any B cell has the capacity to make all of the autoantibodies that are characteristic of SLE? Or does the MRL B cell have characteristics that allow it to recognize a greater number of self antigens?

Finally, the T cell is required for full-blown SLE to develop in all strains with spontaneous disease. In all of the strains tested, depletion or inactivation of $CD4^+$ cells is a very effective intervention to prevent the appearance of disease and even to at least partially reverse established lupus (81 ,82 ,112 ,172 ,176 ,177 ,251 ,295 ,305 ,306 ,366 ,369). Recent studies have shown that murine lupus can be prevented by interrupting T- and B cell activation at the level of second signals. After TCR engagement by MHC/peptide, second signals through CD40-CD40 ligand (gp39), through CD28/CTLA4B7-1/B7-2 ligands, or through BlyS-TACI/BCMA are required for the production of cytokines by T cells and of immunoglobulin by B cells. Interruption of these second signals by the administration of antibodies or soluble receptors that prevent ligand interactions, or by knocking out one of the ligands, prevents development of high-titer IgG anti-dsDNA and of nephritis (176 ,177 ,368 ,433 ,434).

Role of Monocytes/Macrophages and Neutrophils in Murine Lupus

Monocytosis and increased cytokine production by monocytes/macrophages are features of the full-blown autoimmune syndrome in BXSB and MRL-Fas(lpr) mice (188 ,313 ,333 ,362 ,375). Monocytes/macrophages probably play important roles in disease. The glomeruli of MRL-Fas(lpr) mice are infiltrated with monocytes/macrophages (477). Expression of IL-1 and thromboxane (which is induced by IL-1), and of TNF-α, are greatly increased in the glomeruli of MRL-Fas(lpr) mice (189 ,477 ,478). Kupffer cells from the livers of MRL-Fas(lpr) mice also secrete high levels of TNF-α (479). Monocytes/macrophages from BXSB mice also release increased quantities of procoagulants (362). The combined effects of these cytokines would contribute to glomerular damage and accelerate disease.

Defects in Clearance of Immune Complexes and Apoptotic Cells

As in humans with SLE, the clearance of circulating immune complexes, and of cells coated with antibodies, and of apoptotic cells is abnormal in murine lupus. The Fc-mediated clearance of radiolabeled, Ig-sensitized RBCs is delayed in NZB, BW, MRL-Fas(lpr), and BXSB mice by the time they reach 6 months of age. Complement receptor-mediated clearance was delayed in MRL-Fas(lpr) mice but not in the other strains (328 ,480). Clearance of heat-aggregated IgG is reduced in old MRL-Fas(lpr) mice, but not old MRL/+/+, BXSB, or BW mice (481).

Abnormalities in Target Organs

It is possible that certain mouse strains are susceptible to SLE in part because the organs that are exposed to immunoglobulin and T cells are predisposed to injury. For example, renal tubular cells in MRL-Fas(lpr) mice probably process antigens (likely to be self) and act as APCs that activate T cells to induce injury (303 ,304). Mesangial cells from these mice also proliferate more vigorously than mesangial cells from nonlupus mice when exposed to growth factors (482).

The Role of Environmental Factors

The micro-environment is important in some strains, especially with relation to sex hormones. In all murine lupus strains except BXSB, disease occurs earlier in females than in males (12). In NZB and MRL-Fas(lpr) mice, the difference in disease onset is only 1 to 2 months. In BW and SNF1 mice, the difference is several months, and the female predominance in these two strains is striking. Disease in BW mice can be dramatically altered by sex hormone manipulation, with estrogens accelerating disease and androgens delaying it (150 ,151 ,152 ,153 ,154 ,155 ,156 ,157 ,158 ,159 ,160 ,161).

Regarding exogenous stimuli, there is evidence that infections can accelerate murine lupus (119 ,173 ,319). This probably results from the formation of additional immune complexes that can add to the immunoglobulin deposits in glomeruli and blood vessels. Polyclonal activation by in vivo administration of LPS (similar to the effects of endotoxin) also can accelerate disease in MRL-Fas(lpr) mice (319); however, these effects are probably minor. Lupus in mice is almost entirely a genetic disease.

Abnormalities of Immune Regulation

All of the abnormalities in murine lupus, whether spontaneous or induced, likely depend on abnormal regulation to persist and cause disease. In some induced models, regulation simply is overwhelmed by providing huge quantities of pathogenic autoantibodies or by making most B cells express a pathogenic antibody that is encoded by a transgene. In other induced models, regulation is dysregulated by activating undesirable idiotypic circuits. The Fas/lpr gene is an excellent example of a single gene that alters the normal regulation of apoptosis, with catastrophic results for the MRL mouse destined for mild, late-in-life lupus. Some regulatory mechanisms, including B cell tolerance, are influenced by sex hormones in some genetic backgrounds; in those mice, disease can be profoundly influenced by the manipulation of hormones. There is evidence that $CD8^+$ inhibitory T cells become dysfunctional as NZB/NZW F1 mice age, but both $CD4^+CD25^+$ regulatory T and inhibitory $CD8^+$ T cells can be induced by tolerizing regimens, and when so induced can prevent or suppress autoantibodies and disease. The complex interactions that are required to regulate autoimmune responses will continue to be identified in the future.

Summary

Multiple abnormalities are required for a mouse to develop lupus-like disease. These include disturbances in the function of hematopoietic stem cells, DC, B lymphocytes, T lymphocytes, and phagocytic cells. In spontaneous SLE, the abnormalities are determined primarily by genetic influences; most require multiple genes, which are provided by both parents of a susceptible strain. Three accelerating genes, lpr, gld, and Yaa, are not sufficient to cause disease unless they are engrafted onto a host that is genetically susceptible to autoimmunity. The most important results of the abnormalities are production of pathogenic subsets of autoantibodies and immune complexes, which depend on both abnormal B cell repertoires and unopposed T-cell help. Environmental factors may accentuate these abnormalities but are of minor importance.

Therapeutic Interventions in Murine Lupus

A major advantage of each mouse model of SLE is its availability for studies of therapeutic interventions. These interventions are strategies to (a) provide general immunosuppression, (b) eliminate or inactivate helper T cells, (c) inactivate pathogenic B cells, (d) activate suppressor networks, (e) kill autoreactive B cells, (f) activate DC, (g) alter cytokines, (h) replace stem cells, (i) alter generation of eicosanoids, (j) alter immunoregulation via sex hormones, and (k) alter tissue damage in target organs. Table 18-9 summarizes interventions.

All successful interventions are most effective when they are introduced before the development of full-blown clinical lupus. The most interesting ones also are effective in mice with established disease.

Strategies That Are Widely Immunosuppressive

Cytotoxic and immunosuppressive drugs that are standard in the management of SLE in patients have been studied in murine models of lupus. These include glucocorticoids, azathioprine, cyclophosphamide, methotrexate, cyclosporine, and newer cytotoxics such as mycophenolate mofetil, rapamycin, and others not yet available for human therapy (e.g., 15-deoxyspergualin and dimethylthiourea), and total lymphoid irradiation. Glucocorticoids suppress hemolysis and prolong life in NZB mice (483). In BW mice, murine chronic GVHD, and MRL-Fas(lpr) mice, immunosuppressive agents suppress IgG anti-dsDNA, proteinuria, glomerular immunoglobulin deposits, and nephritis, with resultant prolonged survival (131 ,358 ,483 ,484 ,485 ,486 ,487 ,488 ,489 ,490 ,491 ,492 ,493 ,494 ,495 ,496 ,497 ,498 ,499 ,500 ,501 ,502 ,503 ,504 ,505 ,506 ,507 ,508 ,509 ,510 ,511). They are effective even in animals with established nephritis although better when introduced before clinical disease appears. Figure 18-2 shows the effects on survival of strategies from comparable studies.

Azathioprine as a single agent does not prolong survival in NZB, BW, or chronic GVHD mice. Added to glucocorticoids and/or cyclophosphamide, however, the combination is more effective than any single drug alone (486 ,487 ,489).

As a single drug intervention, cyclophosphamide is superior to glucocorticoids or azathioprine in suppressing nephritis and IgG autoantibodies, and it prolongs life in NZB, BW, and chronic GVHD mice (147 ,486 ,490 ,491 ,492 ,493 ,494 ,495 ,496). It is equally effective whether given daily or intermittently (Fig. 18-3). In combination with glucocorticoid, it suppresses disease in

MRL-Fas(lpr) mice (490); combinations of cyclophosphamide and another immunosuppressive drug such as glucocorticoid or FK506 are more effective than either drug alone (490 ,498). Administration of cyclophosphamide in any regimen is associated with substantial increases in malignancies, and in some colonies azathioprine also has this effect (147 ,489 ,495). Any combination therapy that includes cyclophosphamide suppresses lupus nephritis effectively (490).

Table 18-9: Therapeutic Interventions in Murine Lupus

Intervention	Strains Studied	Effects
Immunosuppressive regimens		
1. Glucocorticoids	NZB, BW, MRL/lpr, BXSB, chronic GVH	Prolong survival Suppress GN Suppress autoantibodies Suppress T abnormalities
2. Cyclophosphamide	Same as 1	Same as 1
3. Azathioprine	BW, chronic GVHD	Not effective as single drug; effective in combination
4. Combinations 1–3	BW	More effective than one drug alone
5. Mycophenolate mofetil	BW, MRL/lpr	Decreases autoAb and nephritis, including glomerulosclerosis
6. 15-Deoxyspergualin	MRL/lpr, BXSB	Suppresses B activity Suppresses lymphoproliferation Suppresses CIC, anti-DNA Suppresses GN
7. Cyclosporin A	MRL/lpr, BXSB, BW	Suppresses lymphoproliferation No suppression of anti-DNA, CIC Suppresses GN, arthritis Prolongs survival
8. FK506	MRL/lpr	Prolongs survival Suppresses lymphoproliferation Suppresses anti-DNA Suppresses nephritis
9. Total nodal irradiation	BW	Prolongs survival No suppression of anti-DNA Suppresses GN Reduction of T cell help for months, suppression for weeks
Inhibition of T cells		
1. Anti-L3T4 (anti-CD4)	BW, MRL/lpr, BXSB	Prolongs survival in pre-dz and post-dz mice Depletes or inactivates $L3T4^+$ T, suppresses accumulation of $CD8^+$ T, B, and monocytes in lymphoid organs and kidneys Suppresses anti-DNA Suppresses GN
2. CTLA4-Ig	BW	Suppresses autoAb and GN if given before disease begins. Effective after nephritis onset if combined with cyclophosphamide or anti-CD40L
3. Anti-CD40L	BW	Suppresses autoAb and GN with reduced expression of TGF-b, IL-10 and TNF-α in kidneys, better effects combined with CTLA4-Ig
4. Anti-CD137	BW	Suppresses autoAb and nephritis
5. Anti-Ia	BW	Anti-IAz-prolongs survival Suppresses anti-DNA Suppresses GN Anti-IAd less effective
Inhibition of B Cells		
1. Anti-idiotypes	BW, MRL/lpr	Prolong survival Suppress anti-DNA Suppress GN
2. Deplete/diminish B-1 B cells	BW	Suppress anti-DNA and GN (introduce Xid gene, give B cell superantigen)
3. TACI-Ig	BW. MRL/lpr	Suppresses anti-DNA and GN (or ad-encoded TACI)
4. Ig peptide minigenes	BW	Induce $CD8^+$ T cells that ablate anti-DNA B cells and suppress GN
Induction of Immune Tolerance in T and/or B cells		
1. LJP394 (Riquent)	BXSB	Suppresses anti-DNA and GN
2. Peptides from Ig	BW	Suppresses autoAb and GN: Induces $CD4^+CD25^+$ and $CD8^+$ regulatory/inhibitory T cells
Id-induced lupus		
3. Peptides from histones	SNF1	Suppresses autoAb and GN: Induces $CD4^+CD25^+$ and $CD8^+$ regulatory/inhibitory T cells
4. Peptide	BW	Suppresses anti-DNA, induces $CD4^+$ IL-10 secreting regulatory T cells from D1 protein of Sm antigen
Manipulation of Cytokines:		
1. Inhibit IFN-γ	BW, MRL/lpr	Suppresses autoAb and GN
2. Inhibit IL-4	BW, NZM.2410, NZM.2328	Decreases glomerulosclerosis
3. Inhibit IL-10	BW, MRL/lpr	Decreases autoAb and GN (including with CRP)
4. Inhibit IFN-α	NZB	Decreases autoAb and GN
5. Inhibit TNF-α	BW	Mixed results—see text
6. Inhibit TGF-ß	BW	supress chronic renal lesions
Replace bone marrow stem cells with Allogeneic or T-depleted syngeneic cells:	MRL/lpr, BW, BXSB	Diminishes disease
Reduce Eiconasoids (low calorie/fat diet, PGE, omega-3 fatty acid enriched):	NZB,BW,MRL/lpr,BXSB	Delays and diminishes disease
Sex hormone therapies		
1. Estrogens, castration	BW, MRL/lpr, BXSB	Accelerate male dz Increase IgG anti-DNA Increase nephritis Decrease survival Dramatic in BW, modest effects in MRL/lpr, no effect in BXSB males
2. Androgens	BW, MRL/lpr	Suppress female dz plus castration or antiestrogens Prolong survival Delay IgG anti-DNA Delay nephritis Dramatic in BW, modest effects in MRL/lpr females
3. Anti-sense nucleotides	BW	Diminish anti-DNA and GN for G proteins
4. Indole-3-carbinol	BW	anti-estrogen effect, prolongs survival

Methotrexate delayed the appearance of proteinuria and prolonged survival (without decreasing anti-DNA) in BW and MRL-Fas(lpr)mice, but it did not affect disease in NZW × BXSB F1 males (497). A different antifolate, MXX-68, was as effective as methotrexate in delaying nephritis in MRL-Fas(lpr) mice (499).

The effects of cyclosporin A (Cy-A) in MRL-Fas(lpr), BXSB, and NZB mice also have been studied. Cy-A is highly effective in suppressing lymphoproliferation; the DN T cells that are associated with Fas/lpr do not expand (500 ,501 ,502 ,503). Cy-A in high doses can suppress the synthesis of anti-DNA in vitro (503). However, B cell hyperactivation with production of high levels of Ig, circulating immune complexes, rheumatoid factors, and anti-DNA was not suppressed when the drug was given in vivo (501 ,502). The effects on nephritis were variable. One group reported no suppression of nephritis and no improvement in survival for either MRL-Fas(lpr) or BXSB mice (501), whereas others reported reduction of nephritis and prolonged survival (502). Apparently, renal damage can be suppressed without diminishing B cell

activation and autoantibody synthesis, suggesting that autoantibodies alone may be necessary, but not sufficient, for the development of lethal lupus nephritis. FK506 was given to young MRL-Fas(lpr) mice; it prevented lymphoproliferation and nephritis and also reduced titers of anti-dsDNA (504); in another study FK506 was more effective in combination with cyclophosphamide (498).

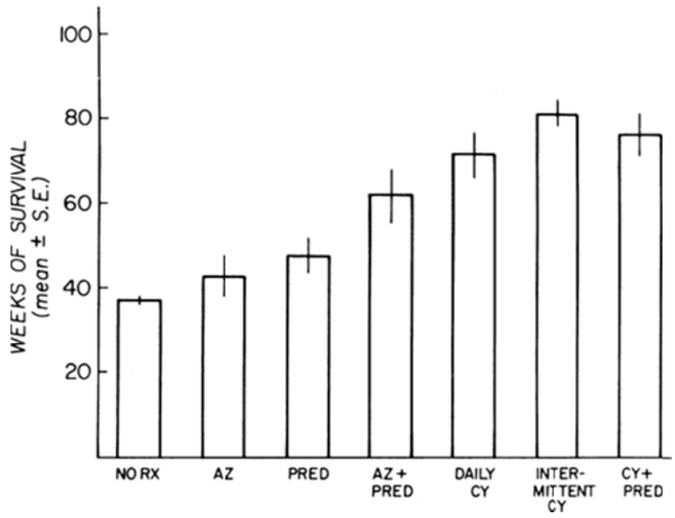

Figure 18-2. Survival in (NZBxN2W) F1 female mice treated from 6 weeks of age with daily oral doses of azathioprine (AZ), prednisolone (Pred), cyclophosphamide (Cy), or combination therapies. Bars indicate mean weeks of survival; vertical lines are 1 SEM. Survival was significantly better in Pred vs. No Rx, AZ plus Pred vs. AZ alone, Pred alone, or no RX, and best in all groups receiving Cy, whether daily or intermittent. (From Hahn BH, Shulman LE. Autoantibodies and nephritis in the white strain (NZW) of New Zealand mice. *Arthritis Rheum* 1969;12(4):355–364.)

A recently developed cytotoxic drug, 15-deoxyspergualin, suppresses immune complex formation, anti-DNA, nephritis, and lymphoproliferation in MRL-Fas(lpr) and male BXSB mice (358 ,505). It has been administered primarily to mice before onset of florid disease. It was also effective in treating the immune thrombocytopenia characteristic of (NZW × BXSB)F1 mice (506). Another newer cytotoxic drug, dimethylthiourea, has been used to treat BW mice (507). When given before disease onset, it is effective at preventing nephritis.

Mycophenolate mofetil has been studied in BW and MRL-Fas(lpr) mice. Mycophenolate is an inhibitor of inosine monophosphate dehydronase, thus inhibiting guanosine nucleotide synthesis. T and B lymphocytes depend on this pathway for their purine synthesis, whereas other types of cells have alternate pathways. Therefore, mycophenolate is relatively specific for suppression of lymphocytes, in contrast to the other drugs discussed above. In both murine lupus strains development of nephritis was suppressed (and in most levels of autoantibodies and total numbers of lymphocytes were also suppressed). In comparison to cyclophosphamide, the numbers of cells infiltrating renal tissue was less with cyclophosphamide (508 ,509 ,510 ,511). Mycophenolate has benefits other than reducing autoantibodies levels (it is particularly effective at reducing IgG2a) (512). In particular, administration of mycophenolate suppresses oxidative damage in kidneys: it decreases renal cortical expression of iNOS and urinary nitrite production, along with reducing glomerular sclerosis (513). In support of this idea, a study in which mycophenolate was combined with a cyclooxygenase-2 inhibitor to reduce thomboxane A2 production (an eiconosoid that promotes ischemia), survival of lupus mice was better with combination therapy (514).

Administration of paclitaxel to BW females resulted in significantly prolonged survival, reduced levels of anti-DNA, and delayed onset of nephritis (515).

Several Asian herbal preparations act as general immunosuppressants. Some of these have been effective in suppressing various manifestations of lupus in MRL-Fas(lpr) mice (516 ,517).

Total nodal irradiation has been studied in murine as well as human SLE(496 ,518 ,519 ,520 ,521 ,522). Irradiation of BW or MRL-Fas(lpr) mice, even after clinical disease is established, results in prolonged survival and markedly diminished nephritis, which is associated with decreased serum levels of anti-DNA. In MRL-Fas(lpr) mice, lymphoproliferation is reduced (520 ,522). Both suppressing and enhancing cell circuits are suppressed for a few weeks after therapy is stopped, but helper circuits return to supernormal, with increased antibody production and proliferation to antigens and mitogens. However, the mice are protected from recurrent high levels of ANA production and from disease for several months after this help appears, despite the fact that suppressive circuits cannot be demonstrated. In one study comparing total node irradiation to cyclophosphamide therapy in BW mice, irradiation was superior in prolonging life, because the incidence of malignant tumors was lower (521).

In summary, immunosuppressive regimens that include glucocorticoids and/or cyclophosphamide, or total nodal irradiation, are impressive in their ability to reverse established nephritis at least partially. The other approaches, such as interfering with synthesis of IL-2, are either more effective when done before clinical organ damage appears or have not been adequately studied in established disease.

Strategies That Deplete or Inactivate T Cells

Because $CD4^+$ helper T cells amplify autoantibodies production and are required for the development of full-blown SLE in all SLE mouse models that have been studied to date, elimination or inactivation of those cells is highly effective in preventing disease, and even in partially reversing it once clinical nephritis is manifest. Administration of antibodies to CD4 prolongs survival, suppresses IgG anti-dsDNA and other autoantibodies, and suppresses nephritis and lymphoproliferation in NZ-derived, MRL-Fas(lpr), and BXSB mice, and even in normal mice that have been induced to express antiphospholipid antibodies (81 ,82 ,112 ,172 ,251 ,295 ,306 ,366 ,460). Anti-CD4 prolongs survival in BW mice with established nephritis (172). Long-term benefits require continual, repeated treatments throughout the lifetime of the mouse.

Apparently, $CD4^+$ cells are not entirely eliminated, or their numbers are repopulated (even in the absence of a thymus), so that autoantibodies and disease eventually appear if the treatment is stopped (523).

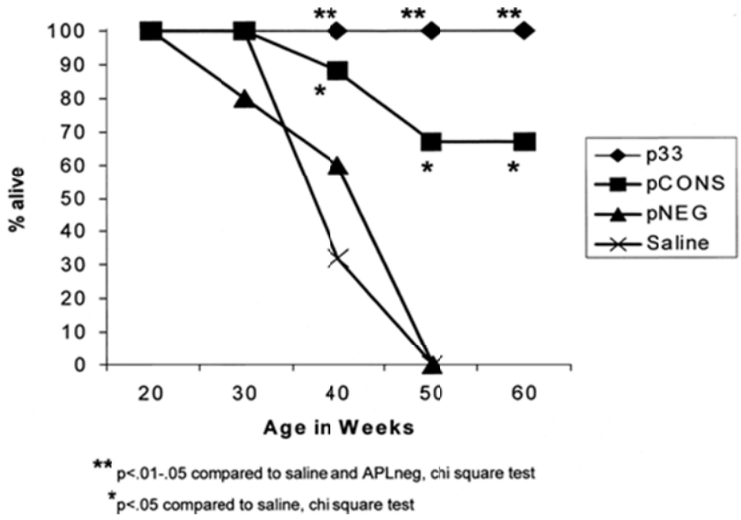

Figure 18-3. Effect of immune tolerance to peptides from autoantibody molecules on survival in (NZB/NZW) F1 mice. BW females were treated from the age of 12 weeks with saline (-×-), a negative control peptide that binds major histocompatibility complex (MHC) class II I-Ed but does not cause T cell activation (pNEG: -Δ-), a wild Ig peptide stimulatory for BW T cells, (p33: -◊-), or a synthetic peptide based on T cell stimulatory Ig sequences (pCONSENSUS or pCONS: -□-) until 60 weeks of age. Peptides were administered as tolerogens, high doses intravenously once a month. Each group contains 5 to 15 mice. Note that all mice in the saline and pNEG groups were dead by 50 weeks of age, whereas 70% to 100% of mice tolerized with peptides that are stimulatory for T cells were still alive. Survival was significantly longer in the effectively treated groups, p <0.01 to 0.05 in the p33 group compared to saline and pNEG by chi square test, p <0.05 comparing pCONS to saline. Autoantibodies and cytokine increases in interferon-γ (IFN-γ) and interleukin-4 (IL-4) were all significantly delayed in the tolerized groups. These mice mounted normal T and B cell responses to immunization with HEL, an external foreign antigen. This illustrates a new approach to achieving specific immune suppression of undesirable autoantibodies.

The monoclonal antibody used in all of these studies is a rat antimouse L3T4 (CD4); it has the advantage of inducing tolerance to itself in the recipient by preventing antibody responses that require T-cell help (112 ,524 ,525). In earlier studies using antibodies against lymphocytes or thymocytes or $Thy\text{-}1^+$ cells ($CD2^+$ in humans), results were often obscured by the development of inactivating antibodies and of serum sickness nephritis caused by the immune response to the antilymphocyte globulin (526 ,527 ,528 ,529). The rat anti-L3T4 monoclonal antibody is cytotoxic to helper T cells and deletes them from the repertoire. The F(ab)2 fragment of the monoclonal antibody is not cytotoxic, because it cannot fix complement, but it inactivates $L3T4^+$ T cells and is as effective as the whole antibody molecule in preventing the development of anti-DNA and nephritis in BW mice (524). In addition to the predictable effects of anti-L3T4 on diminishing T-cell help and autoantibodies formation, non-$L3T4^+$ cells that infiltrate renal and lymphoid tissue as lupus evolves also are influenced. $CD8^+$ T cells and $B220^+$ B cells, as well as $CD4^+$ T cells, are all diminished (525). This suggests a central role for $CD4^+$ T cells in the evolution of activated $CD8^+$ and B cells. In contrast to the benefit of anti-CD4 in (NZW × BXSB)F1 mice, administration of anti-CD8 worsened disease (394).

Anti-CD4 therapy of MRL-Fas(lpr) mice is particularly interesting, because the lymphoproliferative component of their disease is dominated by $B220^+$ $CD4\text{-}CD8^-$ TCR α/ß cells. However, the autoantibodies, arthritis, nephritis, and central nervous system (CNS) disease depend on $CD4^+$ cells (295). Treatment of MRL-Fas(lpr) with a combination of anti-CD4 and anti-CD8 abrogates most disease manifestations. However, treatment with anti-CD4 alone suppresses autoantibodies, proteinuria, histologic nephritis, arthritis, and CNS disease, but does not affect lymphocytic proliferation and actually worsens lacrimal gland destruction (283 ,295 ,306). Nonmitogenic anti-CD3 also reduces mortality and adenopathy in MRL-Fas(lpr) mice (530). The BW mouse has a predictable response, because its disease depends primarily on $CD4^+$ cells: administration of anti-CD8 to tolerized BW mice

depleted $CD8^+$ cells but did not influence autoantibody titers, nephritis, or survival (531). Although anti-CD4 therapy is remarkably effective in murine lupus, its use in the human disease has produced disappointing results. Perhaps by the time a patient is diagnosed, desirable immune regulation has developed and is dependent in part on $CD4^+$ cells, so eliminating T-cell help also eliminates T-cell regulation.

BW mice also have been bred with nude mice to produce BW-nu/nu offspring. Nu/nu homozygotes are athymic and develop T-cell repertoires that are small in number and uneducated in the thymus. Without T-cell help, BW-nu/nu mice have prolonged survival that is associated with decreased levels of IgG anti-DNA and little development of nephritis or lymphoproliferation (532 ,533). However, the animals are not completely disease-free and develop some autoantibodies.

To disable activated $CD4^+$ T cells rather than all $CD4^+$ cells, there has been recent interest in interfering with second signals. T cells receiving only one signal (binding of their TCR) usually undergo activation only if they receive second signals via CD28/CTLA-4 interacting with B7–1 and B7–2 (CD80 and CD86), or CD40 interacting with gp39 (CD40L, CD154). Several groups have investigated the effect of disabling B7–1 and/or B7–2. Antibodies to B7–1 plus B7–2 (or to gp39) interrupt signaling, as does soluble CTLA-4–Ig, which binds B7–1 and B7–2 so they cannot interact with CD28 and deliver a second signal. Such treatments are effective in delaying disease in BW and BXSB mice (176 ,177 ,534). Gene deletion of B7.1 worsened nephritis in MRL-Fas(lpr) mice, whereas deletion of B7.2 reduced kidney pathology: disabling both molecules by treatment with antibodies to both B7.1 and B7.2 suppressed disease (535). In BW mice, one dose of adenovirus containing CTLA-4–Ig reduced numbers of activated T cells and affected disease as long as the protein was present. B cells requiring T-cell help were impaired, although there was no effect on intrinsic B cell abnormalities (536). Blockade of CD28-B7 interactions with soluble CTLA-4-Ig fusion protein has been beneficial in other, nonlupus animal models of autoimmunity. For example, gene therapy with adenovirus containing CTLA-4-Ig prevented induction of collagen-induced arthritis (537). Recent work in which CTLA4-Ig was administered to lupus mice showed that it delayed autoantibodies production if given prior to disease onset, but was less effective in established disease. However, when combined with anti-CD40L (anti-CD154), TACI-Ig, or cyclophosphamide, there was dramatic suppression of autoantibodies and healing of renal lesions. Induction therapy with the combination of CTLA-4Ig and cyclophosphamide arrested the progression of murine lupus nephritis and precluded the need for additional therapy—an experiment very exciting for its potential implications in the therapy of human lupus nephritis (180 ,538 ,539 ,540).

Impairment of CD40/gp39(CD40L) interactions has also been studied in murine lupus. BW mice treated with anti-CD40L had reduced anti-DNA levels, reduced proteinuria, and prolonged survival (178). Treatment with anti-CD40L also prolonged survival in SNF1 mice even if started after nephritis was clinically evident (179). However, anti-CD40L is effective in a higher proportion of mice if administered before nephritis begins, but it can suppress established renal disease in subsets of older mice which repond with rapid reductions in renal mRNA for TGFbeta, IL-10, and TNF-α (541). One group reported that better clinical results are obtained in BW mice by blocking both CD28/B7 interactions using CTLA-4–Ig, and CD40/gp39 interactions using anti-gp39. In fact 10 months after a 2-week course of both therapies, 70% of mice were alive compared to 0% to 18% of mice treated with only one of the agents (180). It is likely that combination therapy will be more useful in human disease as well, since there are several routes to B cell production of autoantibodies.

Finally, inhibition of MHC class II (thus blocking the first signal to $CD4^+$ T cells) has been effective in treating murine lupus. One group studied the efficacy of antibodies to I-A in murine lupus (542). NZB/NZW F1 mice express I-A and I-E MHC class II molecules with two alleles, d and z. Administration of antibodies directed against Az suppressed production of anti-DNA and development of nephritis in BW mice. Anti-I-Ad was somewhat immunosuppressive, but less effective than anti-I-Az. Knockout of MHC class II in MRL-Fas(lpr) mice prevented the development of autoantibodies and nephritis, but not of lymphoproliferation (305). A safer strategy to block MHC-peptide activation of TCR is to provide tolerizing peptides in MHC class II molecules, which is discussed below. A report of methimazole treatment benefiting BW mice speculated that the effectiveness depended on downregulation of MHC class I molecules by the drug (543), suggesting that class I activation of $CD8^+$ cells is also important in development of full-blown murine lupus.

In more recent work, anergy was induced in BW $CD4^+$ T cells by administation of anti-CD137 (4-IBB), which blocks another costimulatory molecule required for sustained T cell help, and autoimmunity was dramatically suppressed (544).

Strategies That Prevent B Cell Activation

Many interventions that interrupt B cell development or activation also prevent murine SLE. Introduction of the Xid gene into NZB or BW backgrounds results in near-deletion of B-1 B cells resulting in inability to synthesize normal levels of IgM. In that setting, NZB.xid and BW.xid mice do not develop their characteristic early life, severe lupus (41 ,545 ,546). They are not disease free, however; a few animals develop autoantibodies and nephritis late in life (545). Xid is a mutated nonreceptor tyrosine kinase (BTK); the kinase promotes activation of NF-κB via activation of the B cell receptor, resulting in IgM production (547 ,548). The hyperactivity of SLE B cells is prevented by Xid. In addition, normal B cells may serve a regulatory function that is defective in lupus B cells. Transfer of MHC-matched normal B cells (but not Xid B cells) into nonirradiated BW mice decreases serum IgG autoantibodies levels, delays proteinuria, and prolongs life (549). Direct inhibition of NF-κB by administration of a p50 antisense nucleotide inhibited NF-κB expression, reduced total IgM and IgG synthesis, and reduced antibodies to dsDNA by 90% (550).

If the IgD or IgM molecules on a B cell surface are ligated, that cell cannot undergo class switch to produce IgG. When MRL-Fas(lpr) mice were treated with multivalent anti-IgD that was conjugated to dextran, development of glomerulonephritis was delayed and survival prolonged (551). Interestingly, removing a major antigen, DNA, that delivers first signals to B cell receptors in mice with lupus was not effective. DNAse treatment of BW mice reduced numbers of anti-DNA–secreting B cells for 1 month but did not alter cytokine production, glomerulonephritis, or survival (552).

Manipulation of the idiotypic network by administration of Id or anti-Id can have profound effects on the immune system, and those effects can result in either upregulation or downregulation of autoantibodies. Administration of carefully chosen Ids or anti-Ids in proper doses at the correct time can suppress Id^+ anti-dsDNA and delay the onset of nephritis in BW mice (553 ,554 ,555 ,556 ,557), MRL-Fas(lpr), and SNF1 mice (222 ,558). Treatment with anti-Ids conjugated to cytotoxic compounds such as neocarzinostatin also is effective in suppressing autoantibodies and nephritis in BW mice, particularly if multiple anti-Ids are included in the regimen (556 ,557). Anti-Ids also can suppress in vitro synthesis of autoantibodies by human B cells (559). There are limitations to Id/anti-Id therapies, however. Some anti-Ids upregulate autoantibodies (560), and variations in dose and time of administration to lupus mice can profoundly influence whether immune responses are enhanced or suppressed. Some anti-Ids do not affect antibody synthesis (561). Beneficial responses can be short-lived, abrogated either by the escape of pathogen-enriched Ids from suppression or by the emergence of pathogenic autoantibodies bearing different Ids (553 ,555). Finally, pathogenic autoantibodies can express different Ids, so that suppression of multiple public (and possibly some private) Ids may be required for prolonged efficacy. The efficacy of anti-Id therapy in mouse SLE is established. A phase I study in nine patients with SLE suggested that anti-Id can be induced by immunizations with a selected idiotype (3E10) without adverse effects (562). Treatment of SLE with intravenous gamma globulin may benefit individuals with SLE by suppressing certain Ids, depending on the anti-Ids in each preparation (563). Mice with induced antiphospholipid syndromes have benefited from the administration of intravenous gamma globulin (456).

Interruption of the B cell growth cytokine IL-4 by administration of soluble IL-4 receptor suppresses autoantibodies production in murine lupus (429).

MRL-Fas(lpr) B cells respond to IL-1 by secreting Ig, and the level of IL-1 mRNA is elevated in the kidneys of MRL mice with nephritis. Infusion of human recombinant IL-1 receptor antagonist into mice with nephritis suppressed circulating IL-1 but did not change the disease (564). Perhaps earlier treatment would be more effective.

Recently, a new member of the TNF-α family has been described that is a coreceptor for second-signal B cell activation following BCR ligation. That molecule, BlyS (also called BAFF, TALL-1, and zTNF4) is secreted by monocytes and binds its receptors TACI, BCMA, and BAFF-R on B cells (435 ,565 ,566 ,567). Since elimination of activated B cells is a reasonable therapeutic goal in murine and human SLE, a phase II trial of anti-BLyS is in progress in human SLE at the time of this writing. Therefore, we will review some information about this molecule. BLyS and a related molecule, April, are made by myeloid cells, stromal bone marrow cells, and T cells. BlyS (also called BAFF) and April are released as soluble molecules and can bind to receptors on B cells. Three such receptors have been defined: TACI, BCMA and BAFF-R. The first two receptors can bind both BlyS and April; BAFF-R binds only BlyS. The consequence of binding to TACI and BCMA is that B cells have longer survival and prolonged activation. B cells in the marginal zone of spleen (which can activate naïve T cells in the absence of antigen) are particularly expanded. In approximately half of patients with SLE, rheumatoid arthritis, or Sjogren syndrome, Blys levels in the circulation are high. A fusion protein of TACI, soluble TACI-Ig, has been used in vivo in mice to bind soluble BlyS and April. This therapy has been effective in delaying onset of autoimmunity in BW mice (566). Administration of adenovirus-encoded soluble TACI reduced glomerulonephritis and proteinuria in MRL/lpr mice but was ineffective in BW mice because of appearance of antibodies to TACI (568). In contrast, in the presence of T cell blockade with CTLA4-Ig, TACI-Ig was highly effective in BW mice: it depleted B cells past the T1 stage, decreased numbers of activated and memory $CD4^+$ T cells, and delayed proteinuria in spite of high titers of autoantibodies that appeared after therapy was stopped (540). Interesting experiments introducing the BlyS/BAFF gene into congenic mice with C57Bl/6 background expressed with the lupus-promoting gene regions Sle1 or Nba2 suggested that BAFF contributed to severe glomerular disease (but not tubulointerstitial), probably unrelated to anti-DNA, antinucleosome and anti-gp70. Whether other autoantibodies are promoted by BlyS/BAFF, or whether there is another mechanism that damages glomeruli remains to be determined (569).

Induction of Tolerance in T and B Cells

There are several mechanisms of immune tolerance: ignorance, anergy, deletion, receptor editing (in B cells), and active suppression. One can induce tolerance in T or B lymphocytes in lupus mice by inhibiting the first activating signal (i.e., binding the TCR or BCR with the peptide or antigen it recognizes), without a second signal (via CD28, CD40, CD137 or BlyS/BAFF), thus inducing anergy. Or, tolerance results by inducing apoptosis in autoreactive cells (deletion). Finally, one can induce regulatory cells to control autoimmunity. Induction of tolerance to autoantigens in either helper T or B cells in individuals with SLE would abrogate production of pathogenic antibodies. Several laboratories have developed strategies to tolerize mice with lupus to DNA and related antigens (570 ,571 ,572 ,573 ,574 ,575). Mice so treated have significant delays in the appearance of autoantibodies and nephritis. For example, intrathymic inoculation of Hl-stripped chromatin into BXSB males significantly reduced T-cell proliferation to nucleosomal antigens, and production of IgG

antichromatin, anti-dsDNA, and anti-ssDNA, for 8 to 10 weeks (574).

Recently there has been success in both human and murine SLE in tolerizing B cells to a molecule containing short nucleotides displayed on a tetrameric scaffold—a compound called LJP 394, or Riquent (575 ,576). BXSB mice treated with this tolerogen have delayed appearance of IgG anti-dsDNA and nephritis, as well as significantly prolonged survival. In some patients with SLE who have antibodies to DNA that bind LJP394 with high avidity, there is good clinical response to repeated intravenous injections of LJP394. Quantities of anti-DNA drop significantly, and there are fewer flares of renal disease compared to a placebo-treated control group (577). Another strategy for cross-linking B cells to inactivate them is to administer DNA/anti-DNA soluble immune complexes. That has been effective in improving survival of MRL-Fas(lpr) mice (578).

In BW mice, repeated tolerization with monthly intravenous doses of a synthetic peptide based on T helper determinants in the VH region of murine antibodies to DNA, or of combined wild immunoglobulin-derived peptides, produced dramatic delays in nephritis and prolonged survival (579 ,580 ,581). Figure 18-3 shows results of one series of experiments. Similarly, tolerization to helper-T-cell–activating peptides from the histone moieties of nucleosomes reduces autoantibodies formation and delays Ig deposition in glomeruli in (SWR × NZB)F1 mice (582). In all these studies, peptides that are both T-cell and B cell epitopes, and induce tolerance to first signals in both T and B cells, were the most effective in delaying clinical disease. One group has reported that repeated oral administration of low doses of whole kidney extract reduced IgG1 and IgG3 anti-dsDNA antibody levels, reduced numbers of inflammatory cells and expression of IL-4 and Il-10 in kidney tissue (while increasing expression of IL-1, IFN-γ, and TNF-α), and prolonged survival (583). This is interesting and may depend on timing of the oral preparation, since aged BW mice have low intestinal IgA levels and are quite resistant to oral tolerance, which usually depends on the production of regulatory and inhibitory T cells in the mucosa-associated lymphoid tissue (584).

Strategies That Activate Suppressor Networks

Most experts suspect that one of the defects in murine and human SLE is an absence of normal suppressive immunoregulatory networks. In normal mice, regulatory T cells develop in both thymus and periphery; they can be $CD4^+$, $CD8^+$, or double negative; some secrete TGF-ß, others IL-10, and still others suppress effector cells by contact (585). These cells have the capacity to prevent autoimmunity and probably function to do so in most normal individuals. Vaccination of mice with disease-inducing T cells, or with certain peptides, can activate suppressive networks, with at least some of the regulatory cells ($CD4^+$) recognizing the TCR of the disease-upregulating T cells. De Alboran et al. (586) inoculated young MRL-Fas(lpr) mice with irradiated cells from the diseased lymph nodes of older MRL-Fas(lpr) mice; peripheral T cells were obtained that protected against disease in adoptive transfer experiments. Normal B cells may also serve a regulatory function; transfer of MHC-matched normal B cells into nonirradiated BW mice decreased serum IgG autoantibodies levels, delayed proteinuria, and prolonged life (549). It is likely that attempts to induce regulatory networks in SLE will be successful in the near future.

Based on these observations, there is currently great interest in devising strategies to induce regulatory/inhibitory T cells to prevent autoimmunity and to control cancers. In the tolerance therapies discussed above, there are multiple simultaneous processes that suppress autoimmunity. For example, in the tolerance induced in BW mice by administration of a soluble 15-mer artificial peptide based on T-cell epitopes in anti-DNA, $CD4^+$ T cell help is anergized, but at least two subsets of regulatory/inhibitory T cells are induced—$CD4^+CD25^+CTLA4^+$ antigen specific cells that suppress B cells making anti-DNA by direct contact, and $CD8^+$ supressors which prevent proliferation of $CD4^+$ helper T cells via secretion of TGF-ß (187 ,198). Similarly, administration of nanomolar quantities of histone peptides which contain T cell epitopes suppress disease in SNF1 mice, at least in part by inducing $CD4^+CD25^+$ regulatory and $CD8^+$ inhibitory cells—each of which depends on secretion of TGF-ß for activity (187). Administration of human Ig CDR1-derived peptide (also containing T-cell epitope) to BW mice also suppresses autoantibodies and nephritis and induces regulatory $CD4^+CD25^+$ T cells (587). A different regulatory T cell was induced in BW mice by IV injection of the D1 protein from Sm antigen; those cells were $CD4^+$ and secreted IFNgamma and IL-10 (but not TGF-ß); they suppressed helper T-cell proliferation and B cell synthesis of anti-DNA (588). The classical $CD4^+CD25^+$ regulatory T cells are characterized by expression of Foxp3, a DNA-binding protein, which may contribute to protection of the regulatory/inhibitory cells from apoptosis (187 ,198). Strategies to induce such cells are in progress for control of many autoimmune diseases.

Fan and Singh used a minigene vaccination approach to elicit cytotoxic/regulatory $CD8^+$ T cells (589). They showed that the impairment in the activation of $CD8^+$ regulatory T cells can be overcome in BW mice by administering plasmid DNA vectors that encode MHC class I-binding peptides. These minigenes encoding single or multiple peptides preferentially induced $CD8^+$ T cells that can kill anti-DNA B cells and suppress glomerulonephritis in BW mice.

Therapeutic Strategies Employing Cytokines

Manipulation of cytokines that affect T cells, B cells, or target tissue alters murine lupus. T cells from virtually all SLE mice develop defects in the production of IL-2 and the presentation of IL-2 receptors on their surfaces. Consistently, treatment of MRL-lpr mice with the human IL2 gene delivered via live vaccinia recombinant viruses or with the murine IL2 delivered via Salmonella typhimurium suppresses glomerulonephritis, autoantibodies production and lymph node enlargement (590 ,591). However, intramuscular injections with cDNA

expression vectors encoding Il2 gene increased autoantibody production in MRL-Fas(lpr) mice (314), and treatment with drugs that inhibit IL-2, such as cyclosporine and FK506, are beneficial (discussed earlier). Rapamycin has some effects that are similar to those of Cy-A and FK506—it prolongs life and reduces lymphoproliferation and nephritis in MRL-Fas(lpr) mice (592).

Interleukin-4 (IL-4) is a multifunctional cytokine. Although most studies have focused on the B cell stimulatory and Th2 promoting properties of IL-4 in the development of autoantibodies and autoantibody-mediated diseases, a few reports suggest a T-cell suppressor role for this cytokine in lupus. Since these properties of IL-4 may sometimes result in opposing outcomes, amplifying or inhibitory, on overall B cell functions, it is not surprising that a few studies have found no role for IL-4 in the development of autoantibodies and lupus. Evidence for a more novel role for IL-4 in the development of lupus nephritis comes from recent studies, which suggests that IL-4 may directly promote extracellular matrix deposition in the glomeruli. Consistent with this idea, blockade of IL-4 by antibody or drug treatment or of its signaling by inactivation of the Stat6 gene ameliorates glomerulosclerosis and delays or even prevents the development of end-stage renal disease, despite the presence of high levels of IgG anti-dsDNA antibodies (236 ,593). Thus, IL-4 may serve multiple roles in the development of lupus: it may enhance autoantibodies production via its direct B cell effects, protect against autoimmunity via its T-cell suppressor effect, or perpetuate tissue damage via its direct effects on target organs (594).

Studies of cytokine mRNA in BW and MRL-Fas(lpr) mice with established clinical lupus suggest that both Th1 and Th2 subsets are activated. In BW mice, the largest population of pathogenic anti-DNA are IgG2a, an isotype that depends on IFN-γ (from Th1 cells) for its synthesis. There are high levels of mRNA for both IFN-γ (i.e., a Thl cytokine) and IL-10 (i.e., a promoter of Th2-cell development) in lymphoid tissues. Inhibition of IL-10 by continuous administration of anti–IL-10 significantly delays the onset of lupus in BW mice (184), probably by interfering with the generation of IL-6 (i.e., a B cell growth factor), because simultaneous administration of anti–IL-6 abrogates the benefit of anti–IL-10 and administration of IL-6 worsens disease (183). In MRL-lpr mice, however, IL-10 deficiency exacerbates lupus manifestations, whereas administration of rIL-10 reduces IgG2a anti-dsDNA autoantibodies production presumably through inhibition of pathogenic Th1 cytokine responses (595). In addition to increases in Th1 and Th2 cytokines in lupus mice, pro-inflammatory cytokines, including IL-1 and TNF-α, that are derived primarily from monocyte/macrophages are increased (313). IFN-γ is a cytokine of central importance in several strains of murine lupus. Administration of this cytokine worsens murine SLE in BW mice; administration of antibodies to IFN-γ or of soluble IFN-γ receptors to BW mice before disease begins significantly prolongs survival and diminishes immunoglobulin deposition and lymphocytic infiltration of kidneys (130). In MRL-Fas(lpr) mice, antibodies to IFN-γ do not alter disease (596), but lowering serum levels of IFN-γ with IFN-γ R/Fc molecules was effective (597). Gene therapy of MRL-Fas(lpr) mice with intramuscular injections of plasmids containing complementary DNA (cDNA) encoding IFN-γ R/Fc molecules resulted in reduced serum levels of IFN-γ, and reduced levels of autoantibodies, lymphoid hyperplasia, and glomerulonephritis, with prolonged survival. Treatment after mice had established nephritis was also effective (597). Genetic deletion of IFN-γ receptor significantly delayed nephritis in BW mice, although the mice developed lethal lymphomas at 1 year of age (598).

Other cytokines have been studied as therapeutic agents in murine lupus. The response of MRL-Fas(lpr) mice to granulocyte colony-stimulating factor (G-CSF) was complex: chronic administration of low doses accelerated nephritis, whereas high doses prolonged survival and prevented inflammation in glomeruli even in the presence of Ig deposits (599). Inhibition of IL-4 in mice transgenic for IL-4 prevented the glomerulosclerosis that occurs in those transgenics (600). Another strategy for changing regulation is to provide large quantities of cytokines. Gene therapy in which cytokines in vectors were injected intramuscularly into MRL-Fas(lpr) mice once a month showed that IL-2 accelerated disease, whereas TGF-ß suppressed it (314). TGFbeta, a cytokine required for generation of regulatory/suppressive T cells early in immune responses, but also involved in promoting glomerular sclerosis in later disease, was inhibited by administration of the angiotensin-converting enzyme (ACE) inhibitor, captopril, to BW or MRL/lpr mice either before or after proteinuria appeared. Treatment delayed proteinuria in pre-morbid mice and reduced chronic renal lesions in older mice, without affecting autoantibodies production. Expression of both TGF-beta1 and TGF-beta 2 isoforms were reduced in the kidneys and IL-4 and IL-10 were reduced in spleen cells (593). It is likely that timing and quantities of TGF-ß are critical in obtaining either immunosuppression or suppression of sclerosis. TNF-α plays a major role in inflammation and immune responses (192). BW mice produce abnormally low quantities of TNF-α, which is a defect that correlates with an unusual restriction fragment length polymorphism in the TNF-α gene (190 ,601). Initial studies reported that administration of normal recombinant TNF-α delayed the development of nephritis (190). The ability of the recombinant molecule to suppress established nephritis and prolong survival in BW mice was shown, but the benefit was lost after a few months. Another study reported that low doses of TNF-α accelerated nephritis (191). Treatment of normal mice with TNF-α reduced the ability of monocytes to support lymphocyte proliferative responses to mitogens, and it inhibited both T-cell cytotoxicity and NK cell activity (192). Such effects, if they occur in autoimmune mice, should confer substantial benefit. Bindarit is a propanoic acid derivative that inhibits the chemokine monocyte chemotactic protein-1 (MCP-1) as well as TNF-α production by activated monocytes and macrophages. Treatment of BW mice with bindarit did not reduce autoantibody titers but delayed proteinuria and prolonged survival, even better than did treatment with cyclophosphamide (602 ,603).

Recently there has been great interest in the role of type I interferons, particularly IFN-α, in promoting SLE, and therefore great interest in strategies that inhibit that cytokine. Evidence that IFNalpha is important in SLE includes: (1) a major lupus-promoting gene in the Nba2 region from NZB mice, Ifi202, is regulated by interferons and once upregulated probably protects B cells from apoptosis (60), (2) peripheral blood cells from patients with SLE display a genomic signature with striking upregulation of genes controlled by IFN types 1 and 2 (604), (3) administration of IFN-α to young BW mice accelerates disease (209), and 4) type I interferon receptor deficiency reduces autoimmunity in NZB mice (208). It is possible that IFN-α is the “boss” cytokine in SLE, in the same manner that TNF-α is the “boss” cytokine in rheumatoid joints. Important advances in this area can be expected between now and the next edition of this text.

New strategies that alter cytokines in BW mice involve administration of C-reactive protein (CRP) or suppressive oligonucleotides. There is a discussion of them in the section on cytokines, but they have effects on the interactions between innate immunity and adaptive immunity that may be equally important. CRP interaction with apoptotic materials facilitates their phagocytosis and clearance (194). It is likely that this process must be intact to reduce the quantitative level of autoantigen presentation by apoptotic cells and bodies that can stimulate autoantibody formation. Suppressive oligonucleotides expressing TTAGGG motifs impair the activation of DC and macrophages and therefore their release of interferons, TNF-α and IL-12, all of which are pro-inflammatory. Administration of CRP to BW mice delayed proteinuria but not anti-DNA formation; the benefit depended on IL-10 (196). BW mice transgenic for human CRP expressed the protein primarily in renal tissue; deposition of IgM and IgG and glomerular damage were reduced (197).

In summary, cytokines appear to have multiple effects on the development of lupus disease. Some cytokines, such as TGF-β and IL-4, appear to have suppressor effects in early stages of disease by modulating immunity, whereas the same cytokines might exacerbate late stages of disease by promoting local tissue repair and remodeling. This is clearly represented in manipulations in which autoantibodies production persists, but renal damage is diminished, thus separating these as two disease manifestations. Doses and timing of cytokine manipulation are critical to outcome, and may be achieved by many different mechanisms.

Strategies to Replace Stem Cells

An important question in SLE is whether replacement of bone marrow stem cells with allogeneic cells from MHC-compatible normal mice (378), or with syngeneic cells that have been depleted of T cells (325), will prevent (or at least delay) disease. Immunoablated MRL-Fas(lpr) mice receiving bone marrow from MRL+/+ or other H-2–matched strains developed mild rather than the usual marked lymphoproliferation; survival was prolonged (605). Interestingly, immunoablation with radiation or high-dose cyclophosphamide followed by transfer of T-cell–depleted syngeneic MRL-Fas(lpr) marrow also prolonged survival (325). In BW mice, transfer of bone marrow–derived pre–B cells from normal donors also suppressed autoantibody production (204). Such stem cells can even be provided from human cord blood (606). Bone marrow stem cell transfer also has benefited BXSB mice (607). These data provided background for the recent studies of immunoablation followed by autologous stem cell transplantation in patients with SLE, several of whom had impressive improvement (608).

Strategies to Alter Generation of Eiconasoids: The Role of Diet

Because inflammation in murine SLE is mediated by multiple molecules, including products of arachadonic acid (AA) metabolism, there has been interest in deviating the products of AA toward less proinflammatory metabolites than the leukotrienes and thromboxanes. This can be done by giving PGE or analogues, or by altering diets. The administration of PGs to BW mice influences their SLE. Repeated injections of PGE1 suppress nephritis and prolong survival (609 ,610 ,611). Two days of treating MRL-Fas(lpr) mice with a PGE analogue, misoprostol, reduced renal cortical IL-1 mRNA levels but not leukotrienes (612).

Dietary factors have a major influence on murine lupus. Calorie reduction alone, to approximately 40% of the usual laboratory mouse dietary intake, significantly prolongs survival and suppresses lymphoproliferation, autoantibody production, increases in Thl and Th2 cytokine production, and nephritis in NZB, BW, MRL-Fas(lpr), and BXSB mice (148 ,613 ,614 ,615 ,616 ,617). Restriction of dietary fat seems to be more important than restriction of protein. Diets that are rich in unsaturated fats and in omega-3 fatty acids, such as fish oil, flaxseed, menhaden oil, and eicosapentanoic acid, are associated with improved survival and markedly less lymphoproliferation, autoantibodies production, nephritis, and vasculitis in NZB, BW, and MRL-Fas(lpr) mice (613 ,615 ,618 ,619 ,620 ,621 ,622 ,623 ,624 ,625 ,626 ,627 ,628). In contrast, diets that are enriched in saturated fats and in omega-9 and omega-6 fatty acids are associated with reduced survival, enhanced oncogene expression, and severe lymphoproliferation (613 ,615 ,618 ,625 ,629).

The most likely explanation for the profound effects of diet in murine lupus relate to the conversion of dietary fats to various AA metabolites (i.e., PGs and leukotrienes). Presumably, the omega-3 fatty acids are precursors of molecules that are less inflammatory and/or immunostimulatory than the products of omega-9 and omega-6 fatty acids. Omega-3–rich diets lower the production of leukotriene B4 and tetraene peptidoleukotrienes by peritoneal macrophages, presumably reducing inflammation (628). Additionally, they increase antioxidant enzyme gene expression and decrease tissue levels of proinflammatory cytokines such as IL-6 and TNF-α (148 ,629).

Dietary factors that are unrelated to lipids also influence murine lupus. BW mice that are raised on a casein-free diet have diminished anti-DNA and nephritis and improved

survival (630). The mechanism of this effect is not known. Alfalfa seeds fed to cynomolgus macaque monkeys were associated with the development of autoimmune hemolytic anemia and ANA (631). When the seeds were autoclaved before administration, however, the disease did not occur (632). Several investigators have attributed this phenomenon to the presence of L-canavanine, which is a nonprotein amino acid, in alfalfa. L-Canavanine is immunostimulatory and increases the proliferation of lymphocytes to mitogens and antigens (633 ,634). The importance of this finding in human SLE, however, is unknown.

Strategies That Manipulate Sex Hormones

The influence of sex hormones on murine lupus is highly variable, depending on the strain. Hybrid mice that are derived from NZ backgrounds, especially BW mice, are exquisitely sensitive to the effects of sex hormones. Females are protected from severe early life lupus by castration plus androgenic hormone, or by antiestrogens(150 ,153 ,154 ,155 ,156 ,635). Estrogens worsen their disease, probably through toxic effects as well as immunostimulation (152). Males develop early-onset severe SLE rather than their usual late-onset disease if they are castrated and treated with estrogenic hormones or antiandrogens (150 ,155 ,156). Whether this relates to the modification of immune responses by sex hormone receptors in immune cells or to modification of gene expression is unclear. In contrast, male BXSB mice develop rapid-onset, early-life lupus whether or not they are castrated or receive sex hormones (156). MRL-Fas(lpr) mice are intermediate between BW and BXSB; that is, estrogenic hormones tend to worsen and androgenic hormones to suppress disease manifestations, but the effects are less dramatic than in BW mice (156). In fact, the effects of estrogen in MRL-Fas(lpr) mice are to worsen renal disease but to lessen vasculitis and sialadenitis (636). This could result from the simultaneous stimulation of antibody responses and suppression of T-cell– and NK-cell–mediated immunity (160), but the effects of sex hormones are doubtless more complicated than that. Studies in normal mice transgenic for Ig genes that encode anti-DNA show that estrogen affects B cell tolerance and permits survival of autoreactive B cells (161). Administration of tamoxifen to MRL-Fas(lpr) mice and to BW mice reduces renal damage and prolongs survival (637). Prolactin administration worsens lupus in BW mice, whereas bromocriptine suppresses it (157 ,158 ,159 ,638). New strategies that reduce sex hormone levels include administration of antisense oligonucleotides to Galpha(Q/11) which inhibits the G proteins required to transmit the effect of gonadatropin-releasing hormone. BW mice receiving the anti-sense oligonucleotides had reduced levels of autoantibodies and proteinuria, with inhibition of IL-6 production (639). A diet enriched in indole-3-carbinol, an anti-estrogen abundant in cruciferous vegetables, was given to BW mice, and resulted in lower levels of anti-DNA, less severe nephritis, and dramatic improvement in survival (640). Studies in this interesting area are likely to expand in the next few years.

Strategies That Protect Target Organs from Damage after Immunoglobulin Deposition

Protecting tissue from damage induced by deposition of immunoglobulins, rather than altering immunoglobulin production, is another strategy for treating lupus. For example, administration of NG-monomethyl-l-arginine, which suppresses nitric oxide production, reduces the severity of arthritis and nephritis in MRL-Fas(lpr) mice (641). High quantities of inducible nitrous oxide synthetase (iNOS) are expressed in kidneys of MRL-Fas(lpr) mice after nephritis begins; administration of linomide significantly decreases iNOS mRNA levels and prevents development of nephritis (642). Similarly, administration of aminoguanidine reduced glomerular expression of both iNOS and TGF-ß mRNA in BW mice: this effect was associated with less glomerulosclerosis (643). Antibody MEL-14, which blocks the homing of lymphocytes to lymph nodes, suppressed adenopathy in MRL-Fas(lpr) mice; it did not alter autoantibody production (644). Combined treatment with antibodies to LFA-1α and ICAM-1 reduced Ig and C3 deposition in glomeruli and prolonged survival in mice treated after induction of chronic GVHD (645). Inhibition of thromboxane A and endothelin receptors reduced histologic renal damage, hypertension, and proteinuria in BW mice (646 ,647). Administration of antibodies to ICAM-1 protected MRL-Fas(lpr) mice from skin vasculitis and behavioral abnormalities that occurred in the controls (648). Administration of heparin or a heparinoid prevented binding of nucleosome/antinucleosome immune complexes to glomerular basement membrane of BALB/c mice and delayed proteinuria and histologic glomerular damage in MRL-Fas(lpr) mice for several weeks (649). Another method to prevent damage is to inhibit development of activated terminal components of complement proteins. Administration of a monoclonal antibody specific for the C5 component of complement blocks cleavage of C5 and generation of C5a and C5b-9. Continuous therapy with anti-C5 for 6 months reduced nephritis and increased survival in BW mice (650). Finally, deposition of immune complexes in glomeruli can be prevented by administration of a soluble peptide selected from a peptide display library for reaction with a mouse monoclonal pathogenic anti-DNA (but not with nonpathogenic monoclonals) (651).

Miscellaneous Interventions

Several additional strategies that affect murine lupus should be noted. Exposure of BW mice to ultraviolet (UV)A light, especially if the mice are shaved to maximize the exposure, was associated with prolonged survival, reduced lymphoproliferation, and suppression of anti-DNA antibodies (652). In contrast, exposure of BXSB mice to UV light that included UVA and UVB, and was reproduced with UVB alone, exacerbated disease (653).

Disease in MRL-Fas(lpr) mice has been successfully suppressed by the administration of cholera toxin (654) and of

a platelet activating-receptor antagonist (655). Administration of a single dose of thalidomide to NZB, MRL/n, and MRL-Fas(lpr) mice reduced the production of IgM and/or IgG, probably by reducing the numbers of $CD5^+$ B cells (656). Treatment of BW mice with lithium chloride prolonged survival through unknown mechanisms (657). The value of these strategies (and of others not mentioned here) will depend on whether these findings can be confirmed and the role of these compounds in altering disease elucidated.

Lupus in Domestic Animals

Spontaneous lupus-like disease has been reported in several animal species other than mice, including dogs, cats, rats, rabbits, guinea pigs, pigs, monkeys, hamsters, and Aleutian minks (658 ,659 ,660 ,661 ,662 ,663 ,664 ,665 ,666 ,667 ,668 ,669 ,670 ,671 ,672). The largest body of literature addresses SLE in dogs.

Spontaneous Canine SLE

The canine lupus model is particularly interesting because of its clinical similarity to human SLE. Like human SLE, canine lupus is a chronic disease with alternating periods of remission and relapse. In contrast, such a cyclic evolution is not observed in mice with lupus, where the disease steadily progresses to its terminal stage (673). Frequent manifestations in canine lupus include fever, polyarthritis (91%), glomerulonephritis (65%), mucocutaneous lesions (60%), lymphadenopathy, and splenomegaly (671). Other infrequent manifestations include hemolytic anemia, thrombocytopenia, and clotting (667 ,669 ,670 ,671 ,672 ,674 ,675 ,676). Bullous, discoid, and systemic type skin lesions can occur. The predominant autoantibodies, occurring in more than 90% of dogs with SLE, are ANAs and antibodies directed against individual histones. Antibodies against ssDNA, dsDNA, Ro/SSA, Sm, RNP, lymphocytes, and platelets are found, but in less than 30% (664 ,670 ,675 ,676 ,677 ,678 ,679). The H130 Id that is characteristic of anti-DNA from MRL-Fas(lpr) mice has been found on anti-DNA in dogs (680). Effective interventions include glucocorticoids, levamisole, apheresis, and tetracyclines. Most dogs respond.

In dogs, the disease can be sporadic or familial. A colony of dogs particularly susceptible to SLE was created by breeding a male and female German shepherd, each of which had SLE. As healthy sires were introduced to mate with F1 and F2 generations, the disease prevalence declined (666 ,667). There is a genetic association with MHC, as in mice and in humans (reviewed in (681)). The DLA-A7 MHC class I gene confers a relative risk of approximately 12 for SLE whether it is found in sporadic or familial disease; DLA-Al and B5 are negatively correlated with disease (668).

Because of concern that SLE may be transmitted by viruses, studies have been done to determine whether SLE in humans is more common among owners of dogs with SLE. A study of 83 members of 23 households with 19 dogs that had high-titer ANAs showed no excess in the number of cases of human SLE (682).

Induced Model of Canine SLE

Normal dogs immunized with heparan sulphate, the major glycosaminoglycan of the glomerular basement membrane, develop antinuclear antibodies, proteinuria and skin disease, and marked deposition of IgM and C3 in the dermal–epidermal junction of skin (683). Cutaneous signs associated with SLE included alopecia, erythema, crusting, scaling, and seborrhoea. Three of eight dogs showed lameness. Therefore, the heparan sulfate-immunized dog can be useful as a canine SLE model for studying immune-mediated skin disease and autoimmunity.

SLE in Cats, Monkeys, and Horses

SLE in cats usually is a spontaneous disease (684). Analyses of clinical features in 22 cases showed that glomerulonephritis (in 10 cases), neurological signs (in 9 cases), arthritis (in 9), anemia (in 8) and dermatological signs (in 7 cases) were frequent manifestations (685). Other manifestations included fever, lymphadenopathy, mucocutaneous ulcers and thrombocytopenia. In addition to spontaneous diseases, there has been interest in a series of experiments in which the administration of propylthiouracil to cats induces autoantibodies and autoimmune hemolytic anemia (686).

SLE in monkeys can be induced by feeding macaques alfalfa seeds, probably because of the immunostimulatory properties of the L-canavanine nonprotein amino acid that the seeds contain (631 ,632 ,633 ,634).

SLE is rarely reported in horses. In these cases, horses were reported to manifest with polyarthritis, proteinuria, thrombocytopenia, and a positive ANA in one case (687) and weight loss, Coombs' positive anemia, alopecia, ulcerative glossitis, generalized lymphadenopathy, and skin inflammation with dermoepidermal Ig deposits on biopsy in another case (688).

Attempts have been made to induce SLE in animals by transferring plasma from patients with SLE. Histologic evidence of glomerulonephritis was produced by repeated infusions of human plasma containing LE factors into healthy dogs in one set of experiments (689) but not in another (690). Similar experiments were unsuccessful in guinea pigs.

Efforts to induce lupus-like disease in various animals by administering lupus-inducing drugs have been largely unsuccessful. Hydralazine and procainamide have been given to dogs, guinea pigs, swine, and rats, but with little evidence of autoimmune responses (691). On the other hand, immunization of rabbits, mice, and baboons with protein or oligopeptide autoantigens (from Sm B/B8, Ro 60-kd peptides, and La/SS-B) have induced epitope spreading, antinuclear antibodies and proteinuria in a proportion of animals (466 ,467 ,468 ,469 ,470). Differences in proportions of animals that develop autoantibodies in different experiments may reflect differences in environmental stimuli to which animals are exposed in different laboratories.

Finally, dogs have been studied for evidence of vertical transmission of infectious agents that cause SLE. In breeding studies performed by Lewis and Schwartz (665), the incidence of positive LE-cell tests in inbred backcrosses and outcross

matings was not consistent with any conventional mechanisms of inheritance. The investigators concluded that the results could be explained by vertical transmission of an infectious agent in a genetically susceptible individual. Cell-free filtrates of tissues from seropositive dogs also have been injected into newborn mice (665), and these mice developed ANAs and, in some cases, lymphomas. Passage of cells or filtrates from the tumors to normal newborn puppies resulted in ANA production or positive LE-cell tests. C-type RNA viruses were identified in the tumors. In cats, autoimmunity is highly associated with the feline leukemia virus (662). It may be that autoimmune disease similar to human SLE is more closely linked to viral infections in dogs and cats than in humans.

Use and Analysis of Animal Strains for Lupus Research

A variety of animal strains develop lupus-like disease, each with particular clinical manifestations and pathogenesis, representing different stages or subsets of SLE. Whereas selection of the model that truly represents human SLE remains debated, investigators have chosen models based on the clinical manifestation or phenomena of interest within the lupus autoimmune spectrum. For example, NZB/Bl mice may be most suitable to study autoimmune hemolytic anemia; BW and NZM strains have been extensively studied for anti-dsDNA antibody production, T cell autoreactivity and typical progressive lupus nephritis. MRL-lpr/lpr mouse, on the other hand, can serve as a model for a more multisystem disease such as lupus dermatitis, arthritis, fulminant interstitial nephritis, and lymphadenopathy and autoantibodies against multiple antigens. Although not extensively investigated, canine SLE may serve as a model for relapsing-remitting disease with clinical manifestations that more closely mimic human SLE. Induced SLE models such as hydrocarbon oil-induced lupus in otherwise normal mouse strains may be particularly useful in investigating the role of various genes in the pathogenesis of lupus using gene-targeted strains that are not normally lupus-prone, as it may save the 2 years or more that is required to backcross the null genotype from the stock strains (usually C57BL6/Sv129) on to the lupus-prone backgrounds. Materials and methods required for analyses of autoantibodies and disease profile are nicely outlined in a recent review (5).

Translation of Lessons from Animal Models in to Human Disease: Successes and Failures

Parallel developments in human disease observations and animal model investigations have helped in tracing some pathogenetic steps leading to manifestations of lupus. Some observations made in animal models are already being translated onto human clinical trials. For example, more than 30 years ago, Borel et al. showed that it is possible to prevent lupus in an animal model by inducing tolerance to denatured DNA (570). About 15 years later, this finding was translated onto human disease by showing that a DNA-human IgG conjugate inhibits the formation of anti-dsDNA in vitro by lymphoid cells from SLE patients (692). Such studies eventually led to a clinical trial to evaluate a dsDNA-directed B cell tolerogen, a synthetic molecule with the ability to bind dsDNA antibodies leading to anergy or apoptosis of B cells, which has shown delayed renal flares and reduction of anti-dsDNA antibodies in a subset of SLE patients (577 ,693). Similarly, identification of anti-DNA Ig-derived peptides as T cell epitopes in murine lupus in early 1990s led to a clinical trial using anti-DNA Ig-derived peptides in human SLE by Teva Pharmaceuticals. However, past experience has taught us that rush to clinical trials must not occur without full realization that the biological basis by which an intervention may suppress disease in an animal model may not be directly translatable to humans. Thus, while the full elucidation of human lupus pathogenesis and the most effective therapies to suppress or prevent disease will clearly require intense investigations using current model systems and development of newer models that are more representative of human disease, a more intensive effort will be needed to translate this knowledge towards the development of disease stage-specific biomarkers and treatments for human disease.

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Chapter 19

Pathogenesis of Atherosclerosis in Lupus

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Introduction

Premature atherosclerosis (ATH) is a major comorbid condition in systemic lupus erythematosus (SLE). While typical features of SLE such as nephritis and vasculitis have been the traditional focus of treatment, the identification of comorbid conditions such as atherosclerosis has become more important as the treatments for SLE improve and patients live longer. In a landmark study, Urowitz et al. described a bimodal mortality curve where early mortality (first 10 years after diagnosis) was primarily because of lupus-related causes and infection, while late deaths (more than 10 years after diagnosis) were primarily attributable to atherosclerotic and thrombotic disease (1). The mortality rate from atherosclerosis in SLE patients has been between 6% to 16% in various recent series (2). The incidence of subclinical atherosclerosis in SLE is also increased; in a recent study using carotid ultrasounds, a 37% prevalence of carotid atherosclerosis was found in lupus patients compared to 15% of controls ($p < 0.001$) (3). Manzi et al. found a prevalence of subclinical carotid atherosclerosis of 40% in their cohort (4). Asanuma et al. also found an increased prevalence of subclinical atherosclerosis when electron beam computerized tomography was used as the screening instrument, with coronary calcification present in 31% of SLE patients compared to 9% of controls (5).

The traditional risk factors of hypertension (2 ,6 ,7), hypercholesterolemia (1 ,7 ,8), diabetes mellitus (1 ,7), older age (2 ,7 ,8), and postmenopausal status (2 ,8) have all been associated with atherosclerotic disease in SLE patients. Case control studies have suggested that independent factors associated with SLE are also risk factors. Manzi et al. compared age-specific incidence rates for coronary artery disease (CAD) for SLE patients and controls from the Framingham Offspring Study (8). Women with SLE between ages 35 to 44 were 50 times more likely to have a myocardial infarction (MI) than controls. In a Canadian cohort after controlling for gender, blood pressure, diabetes, cholesterol, smoking, and left ventricular hypertrophy, the relative risk attributed to SLE for MI was 10.1 and 7.9 for stroke (9). Thus, while SLE patients are subject to the same traditional risk factors as the general population (9 ,10 ,11), these factors do not adequately account for the significantly increased level of cardiovascular disease. The mechanisms of the increased and accelerated atherosclerotic risk for SLE patients remain to be determined. It is likely that multiple mechanisms are operative, including inflammation of arteries, degeneration of intimae and media of arteries, and prothrombotic states (12 ,13). To further understand the pathogenesis of atherosclerosis in SLE, it is important to first develop an understanding of the development of atherosclerosis in the general population, and the role played by inflammation.

Inflammation and the Pathogenesis of Atherosclerosis

For many years, atherosclerosis was regarded as a passive accumulation of lipids in the vessel wall. Recently, however, it has been realized that inflammation plays a role not only in the development of the atherosclerotic lesion, but also in the acute rupture of plaques that occurs during acute myocardial ischemic events (12 ,13). As in the pathogenesis of SLE itself, the interplay of multiple inflammatory mediators, including leukocytes, cytokines, chemokines, adhesion molecules, complement, and antibodies, results in the formation of atherosclerotic plaques (14).

Adhesion Molecules and Leukocyte Recruitment

Atherosclerotic lesions begin with the recruitment of inflammatory cells such as monocytes and T cells to the endothelial wall. First, the endothelial cells are stimulated to express leukocyte adhesion molecules, including E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) (14). These cell-surface proteins are upregulated during periods of inflammation. For example, the expression of adhesion molecules can be induced by pro-inflammatory cytokines such as TNF-α and IL-1, which upregulate leukocyte adhesion molecules in an NF-κB dependent process (14). Also, VCAM-1 can be induced in response to exposure of the endothelial cells to the lipopolysaccharides of Gram-negative bacteria, lysophosphatidylcholine (LPC) and oxidized phospholipids such as oxidized LDL (OxLDL) (15 ,16). Additionally, the expression of ICAM-1 can be induced in response to hemodynamic stress, as in cases of hypertension (17). High Density Lipoproteins (HDL) are capable of inhibiting the

expression of these cell surface adhesion molecules (18 ,19), likely through inhibition of endothelial cell sphingosine kinase, an enzyme that catalyzes a step in the pathway by which TNF stimulates the expression of endothelial cell adhesion molecules (20).

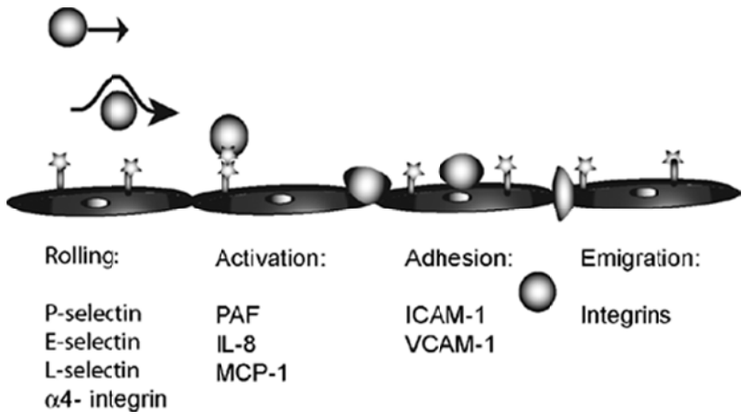

Figure 19-1. Adhesion molecules are involved in the adhesion of monocytes to activated endothelial cells. Monocytes randomly contact endothelial cell adhesion molecules. Selectins such as E-selectin slow the monocyte and induce rolling of the monocyte along the endothelial surface. Next, the monocyte firmly attaches to the surface by binding to vascular cell adhesion molecule 1 (VCAM-1) or intercellular adhesion molecule 1 (ICAM-1), which interact with integrins on the monocyte surface. Once the monocyte is tightly bound, it then migrates between endothelial cells in response to MCP-1.

The process of leukocyte adhesion occurs in several steps. First, E-selectin mediates the rolling and loose tethering of leukocytes on the endothelial cell surface, before they are more tightly bound by VCAM-1 and ICAM-1 (Fig. 19-1) (21). The importance of these adhesion molecules in the development of atherosclerosis is highlighted by the fact that atherosclerosis-prone apoE deficient mice who are also deficient in E-selectin develop fewer plaque lesions (22). Also, soluble levels of VCAM-1 can be detected in the systemic circulation, and elevated levels of this adhesion molecule have been found in humans with coronary artery disease (CAD) (23 ,24). In one cross sectional carotid ultrasound study of SLE patients, however, neither levels of soluble VCAM-1 nor ICAM-1 were significantly associated with carotid plaque (3).

Leukocyte Migration into the Intima

After leukocytes adhere to the cell surface, they migrate through the endothelium and into the intima (14). This transmigration is influenced by several factors; first, several chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1) are produced by the endothelial and smooth cell layers (25). The expression of MCP-1 in smooth muscle cells and endothelial cells can be induced by both cytokines such as TNF-α and IL-1 and by OxLDL (25 ,26 ,27). Additionally, some of the components released in the complement cascade, such as C5a, are strongly chemotactic for monocytes, and can also stimulate endothelial cells to express MCP-1(25 ,28). Conversely, normal HDL inhibit the expression of MCP-1(20). The importance of MCP-1 in the development of the atherosclerotic plaque is emphasized by the fact that atherosclerosis-prone mice who are also deficient in MCP-1 or its receptors have drastically reduced atherosclerotic plaque burdens compared to mice with normal MCP-1 levels (29 ,30). Elevated circulating levels of MCP-1 are also positively related to increased carotid artery intimal-medial thickness intimal-medial thickness (IMT) in humans (31).

Low-Density Lipoproteins and the Development of Foam Cells

Next, low-density lipoproteins (LDLs) are transported in a concentration dependent manner into artery walls (32). These LDLs become trapped and bound in the extracellular matrix of the subendothelial space (33), and seeded with oxidant waste products from nearby artery wall cells. These trapped and seeded LDLs then accumulate additional reactive oxygen species from nearby artery wall cells. When a critical threshold of reactive oxygen species is reached, LDL phospholipids become oxidized, resulting in the formation of specific pro-inflammatory oxidized lipids (POVPC, PAPC, PGPC, and PEIPC). When endothelial cells (34) are exposed to these pro-inflammatory oxidized lipids, they release cytokines such as monocyte chemotactic protein-1, macrophage colony-stimulating factor (M-CSF), and growth-related oncogene (GRO), resulting in monocyte binding, chemotaxis, and differentiation into macrophages (34). (HDL) cholesterol are capable of inhibiting the transmigration of leukocytes in response to OxLDL (35). The OxLDL are phagocytized by infiltrating monocytes/macrophages, which then become the foam cells around which atherosclerotic lesions are built (32).

Monocytes and T cells infiltrate the margin of the plaque formed by foam cells. Muscle cells from the media of the artery are stimulated to grow (36). These muscle cells encroach on the lumen of the vessel and ultimately lead to fibrosis, which can render the plaques brittle. The occlusion that results in myocardial infarction can occur when one of these plaques ruptures, or when platelets aggregate in the narrowed area of the artery (36).

Inflammatory Changes in the Endothelium

As noted above, changes in the endothelium can accelerate the formation of the atherosclerotic plaque. For example, when exposed to oxidized LDL, interleukin-1, and tumor necrosis factor (TNF), the vascular endothelium undergoes a series of inflammatory changes, resulting in endothelial cell activation (ECA) (37). When ECA occurs, there is an up-regulation of leukocyte adhesion molecules such as VCAM-1, ICAM-1, and E-selectin (37). There is a loss of vascular integrity, and the endothelium becomes leaky. Additionally, ECA can result in prothrombotic changes, including enhanced plasminogen activator inhibitor type-1

release, the production of platelet activating factor, and the expression of tissue activating factor (37). Chemoattractant cytokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6), and interleukin-8 (IL-8) are also expressed (37). Thus, exposure to OxLDL induces a cascade of pro-inflammatory, pro-atherogenic changes in the endothelium (38).

HDL Clears OxLDL from the Endothelium

There are many mechanisms designed to clear OxLDL from the subendothelial space, such as macrophage engulfment using scavenger receptors (39 ,40), and enhanced reverse cholesterol transport mediated by HDL (41 ,42). In addition, HDL has also been shown to both prevent and reverse LDL oxidation (43 ,44). When Watson and colleagues incubated LDL with HDL, there was an 80% decrease in the biological activity of the LDL (45). Both HDL and its major apolipoprotein constituent, apolipoprotein A-1 (apoA-I) have been shown to prevent LDL oxidation (34 ,46). In addition to the enhancement of reverse cholesterol transport (41 ,42 ,47 ,48), HDL and apoA-I exert their beneficial effects by removing reactive oxygen species from LDL, thus preventing the oxidation of LDL and subsequent recruitment of inflammatory mediators (34 ,49). In addition to apo A-I, HDL contains several enzymes that can prevent or destroy the formation of the oxidized phospholipids in OxLDL that induce the inflammatory response (50). These enzymes include paraoxonase (51), platelet-activating factor acetylhydrolase (PAF-AH) (45), and lecithin-cholesterol acyltransferase (LCAT) (52) (Fig. 19-2).

Thus, it is not solely the amount of HDL present that determines atherosclerotic risk, as HDL function is equally significant (17). For example, during the acute phase response HDL can be converted from their usual anti-inflammatory state to pro-inflammatory. Van Lenten and colleagues studied HDL taken from humans before and after elective surgery (53). Before surgery, HDL were able to inhibit LDL oxidation and LDL-induced monocyte chemotactic activity in a human artery wall cell coculture model. Three days after surgery, however, at the peak of the acute phase response, HDL promoted LDL oxidation and monocyte chemotactic activity; HDL returned to an anti-inflammatory state 1 week after surgery (53). Thus, acute phase HDL can be described as pro-inflammatory. Van Lenten et al. also found that levels of anti-inflammatory components of HDL such as apoA-I and HDL-associated paraoxonase activity were reduced in the acute phase response (53). Additionally, acute phase HDL was greatly enriched in acute phase reactants such as serum amyloid A (53). Thus, HDL can be described as a “chameleon like lipoprotein;” anti-inflammatory in the basal state and proinflammatory during the acute phase response (32) (Fig. 19-3). This acute phase response, however, can also become chronic (54), and may be a mechanism for HDL dysfunction in SLE.

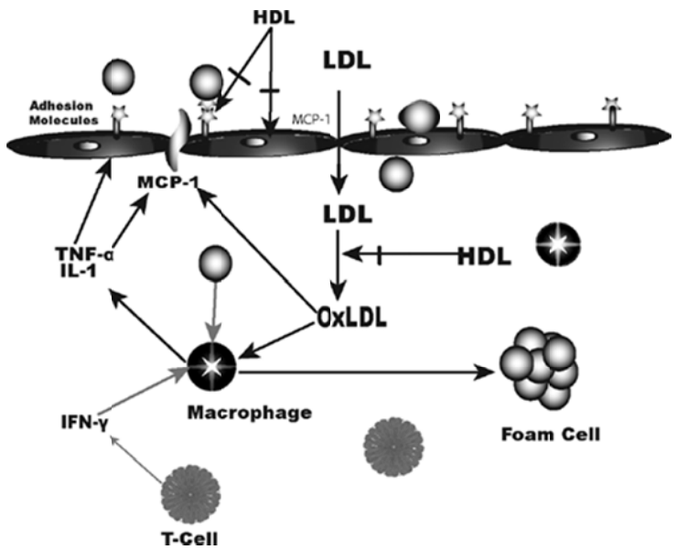

Figure 19-2. Atherosclerosis is an inflammatory disorder that is initiated by the interplay of cytokines, lipids, oxidation products, and leukocytes. The process begins when LDL enters and is trapped in the arterial intima. LDL is oxidized and transformed into oxidized LDL (OxLDL). OxLDL then activates endothelial cells to express monocyte chemotactic protein 1 (MCP-1), which attracts monocytes from the vessel lumen and into the subendothelial space. OxLDL then promotes the differentiation of monocytes into macrophages. Macrophages in turn release a variety of chemicals, including cytokines. Of these cytokines, tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) activate endothelial cells to express adhesion molecules that bind monocytes, making them available for recruitment into the subendothelial space by MCP-1. Normal functioning HDL inhibits the formation of oxidized LDL, the expression of endothelial cell adhesion molecules and MCP-1, and promotes the efflux of cholesterol from foam cells.

The Role of Cytokines in Atherosclerosis

T cells, primarily of the Th1 subtype, are also abundant in atherosclerotic lesions, and may play a role in the formation of plaque through the cascade of cytokines that is initiated by their activation (55). Vascular endothelial and smooth muscle cells are important targets for inflammatory cytokines, and these cells can produce additional cytokines when stimulated (13). At least two stimuli for Th1 differentiation are present in the atherosclerotic plaque. IL-12 is expressed by macrophages, smooth muscle cells, and endothelial cells, and is an important stimulus for Th1 differentiation (56). Elevated levels of IL-12 have been found in atherosclerotic plaques (56), and the inhibition of IL-12 using a vaccination technique that fully blocks the action of IL-12 has been shown to decrease atherosclerosis in mice (57). IL-12 production is upregulated in monocytes exposed to oxidized LDL (56).

IFN-γ has also been detected in human plaques (14). It is a powerful growth inhibitor for smooth muscle cells, endothelial cells, and collagen production, and thus promotes plaque instability (58). Additionally, IFN-γ induces the expression of secretory phospholipase A2, leading to the

production of inflammatory lipid mediators such as LPC, PAF, and eicosanoids (59). INF-γ also improves the efficiency of antigen presentation, and leads to increased synthesis of TNF-α and IL-1 (60). All of these actions contribute to the formation of the atherosclerotic plaque, and indeed, in atherosclerosis-prone apoE knockout mice who are also lacking IFN-γ, atherosclerosis is decreased by nearly 60% (61 ,62). The administration of IFN-γ also accelerates atherosclerosis in apo-E knockout mice. Increased levels of both IFN-γ and IL-12 have been found in humans with unstable and stable angina compared with controls (63).

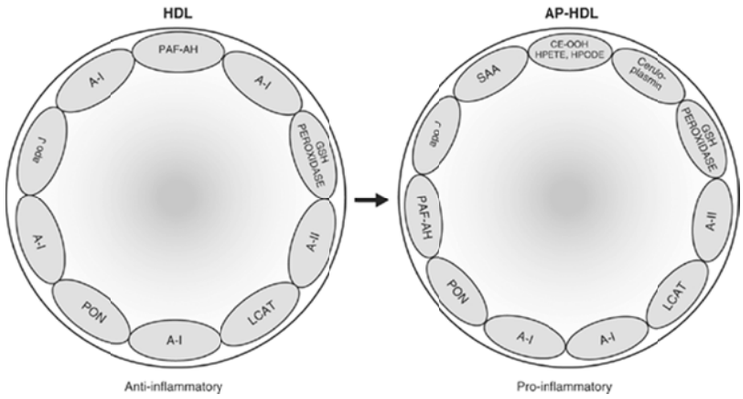

Figure 19-3. The acute-phase (AP) reaction favors the formation of proinflammatory HDL and mildly oxidized LDL. **A,** In the basal state, HDL contains apoA-I and apoJ as well as 4 enzymes, PON, PAF-AH, lecithin:cholesterol acyltransferase (LCAT), and plasma reduced glutathione selenoperoxidase (GSH peroxidase) that can prevent the formation of or inactivate the inflammatory LDL-derived oxidized phospholipids found in mildly oxidized LDL. As a result, in the basal state, HDL may be considered anti-inflammatory. During the acute-phase reaction, A-I may be displaced by the pro-oxidant acute-phase reactant SAA. Another pro-oxidant acute-phase reactant, ceruloplasmin, associates with HDL as does the anti-oxidant acute phase reactant apoJ. PON, PAF-AH, and LCAT decrease in HDL during the acute-phase reaction, and the lipid hydroperoxides HPETE, HPODE, and cholesteryl linoleate hydroperoxide (CE-OOH) increase in HDL. A-II and GSH peroxidase are shown as unchanged during the acute-phase reaction although there are no data on the latter. The net effect of the changes in HDL during the acute-phase reaction is the production of pro-oxidant, proinflammatory HDL particles (AP-HDL). (From Navab M, Berliner JA, Subbanagounder G, et al. HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. *Arterioscler Thromb Vasc Biol* 2001;21(4):481–488.)

As noted above, TNF-α and IL-1 are also present in human atherosclerotic lesions. Like IFN-γ, they also affect smooth muscle proliferation. TNF-α and IL-1 induce local inflammation in blood vessels by stimulating the activation of macrophages (17), inducing the secretion of matrix metalloproteinases (64), and promoting the secretion of cell surface adhesion molecules (14). TNF-α can also upregulate the expression of cell surface adhesion proteins; normal functioning HDL can inhibit this upregulation (65). TNF-α and IL-1 can also inhibit lipoprotein lipase, an enzyme important in the metabolism of triglycerides and very low-density lipoprotein (VLDL) (66 ,67). Additionally, TNF-α and IL-1 enhance production of M-CSF, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) by smooth muscle cells, endothelial cells, and monocytes. These mediators activate monocytes and stimulate their transformation into macrophages and foam cells (68). Inhibition of TNF-α decreased the progression of atherosclerosis in apoE knockout mice (69). Elevated levels of TNF-α may also play a role in the increased risk of atherosclerosis in the general population (70). TNF-α has been linked to vascular injury in both acute and chronic inflammatory conditions. TNF-α has been identified in human endothelial and smooth muscle cells in all stages of atherosclerosis, from early intima thickening to established occlusive atherosclerosis (71 ,72).

In addition to TNF-α and IL-1, the pro-inflammatory cytokine pathway also involves the expression of IL-6 (14). Circulating IL-6 is another cytokine marker that is a strong independent marker of increased mortality in unstable CAD (73). IL-6 stimulates hepatocytes to produce CRP and other markers of inflammation (74). CRP is not only a marker of

acute phase reaction, but may also have a direct effect on leukocyte recruitment and apoptosis in vessel walls (75 ,76). IL-6 is also required for short-term regulation of paraoxonase (PON), an anti-oxidant enzyme present in HDL (77). LDL-derived phospholipids such as oxidized palmitoyl arachidonyl phosphatidyl choline (OxPAPC) induce the expression of IL-6, which in turn down regulates PON mRNA levels (73). Patients with unstable coronary syndromes have elevated levels of both IL-6 and CRP (78 ,79). Additionally, IL-6 has been described as an independent predictor of endothelial dysfunction in rheumatoid arthritis (RA) patients (80).

TGF-β, in contrast, likely plays a protective role against atherosclerotic plaque formation, as discussed in several publications (39 ,70 ,71 ,72 ,73 ,74 ,75 ,76). Thus, the balance of pro-inflammatory and anti-inflammatory cytokines and their interactions with inflammatory cells and lipid components contributes to the formation and maintenance of the atherosclerotic plaque (Table 19-1).

Table 19-1: Some Atherogenic Cytokines and Their Mechanisms

Cytokine	Expressed By	Induced By	Biological Function	Evidence fo Role in ATH	References
MCP-1	Smooth muscle cells, foam cells	TNF-α IL-1 OxLDL Complement Elevated Homocysteine Inhibited by HDL	Chemotactic factor for monocytes	Increased MCP-1 levels related to carotid artery IMT	(25,26,27,29,30,31,37)
TNF-α	Endothelial cells,	IL-6	Activate Macs	Increased	(14,17,64,65,66,67,68,69,70,71,72)
IL-1	Macs, T-cells, foam cells	Inhibited by HDL	Increase adhesion molecules Inhibit LPL Increase SAA Increase MCP-1 Increase CD40L	TNF-α levels increase ATH risk	
IFN-γ	T-cells, foam cells		Inhibit SMCs, EC, collagen Increase plaque instability Increase TNF-α IL-1 Inhibit LPL Prime Macs for activation Increase CD40	Increased IFN-γ in unstable angina	(14,58,59,60,61,62,63)
IL-6	Smooth muscle cells, ECs, foam cells	OxLDL TNF-α IL-1 IFN-γ	Increases SAA Stimulates hepatocytes to produce CRP Regulates paraoxonase	Predictor of unstable angina, endothelial dysfunction	(14,37,73,74,75,76,77)
IL-12	Macs, SMCs, ECs	OxLDL	Th1 differentation	Increased levels seen in ATH plaques	(56,57,63)

ATH, atherosclerosis; CRP, C-reactive protein; EC, endothelial cell; LPL, lipoprotein lipase; Macs, macrophages; OxLDL, oxidized LDL; SMC, smooth muscle cells.

Abnormalities in SLE

Many of the inflammatory mediators described above are also actively involved in the pathogenesis of SLE, and are thus likely to play a role in early atherogenesis. Several of these inflammatory risk factors for atherosclerosis, as well as traditional risk factors, have been demonstrated in patients with SLE.

Dyslipoproteinemia and SLE

Hyperlipidemia is a well established risk factor for coronary artery disease in the general population. Hyperlipidemia is also common in SLE, and has been described in more than 50% of lupus patients (7). Secondary causes of hyperlipidemia, such as the nephrotic syndrome (81) and medications such as corticosteroids (82) do play a role in SLE (83). The disease activity of lupus itself, however, has also been associated with dyslipidemia. High levels of VLDL and triglycerides

(TG) and low levels of HDL have been described as the “lupus pattern,” and are more strikingly noted in patients with active disease (84). Further investigation has suggested that the interplay between blood lipids and chronic inflammation may be one contributing factor to atherosclerosis in SLE (85).

Oxidized Low-Density Lipoproteins

As noted above, the oxidation of LDL is a triggering mechanism in the pathogenesis of atherosclerosis. In fact, elevated levels of circulating oxidized LDL are strongly associated with documented coronary artery disease in the general population (86). Elevated levels of circulating OxLDL have also been described in SLE patients, especially in those with a history of cardiovascular disease (87 ,88). Levels of the oxidized phospholipid OxPAPC have also been associated with thickened IMT on carotid ultrasound (89). Interestingly, renal manifestations of SLE have also been associated with higher levels of circulating OxLDL (88).

Circulating antibodies to OxLDL (anti-OxLDL) have also been described, although the relationship to the development and progression of atherosclerosis is unclear. Elevated levels of antibodies against OxLDL have been described in the general population, and in some studies are predictive of myocardial infarction and the progression of atherosclerosis (90 ,91). Other studies, however, have not found any such correlations (14 ,92). Similarly, the presence of antibodies to OxLDL has been described in subjects with SLE. Anti-OxLDL have also been described in up to 80% of patients with SLE and antiphospholipid antibody syndrome (86 ,93 ,94 ,95). Some studies have demonstrated that autoantibodies to oxidized LDL are more common in SLE patients who have a history of cardiovascular disease than in SLE controls or normal subjects (87), although in two other studies, anti-OxLDL and arterial disease were not associated (96 ,97). Titers of antibodies to OxLDL have also been associated with disease activity in SLE (98).

There is some speculation that the increased risk of thrombotic and atherosclerotic events seen in patients with SLE and antiphospholipid antibodies may be due in part to a cross-reactivity between anticardiolipin and OxLDL (93). Cardiolipin is a component of LDL, (99) and indeed, a cross-reactivity between anti-cardiolipin and anti-OxLDL antibodies has been demonstrated (93 ,94). Additionally, β_2-glycoprotein I (β_2GPI) has been shown to bind directly and stably to oxidized LDL (100). These OxLDL-β_2GPI complexes have been found in patients with SLE and/or APS, and are associated with a risk of arterial thrombosis (101).

Lipoprotein (a)

In addition to oxidized LDL, lipoprotein (a) (Lp[a]) has also been implicated in the pathogenesis of atherosclerosis in both the general and SLE populations (86). Lp(a) is structurally related to LDL, but also contains apo(a) that is covalently linked to apolipoprotein B-100 (102). Lp(a) is rich in cholesterol, and is structurally similar to plasminogen (103 ,104). In the general population, elevated Lp(a) levels have been associated with a two-fold increased relative risk of myocardial infarction (104 ,105). Although the mechanism by which Lp(a) contributes to the pathogenesis of atherosclerosis is not well understood, Lp(a) has been shown to physically associate with pro-inflammatory oxidized LDL (106), and circulating plasma levels of both Lp(a) and OxLDL have been associated with coronary artery stenosis (86).

Several researchers have found elevated levels of Lp(a) in SLE patients (107 ,108 ,109). One study reported that serum Lp(a) levels were increased in lupus patients with renal disease and hypoalbuminemia, and that treatment with corticosteroids reduced the elevated Lp(a) levels (109). Another group reported, however, that Lp(a) levels are not influenced by corticosteroids or disease activity (108). Patients with high serum Lp(a) concentrations also had higher levels of circulating IgM OxLDL-containing immune complexes (110).

High-Density Lipoproteins

As noted above, normal HDL perform many anti-inflammatory, protective functions. HDL enhance reverse cholesterol transport by promoting the efflux of cholesterol from the artery wall (41 ,42 ,47 ,48). HDL and its major apolipoprotein constituent, apolipoprotein A-I (apo A-I), have also been shown to prevent LDL oxidation (34 ,46). They are capable of removing “seeding molecules” from LDL, thus preventing the oxidation of LDL and subsequent recruitment of inflammatory mediators (34 ,49). Additionally, normal anti-inflammatory HDL are capable of inhibiting the expression of cell surface adhesion molecules, (18 ,19), the expression of MCP-1 (20), and the synthesis of platelet-activating factor by endothelial cells (111). HDL also modulate endothelial function, likely by stimulating endothelial nitric oxide production (112 ,113).

Levels of HDL have been found to be reduced in many patients with SLE (107). In one study, HDL levels were reduced in 79% of patients with active SLE and 29% of patients with inactive disease when compared to controls (84). Additionally, decreased levels of HDL have been associated with increased TNF-α and soluble TNF receptors in SLE patients (85). Reduced levels of HDL have also been found in SLE patients with IgG anti-cardiolipin antibodies (114 ,115).

As previously noted, however, it is not solely the amount of HDL present that determines atherosclerotic risk, as HDL function is equally significant (17). Abnormal functioning, pro-inflammatory HDL has been associated with atherosclerosis in the general population. In one study of patients with documented coronary artery disease and normal HDL cholesterol levels, 19 of 20 atherosclerosis patients had pro-inflammatory HDL, compared to 2 of 20 controls (116). HDL function has also been described as abnormal in subjects with SLE. Our group has found that 45% of women with SLE, compared to 20% of RA patients and 4% of controls, had pro-inflammatory HDL that was not only unable to prevent oxidation of LDL but caused increased levels of oxidation (117). In this study, 4 of 4 SLE patients with a history of documented atherosclerosis had

pro-inflammatory HDL, further suggesting that HDL play an important role in the pathogenesis of atherosclerosis.

Abnormalities of the functional components of HDL may also contribute to the pathogenesis of ATH in SLE. Many of these abnormalities have been described in patients with SLE, including apoA-I, paraoxonase, serum amyloid A, and ceruloplasmin, and are described in detail below.

ApoA-I

ApoA-I is the major apolipoprotein component of HDL. Even when apoA-I is isolated from HDL particles, it has been shown to prevent LDL oxidation both in artery wall coculture studies and in cell-free systems (34 ,46). As a component of HDL, apoA-I exerts its beneficial effects through the enhancement of reverse cholesterol transport (41 ,42 ,47 ,48). HDL also removes “seeding molecules” from LDL, thus preventing the oxidation of LDL and subsequent recruitment of inflammatory mediators (34 ,49). Nearly 100% of ApoA-I is carried in plasma on HDL molecules.

Reduced levels of both HDL and apoA-I have been found in SLE patients with IgG anticardiolipin antibodies (95). In the general population, antibodies to apoA-I have been found in up to 21% of patients with acute coronary syndromes who have no other features of autoimmune disease (118). Antibodies to apoA-I have also been described in SLE; in one study, antibodies to apoA-I were found in 32.5% of patients with SLE and 22.9% of patients with primary antiphospholipid syndrome (APS) (115). It is unclear, however, how the presence of these antibodies affects the function of apoA-I in either SLE or acute coronary syndrome patients.

Paraoxonase

Serum paraoxonase (PON) is a serum esterase that is secreted primarily by the liver, and is associated with HDL in plasma. PON has been identified as one of the important components of HDL that prevents lipid peroxidation (119) and blocks the pro-inflammatory effects of mildly oxidized LDL (120). Decreased levels of PON activity have also been associated with atherosclerosis in the general population. One study demonstrated that PON activity in HDL was significantly lower in patients with documented coronary artery disease than in controls (49). Altered levels of PON activity have also been seen in patients with SLE. In one study, PON activity was reduced in SLE and antiphospholipid syndrome patients compared to controls, although there was no reduction in the total antioxidant capacity of the plasma (121). In that study, antibodies against HDL and ß2GPI were associated with the reduction in PON activity (121). Similarly, in patients with anticardiolipin antibodies, PON activity was reduced when compared to healthy controls (122). Although further investigation is necessary, it is possible that the antibodies against HDL and $ß_2$GPI contribute to the oxidation of LDL through a cross-reactive, inhibitory effect on PON activity (121).

Serum Amyloid A

Serum amyloid a (SAA) is an acute phase reactant that may be associated with HDL during periods of acute inflammation, and may serve as a transient cholesterol binding protein (123 ,124). ApoA-I levels bound to HDL decrease by 73% as SAA levels increase in acute inflammatory phase HDL (53). This SAA-HDL is more likely to be rapidly removed from circulation (125), and has decreased enzymatic activity compared with normal HDL (126). Inflammatory cytokines such as IL-1 and IL-6 have been shown to upregulate SAA hepatic synthesis (127 ,128). Additionally, antibodies to apoA-I have been inversely correlated to plasma SAA levels in patients with acute coronary syndromes (118). SAA levels have been shown to be elevated in SLE patients with active disease, although the relationship of SAA levels to other abnormalities of lipid metabolism in SLE has not been explored (129 ,130).

Ceruloplasmin

Ceruloplasmin is another acute phase reactant that can be associated with HDL during periods of acute inflammation. Elevated levels of ceruloplasmin, which normally carries about 95% of plasma copper (131), have been demonstrated in patients with CAD (132). HDL that have been enriched with ceruloplasmin demonstrate altered ability to inhibit LDL oxidation (133). The relationship of ceruloplasmin levels and atherosclerosis has not been described in SLE. One study of 27 SLE subjects and 20 controls did note, however, that serum ceruloplasmin levels were higher in SLE subjects than in controls (134).

Triglycerides

Triglyceride molecules also have been felt to play a role in the pathogenesis of ATH (135). Triglyceride levels are often elevated in patients with SLE (83 ,84 ,136). Several abnormalities in lipid and chylomicron metabolism that may explain these elevated triglyceride levels have been found in SLE. Chylomicrons are the triglyceride-rich lipoproteins that carry dietary lipids absorbed in the intestine (137). Chylomicron triglycerides are broken down by the enzyme lipoprotein lipase (LPL) (138). The chylomicron remnants are then removed from circulation by the liver using apoE as a ligand (138 ,139).

Disturbances in chylomicron metabolism, including decreased lipolysis and slowed chylomicron removal, have been observed in patients with SLE (140). One explanation for the decreased lipolysis of chylomicrons is the presence of antibodies to lipoprotein lipase. One study found that antibodies to LPL were found in 47% of patients with SLE (141). Another study described antibodies to LPL in nearly 40% of SLE patients; triglyceride levels were significantly elevated in the anti-LPL+ group (142). Studies are currently underway to determine if these antibodies are associated with an increased risk of atherosclerosis in SLE patients.

In addition to antibody formation, inflammation may induce other mechanisms that result in the elevation of triglycerides in SLE patients. TNF-α, IL-1, and IFN-γ have all been shown to decrease LPL enzymatic activity (66 ,67 ,143). In lupus patients, a strong positive correlation between TNF-α and plasma triglycerides has been described (144).

Endothelial Function

A number of abnormalities of the vascular endothelium have been described in association with SLE. Endothelial function can be measured by examining endothelium-dependent (flow mediated) dilation (FMD) of the brachial artery in response to reactive hyperemia. FMD is thought to be dependent on the production and release of nitric oxide (37). Endothelial dysfunction has been described as an early abnormality in the development of atherosclerosis (145), and is predictive of subsequent cardiovascular events (146) in the general population. Increased endothelial dysfunction has also been described in women with SLE. In one cohort, SLE subjects had greater endothelial dysfunction that correlated with increased carotid intima-medial thickness, even after controlling for traditional cardiac risk factors (147). One pediatric population with SLE, however, demonstrated normal endothelial function (95).

Antibodies to endothelial cells have also been described in up to 63% of subjects with SLE (148). Patients with these anti-endothelial cell antibodies were also found to have an increased prevalence of vascular lesions (including arterial and venous thrombosis and vasculitis), lupus nephritis, and anticardiolipin antibodies (148). Endothelial cell activation can also be induced by antiphospholipid and anti-$ß_2$GPI antibodies (149).

Circulating endothelial cells (CECs) in patients with SLE are associated with increased levels of complement split products, suggesting that they are a surrogate marker for the injury of the vascular endothelium that is due to complement activation (150). Elevated levels of circulating apoptotic endothelial cells have been described in SLE subjects. Apoptotic endothelial cells in this group were strongly correlated with abnormal brachial artery FMD (151).

Immune Complexes

Immune complexes (IC) have also been described as a risk factor for atherosclerosis in the general population. In one prospective study of 257 healthy men, the levels of circulating immune complexes at age 50 correlated with the future development of MI (152). Other studies have demonstrated increased levels of immune complexes containing both Lp(a) (153) and LDL (154) in patients with CAD. In vitro studies have also suggested that LDL containing immune complexes may play a role in atherogenesis. Macrophages that ingest LDL-IC become activated, and release TNF-α, IL-1, oxygen-activated radicals, and matrix metalloproteinase-1 (154). LDL-containing immune complexes have been examined in several studies of SLE subjects, with varying results. In one study of a pediatric SLE population, there was an increase in levels of IgG LDL-immune complexes in SLE subjects compared to healthy controls, although there was no association with endothelial dysfunction (95). Another study of an adult SLE population, however, demonstrated no difference from controls in levels of IgG or IgM LDL-containing IC (88).

IC containing C1q have also been implicated in atherosclerosis. C1q containing IC can bind to C1q receptors on endothelial cells, stimulating the expression of VCAM-1. C1q-IC may also contribute to atherosclerosis by interfering with cholesterol metabolism in the arterial wall. Cholesterol 27-hydroxylase is a P450 enzyme that is present in arterial endothelial cells and macrophages, and is responsible for the first step of cholesterol metabolism to 27-hydroxycholesterol. 27-hydroxycholesterol is more soluble, and thus more easily transported out of the arterial wall and into the liver. 27-hydroxycholesterol is also anti-atherogenic in other ways, as it can down-regulate cell surface LDL receptors, and suppress smooth muscle cell proliferation (155 ,156). C1q-IC have been shown to inhibit cholesterol 27-hydroxylase, and thus may contribute to atherogenesis (157). No studies to date have examined the relationship of C1q-IC to atherosclerosis in subjects with SLE.

CRP

CRP is an acute phase reactant that is synthesized in the liver in response to interleukin-6. It has been well established as a predictor of cardiovascular events in the general population; when both CRP and cholesterol levels are high, a person's overall risk of developing a future cardiovascular event is increased up to ninefold (74). There is evidence that CRP is not solely a marker of systemic inflammation, but rather may play a direct role in the pathogenesis of atherosclerosis. For example, CRP has been shown in vitro to activate endothelial cells to express ICAM, VCAM, and E-selectin (158). CRP has also been shown to activate complement (159), induce endothelial cells to produce MCP-1 (75), and mediate macrophage uptake of LDL (160). In SLE subjects, however, the role of CRP as a predictor of atherosclerosis is less clear. In one cross-sectional study, Manzi et al. found that CRP was significantly associated with focal plaque, although this effect did not persist in the logistic regression models (4); in a separate cross-sectional study, the same group found that high CRP levels >4 mg/mL were independent determinants of IMT (161). Roman et al., however, did not find an association with plaque and CRP (3).

Homocysteine

Homocysteine is another predictor of atherosclerosis in the general population (162). Homocysteine is a metabolite in methionine production, and may play a direct role in the pathogenesis of SLE through its toxic effects on the endothelium (163). Homocysteine is also prothrombotic (164), and decreases the availability of nitric oxide (165). High levels

stimulate monocytes to secrete MCP-1 and IL-8 (166). The thiolactone metabolite of homocysteine combines with LDL to enhance foam cell formation in vessel walls (167). The molecule releases free oxygen radicals that can damage tissue (168), and there are several prothrombotic actions on platelets and endothelial cells (169). Hyperhomocysteinemia can result from genetic and/or dietary factors. As previously noted, population studies have identified an association between high homocysteine levels and atherosclerosis in the general population (167). Petri has prospectively demonstrated that elevated homocysteine levels may also be a risk factor for the later development of CAD in SLE patients (170). In several studies, elevated levels of homocysteine have also correlated with ATH in SLE (10 ,87 ,171 ,172). In other recent studies of SLE, however, homocysteine has not correlated with evidence of plaque on carotid ultrasound (3 ,5 ,173).

CD40-CD40L Interactions

The overexpression of CD40 ligand (CD40L) has also been implicated in the pathogenesis of atherosclerosis in the general population (174). The interaction between CD40L, expressed on T cells, and its receptor, CD40 on B cells is involved in the mediation of B cell activation and the production of autoantibodies (175). CD40L is also expressed on macrophages, endothelial cells, activated platelets, and macrophages. Ligation can result in the induction of VCAM-1, ICAM-1, and E-selectin by endothelial cells (176), expression of tissue factor by macrophages (177), and the elaboration of cytokines such as interleukin-1SS (178). The overexpression of both CD40 and CD40L has been demonstrated in human atherosclerotic lesions (174). Additionally, healthy women with high levels of soluble CD40L have a higher incidence of cardiovascular events (179). Women with SLE also have abnormally high levels of CD40L (175). Additionally, CD40 expression is upregulated on endothelial cells of women with lupus nephritis (180). In one recent study of SLE, however, CD40 levels did not correlate with evidence of atherosclerosis on carotid ultrasound (3).

Antiphospholipid Antibodies

The role of antiphospholipid antibodies (APL) in atherosclerosis in SLE is controversial. Because these antibodies are associated with an increased risk of arterial and venous clots, it seems logical that these antibodies would also be associated with atherosclerosis. In fact, several studies in men have shown that antiphospholipid antibodies are associated with an increased risk of future myocardial infarctions (90 ,181). Also, in renal transplant patients, the presence of APL was associated with a relative risk of an atherosclerotic event of 2.82 (182). Similarly, patients with primary antiphospholipid antibody syndrome had thicker carotid artery IMT than controls, especially those older than 40 (183). Interestingly, these findings have not been convincingly replicated in SLE studies. Many lupus cohorts have not shown a consistently significant association with APL and atherosclerosis (3 ,4). Coronary calcification scores were associated with APL positivity in a univariate analysis; however, the association was no longer significant when adjusted for age and sex (5).

In a prospective Hopkins Lupus Cohort, 37 of 380 lupus patients developed myocardial infarctions; patients with a positive lupus anticoagulant (LAC) were more likely to develop a myocardial infarction than those without (184). This association, however, was not seen with anticardiolipin antibodies (ACL), and neither LAC nor ACL were associated with subclinical atherosclerosis as assessed by carotid ultrasounds. Conversely, several studies have demonstrated an association between the presence of APL and atherosclerosis in SLE. In the LUMINA study, 34 patients (6.2%) experienced a cardiovascular or cerebrovascular event after entering the cohort (185). The authors did find that APL were an independent risk factor for having an event in this cohort (185). Similarly, a cross-sectional study from England found that APL were associated with plaque seen on carotid ultrasound (147).

Animal studies evaluating the role of APL in atherosclerosis likewise remain contradictory. George and colleagues immunized LDL receptor deficient mice with $ß_2$-GPI and found that fatty streak formation was accelerated (186). They were also able to accelerate atherosclerosis by passively transferring $ß_2$-GPI reactive lymphocytes. In a follow-up study, the authors fed $ß_2$-GPI to LDL receptor deficient mice to induce oral tolerance. They were able to show that the mice that received the $ß_2$-GPI had less atherosclerosis, and that the oral tolerance was successful as assessed by a inhibition of lymph node proliferation to $ß_2$-GPI in these mice (187).

Other authors, however, have suggested the APL may play a protective role in the pathogenesis of atherosclerosis. Immunization of rabbits (188), LDL receptor deficient mice (189) and apoE deficient mice (190) with LDL and/or OxLDL inhibited the progression of atherosclerotic lesions. Furthermore, passive administration of a monoclonal IgG cardiolipin reactive antibody to LDL receptor deficient mice reduced plaque formation (191).

There are a number of mechanisms by which APL could exert their influence on atherosclerosis. As noted in the sections above, APL may cross-react with a variety of compounds that are involved in plaque formation (Table 19-2). Some studies have shown that persons without autoimmune diseases that have elevated levels of both anti-OxLDL and anticardiolipin have an increased risk atherosclerosis (90). Similarly, in one study, both anti-HDL and anti-$ß_2$-GPI antibodies were found in patients with SLE and primary APS (121). Furthermore, APL may participate in the process of atherosclerosis by binding to and activating endothelial cells (149). Additionally, APL have been shown to bind Annexin V, an antithrombotic protein, and in turn decrease the binding of Annexin V to the endothelium. Decreased Annexin V binding was noted in SLE with cardiovascular disease compared with SLE without cardiovascular disease (192). Further studies are needed to explore the contributions of antiphospholipid antibodies to atherosclerosis.

Table 19-2: Potential Cross-Reactivities of Antiphospholipid Antibodies and Factors Affecting Atherosclerosis

Factor	Reference
LDL	(99,191)
OxLDL	(90,93,94,100,101)
HDL	(101,121)
Paraoxonase	(121,122)
Annexin V	(192)
Apo A-1	(101,115)
Endothelial cell activation	(149)
Antiendothelial cell antibodies	(148)

Summary

Atherosclerosis is a complicated inflammatory process characterized by the interactions of numerous different moieties including lipids, enzymes, endothelial cells, cytokines, and peripheral blood mononuclear cells. The prevalence of atherosclerosis is higher in SLE and occurs at an earlier age. The lupus related factors that account for this increased risk are likely numerous and related to the factors described in this chapter. Expanding our understanding of the pathogenesis of atherosclerosis in SLE is critical if we are to improve the quality of care and improve mortality in this vulnerable population.

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Chapter 20

Autoantigens and Defects in Immune Tolerance in Lupus

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Introduction

Presence of autoantibodies against a variety of ubiquitous self-antigens is a hallmark of systemic lupus erythematosus (SLE) (1). While primary impairments in B cells that can produce autoantibodies have been described in lupus, T cell help is paramount for the production of pathogenic autoantibodies (2). Thus, delineation of mechanisms of autoantibody production would require thorough understanding of loss of self-tolerance in both T and B cells. In this chapter, we will introduce concepts of normal immunologic tolerance, and review potential mechanisms that lead to breakdown of tolerance in lupus. We will then describe various autoantigens that have been implicated in the induction of lupus-like autoimmunity. Several groups have used ingenious and arduous approaches to map T cell epitopes (fragments of autoantigens which can activate T cells capable of eliciting or suppressing autoantibody production by B cells) in these autoantigens. Peptides containing these autoantigenic epitopes have been administered in ways that can induce immune tolerance and prevent disease in animal models of lupus. Efforts are underway to translate these findings from model systems into human disease to develop antigen-specific therapies. These and other methods of re-establishing immune tolerance in lupus will also be discussed.

Immune Tolerance

Lymphocyte Homeostasis and Immune Tolerance

The immune system is unique in its ability to maintain a state of equilibrium despite its continuous exposure to self-antigens and mounting an adequate response to a variety of foreign antigens. After responding to an antigen the immune system returns to its original state, so that the numbers and functional status of lymphocytes are reset at roughly the original state. This process known as lymphoid homeostasis allows the immune system to respond to new antigenic challenges. The size and content of the preimmune lymphocyte repertoire are tightly regulated, as new emigrants from the lymphoid organs compete for “space” with resident cells (3). Several groups have tried to define factors that control naïve and memory T-cell homeostasis under lymphoproliferative or lymphopenic conditions (4 ,5). There has been a recent interest in the hypothesis that in lymphopenic conditions, T cells expand to re-establish homeostasis by a process dependent on self-major histocompatibility complex (MHC)-peptide recognition and on the availability of cytokines that can promote the proliferation and survival of lymphocytes. Such lymphocyte expansion is believed to be a normal physiologic process. The chronic recurrence of this process, however, might lead to selection and accumulation of high affinity self reactive T cell clones and ensuing autoimmune disease (6). A recent study indeed provides experimental support for this hypothesis (7). This study showed that autoimmune nonobese diabetic (NOD) mice have reduced number of CD4 T and B cells as compared to control mouse strains. Increasing T cell numbers such as by immunization with complete Freund's adjuvant (CFA) increases B and T cell numbers and protects these mice from autoimmune diabetes. Interestingly, self-reactive T cell receptor (TCR) transgenic T cells expand in the lymphopenic NOD mice, but not in NOD mice “filled” (reconstituted) with syngeneic T cells, in CFA-immunized NOD mice, and in congenic B6.idd3.NOD mice that have normal T and B cell numbers. Thus, lymphopenia and the resulting compensatory homeostatic expansion of effector lymphocytes reactive with self antigens may precipitate autoimmunity (7). Other examples of lymphopenia-induced autoimmunity in rodents include the development of autoimmunity after neonatal thymectomy, discontinuation of cyclosporine treatment or total lymphoid irradiation (8 ,9). Lymphopenia also accompanies human autoimmune diseases, such as SLE and Sjogren' syndrome (10).

Lymphocytes with receptors specific for self-antigens are generated continuously in the body, yet most individuals maintain a state of unresponsiveness to their own antigens, a process referred to as self-immune tolerance. Thus, immune tolerance can be broadly defined as a physiologic state in which the immune system does not react harmfully against the components of an organism that harbors it or against antigens that are introduced to it (11). Harmful responses are prevented by a variety of mechanisms that operate during development of

the immune system and during the generation of each immune response. These mechanisms can be broadly classified into four major groups, which are: (1) Central tolerance, which implies induction of tolerance in developing lymphocytes when they encounter self-antigens in the thymus or bone marrow. This process ensures tolerance to self-antigens that are present in high concentrations in the bone marrow and thymus. This process occurs by induction of apoptosis of self-reactive lymphocytes also known as clonal deletion. (2) Peripheral tolerance is maintained by mechanisms that operate on mature lymphocytes once they exit the primary lymphoid organs. Some self-antigens may not be able to induce central or peripheral tolerance. (3) Clonal ignorance may be the mechanism of tolerance for these self-antigens, which is believed to operate when the self-antigen is sequestered in anatomical sites, which are inaccessible to lymphocytes. (4) Clonal anergy is another mechanism of lymphocyte tolerance in which the lymphocyte is functionally unresponsive following antigen encounter but remains alive for extended periods of time in a hyporesponsive state (12). Self-antigen recognition without costimulatory signals is widely believed to induce lymphocyte anergy. However, the conditions or factors that determine whether a self-antigen can be functionally ignored or induces anergy remain to be fully understood. More importantly, so far we do not know which self antigens can induce which form of self-tolerance or what is the relative contribution of each of these mechanisms in shaping the normal immune repertoire. There is also no proper understanding of what are characteristics of a self-antigen that can lead it to undergo central tolerance, peripheral tolerance, clonal ignorance, or clonal anergy. Nevertheless, substantial progress has been made in unraveling these basic tolerance mechanisms that are common to both B and T lymphocytes. Since current knowledge supports loss of tolerance in both B and T cells in eliciting pathologic autoimmunity, we will discuss them separately (Table 20-1).

Mechanisms Underlying T Cell Tolerance

Tolerance of self-reactive T cells occurs in both a central tolerance mode occurring in the thymus, and in peripheral tolerance mode occurring in the peripheral lymphoid organs. Figure 20-1A depicts the sites of tolerance and potential mechanisms.

Table 20-1: Mechanisms of Self-Tolerance in T and B Cells

	T cells	B cells
Clonal deletion	Yes	Yes
Ignorance	Yes	Yes
Anergy	Yes	Yes
Immune deviation	Yes	
Regulatory T cells	Yes	
Receptor editing		Yes

Thymic Selection

The recognition of self-peptides, in association with self-MHC molecules, presented to differentiating T cells by antigen-presenting cells (APC) present in the thymus results in thymic selection of T cells (11). This thymic selection process ensures that mature T cells are both self-MHC restricted and self-tolerant. When the TCRs on a pre–T cell thymocyte are engaged, the thymocyte can be either positively or negatively selected depending on the balancing effects of several other factors regulating this process. Thymic selection begins at the double positive (DP, $CD4^+CD8^+$) stage in the thymus (when the alpha and beta chain genes are expressed) and beyond. This process has two important outcomes: MHC restriction (positive selection) and central tolerance (negative selection). Thymic cortical epithelial cells function as the effector cells in a process known as positive selection. In positive selection, T cells that bear a TCR that can bind self-MHC are selected to survive and proliferate. T cells that are not positively selected are triggered to undergo apoptosis. Positively selected thymocytes must go through a second phase of selection known as negative selection. During negative selection, any T cell that is presented with antigenic peptide bound to MHC within the thymus is triggered to undergo apoptosis. The self-peptides encountered in the thymus are derived from proteins expressed in thymus as well as other proteins brought to the thymus via the blood stream. Negative selection can apparently be mediated by a variety of different cell types, including thymic dendritic cells (DC) and macrophages. The surviving T cells migrate to the medulla where they continue maturation and finally leave the thymus through the postcapillary venules or efferent lymphatics.

Although thymic selection should enable the deletion of all self-reactive T cells, this process is not perfect because not all peptides that an organism may encounter in the lifetime are presented in the thymus. Other variables such as peptide concentrations, affinity of TCRs or state of APCs in the thymus may all determine whether the threshold for receptor occupancy is reached for the positive or negative selection to occur. Potentially self-reactive T cells that escape central tolerance can still be tamed through several backup mechanisms for maintenance of self-tolerance. These peripheral tolerance mechanisms include antigen-specific unresponsiveness or anergy, immune deviation, and elimination after repeated activation (13). Variables that determine whether peripheral deletion proceeds efficiently include extent of TCR occupancy, affinity of antigenic peptide for the MHC and affinity of TCR for the antigen peptide complex. High antigenic dose and chronic stimulation favor elimination both in CD4 and CD8 T cells. The silencing of T cells upon persistent activation in the periphery may thus represent a continuous process, ranging from the activation to unresponsiveness to deletion, with T cell signal strength and exposure time together determining the outcome. A major mechanism for peripheral deletion of activated T cells involves activation-induced cell death (AICD) via the Fas-FasL pathway, as suggested by studies in mouse models, where mutations in these molecules are

associated with the development of autoimmunity. Mouse deficient in Fas (MRL-FAS$^{lpr/lpr}$ [MRL-lpr]) or FasL (gld mice) develops severe lymphoproliferative autoimmune disease caused by accumulation of activated T cells. Mutations in Fas are associated with autoimmune disease in humans as well (14). Some mouse strains carrying gld or lpr mutations develop arthritis or nephritis, whereas the same mutations on other genetic backgrounds cause only excessive lymphoproliferation (15 ,16 ,17). In humans, not all subjects carrying mutations in Fas or FasL develop autoimmune disease (14 ,18). This suggests that other genetic parameters and additional mechanisms contribute for peripheral tolerance of autoreactive T cells.

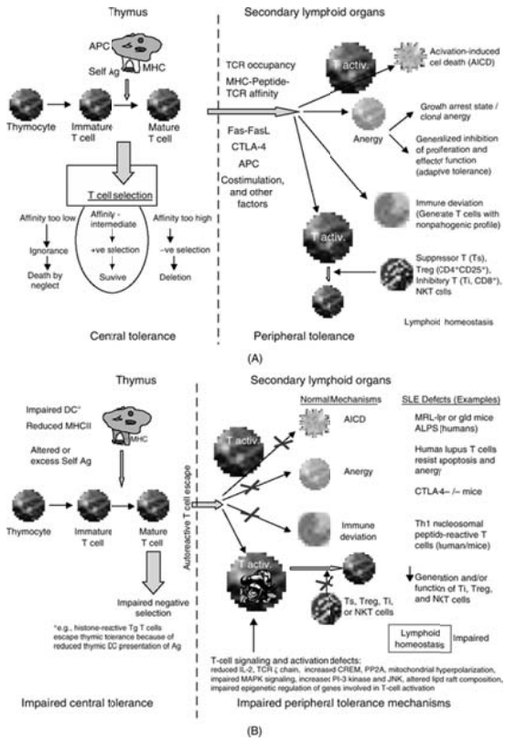

Figure 20-1. T-cell tolerance. **A,** Normal tolerance mechanisms. Immune tolerance is a physiological state in which the immune system does not react harmfully against the components of an organism that harbors it or against antigens that are introduced to it. Self-reactive T cells undergo negative selection in the thymus; those that escape thymic tolerance are subjected to multiple peripheral tolerance mechanisms at many levels. **B,** Breakdown of T-cell tolerance in SLE. T cells may escape negative selection in thymus by impaired presentation of self-antigen by thymic APC. The affinity of self-epitopes can also prevent them from undergoing negative selection. Self-reactive T cells exit the thymus and get activated by self-antigen presented on APC, which induces a hyper-responsive phenotype coupled with resistance to induction of anergy and/or apoptosis. Immune deviation and activation of Treg and Ti cells, which are additional mechanisms to control autoreactive T cells, fail to suppress them, thereby leading to the expansion and survival of autoreactive T cells. SLE T cells also exhibit an overexcitable phenotype which further contributes to increased T-cell activation and TCR mediated signaling.

Another molecule, CTLA-4, has been implicated in peripheral deletion. Whereas ligation of Fas on activated T cells is sufficient for the induction of cell death, CTLA4-induced cell death is dependent on the simultaneous triggering of the TCR. The importance of this pathway is illustrated in studies where targeted disruption of the CTLA-4 gene in mice results in massive accumulation of activated lymphocytes in lymphoid organs, infiltration of multiple tissues with activated lymphocytes, autoimmunity, and fatal multi-organ inflammation (19 ,20 ,21). However, there has been no direct demonstration that the infiltrating lymphocytes actually recognize self-antigens in these mice.

Induction of anergy is another mechanism of lymphocyte tolerance in which the lymphocyte is functionally unresponsive following antigen encounter but remains alive for extended periods of time in a hyporesponsive state (12). Two different forms of T cell anergy have been described. One is principally a growth arrest state that has been termed as clonal anergy and the other represents a generalized inhibition of proliferation and effector functions called adaptive tolerance or in vivo anergy (22). According to the two-signal model of T cell activation versus anergy, the APCs having the ability to offer T cells the prerequisite triggering of TCRs (signal 1) and costimulation (CD28/B7, the signal 2) induce T cell activation. However, not all APCs have the ability to offer T cells both of these signals, and signaling through the TCR alone induces a state of functional unresponsiveness or clonal anergy. This could happen via two pathways: one is the direct inhibition of CD28 signaling by “anergy factors” and the other involves indirect effect on cell cycle progression through growth factors such as IL-2 (23 ,24). Recent work has led to a better understanding of the cell-intrinsic program that establishes T cell anergy. During the induction phase of anergy, “incomplete” stimulation of T cells (TCR triggering without costimulation) leads via calcium influx to an altered gene expression program that includes upregulation of several E3 ubiquitin ligases. When the anergic T cells contact APCs, intracellular signaling proteins are monoubiquitinated and targeted for lysosomal degradation, thus decreasing intracellular signaling and also resulting in decreased stability of the T cell-APC contact (25). Ubiquitin ligases that have been implicated in T cell anergy are c-Cbl, Cbl-b, GRAIL, ITCH, and Nedd4 (26). Interplay of these ubiquitin ligases has been shown to regulate T cell anergy.

Immune Deviation

The immune system has also evolved to have a functional mechanism of tolerance in the face of persistent T cell activation. Skewing of a T cell response into a lineage that does not mediate disease and which prevents development of harmful T cell responses is called immune deviation. In certain autoimmune models, animals have tissue infiltration with autoreactive T cells that bear an activated phenotype but the animals do not develop clinical manifestations of disease (27). Studies have shown that T cells from nonautoimmune animals can respond to autoantigens in vitro as strongly as T cells from the autoimmune-prone animals do (28). In

NOD mice, which spontaneously develop diabetes, presence of Th1 cells in islets was found to be associated with the clinical disease, whereas resistance to disease is associated with predominance of cells producing Th2-like cytokines (29). Similarly, in EAE models the Th1 responses are generally pathogenic, whereas Th2 responses are protective. Specific mechanisms, which allow skewing of T cell immune deviation, are still not clearly understood. Several explanations for the apparent dichotomy have been proposed, including the role of the type of APCs participating in the immune response, modulation of the costimulatory molecules, and signal transduction pathways.

Regulatory T Cells

A number of studies have shown existence of a number of T cell subsets, which can regulate autoimmunity. Such subsets of T lymphocytes are called regulatory T cells. One of the most studied subset of regulatory T cells (Treg) is $CD4^+CD25^+$ cell, also called the natural T_{regs}, which are derived as a functionally mature population from the thymus. Natural Tregs comprise approximately 5% to 10% of the peripheral CD4 T cells in humans and mice. Their importance in the regulation of autoimmunity was initially realized in studies where passive transfer of T cells lacking in $CD4^+CD25^+$ subset into athymic nude mice resulted in spontaneous development of various T cell–mediated autoimmune diseases (30). Recent advances in the identification of markers to identify these cells and in understanding of their function at the molecular level have begun to pinpoint their roles in self-tolerance and autoimmune disease (31 ,32 ,33). As described below in section 2, $CD8^+$ inhibitory T cells that produce TGF-ß prevent or suppress autoimmunity (34) and may play a role in maintaining self-tolerance. Another regulatory T cell population, called natural killer T (NKT) cell, has also been proposed to play a role in the induction of self-tolerance in periphery (35 ,36 ,37).

Mechanism of B Cell Tolerance

As with T cells, tolerance of self-reactive B cells occurs in both a central tolerance mode occurring in the bone marrow, and in peripheral tolerance mode occurring at different stages of maturation of B cells as well as at the level of mature B cells. Figure 20-2 depicts the sites of B cell tolerance and potential mechanisms.

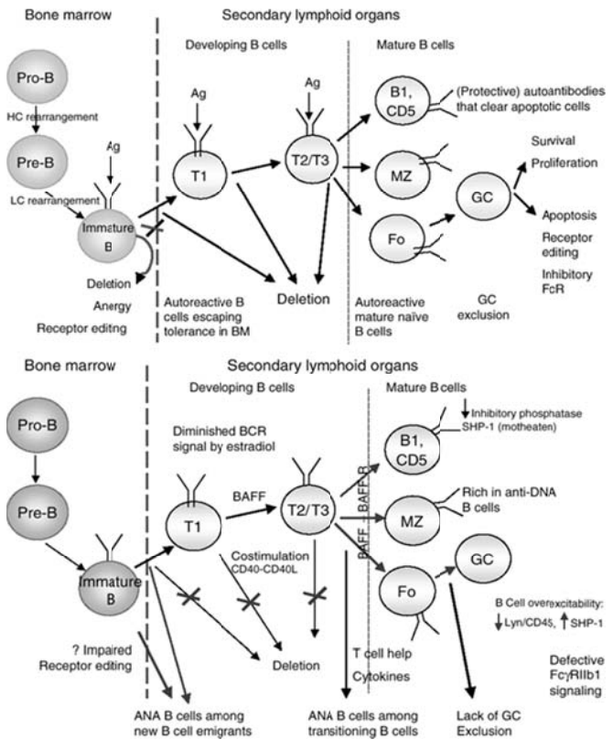

Figure 20-2. B-cell tolerance. **A,** Normal B-cell tolerance checkpoints. There are three distinct tolerance checkpoints in B cells. First, there is an initial checkpoint during the maturation of B cells in the bone marrow; second, there are many checkpoints during B-cell development in the periphery, and finally, there are checkpoints involving mature B-cell subsets. Fo, follicular; GC, germinal center; HC, heavy chain; LC, light chain; MZ, marginal zone; T, transitional. Details on the mechanisms involved at each of these stages are provided in the text. **B,** Breakdown of B-cell tolerance in SLE, as described in section 2.4.

More than half of all newly generated immature B cells in the bone marrow of healthy individuals appear to be polyautoreactive and capable of binding self-antigen including nuclear antigens (38 ,39). Elaborate control mechanisms must therefore exist to remove such potentially autoreactive B cells ensuring self-tolerance. In fact, extensive studies in mouse models and some in humans with regard to B cell selection suggest that there are number of distinct tolerance checkpoints during B cell development and maturation (38 ,40), which can be broadly categorized into three stages. First, there is an initial checkpoint during the maturation of B cells in the bone marrow; second, there are many checkpoints during B cell development in the periphery, and finally, there are checkpoints involving mature B cell subsets (Fig. 20-2A).

The majority of polyreactive and antinuclear antibody B cells are removed at the immature B cell stage in the bone marrow (38). Essentially three mechanisms have been described to silence developing autoreactive B cells in the bone marrow: deletion, anergy, and receptor editing (39 ,41 ,42 ,43). B cell receptor (BCR) signaling strength and the physical nature of the self-antigen (soluble versus membrane-bound) play major regulatory roles in the selection process (44 ,45). Immediately after rearrangement of light chain genes, the newly generated immature B cells

express BCR, which upon contact with the antigen results in maturational arrest of B cells, reexpression of rag-1 and rag-2, and resumption of rearrangement of light chain loci. This process has the potential to delete the initially successfully rearranged light chain gene and create newly arranged light chain gene. This regulatory event is known as receptor editing. It is presumed that immature B cells that are no more self-reactive can now mature and leave the bone marrow. The immature self-reactive B cells are destined to undergo clonal deletion or clonal anergy depending upon the affinity of the antigen to the BCR.

In the periphery, most remaining potentially autoreactive B cells are removed when newly emigrant B cells transition into naïve immunocompetent lymphocytes (38). To develop from the immature state in the bone marrow to the mature naive state in the peripheral lymphoid organs, a B cell must survive several checkpoints (40). The first checkpoint is between the immature cell in the bone marrow and the transitional T1 cell in the spleen. The second is between the T1 and more mature T2/3 state, and the third is between the T2/T3 stage and mature B cells. Tolerance mechanisms are less clear at these checkpoints, and both positive and negative selection mechanisms have been proposed (46 ,47 ,48). Negative selection that is mediated by BCR signaling is generally considered to be a B cell intrinsic property. The transitional B cells can be rescued from negative selection by costimulatory signals. For example, CD40 engagement by CD40 ligand (CD40L) can rescue B cells destined to undergo BCR-mediated apoptosis. Further, B cell activating factor of the tumor necrosis factor family (BAFF) can enhance the survival of transitional B cells.

B cells that escape tolerance mature into immunocompetent B cells having the phenotype of the following B cell subsets, namely B1 B cells, marginal zone (MZ) B cells, short-lived plasma cells, or germinal center–matured long-lived plasma cells. The B1 cells express CD5, are restricted in diversity, and fail to generate a memory population. Self-reactive B1 cells bearing low affinity BCRs normally home to peritoneal cavity (in mice) and produce autoantibodies that are thought to help avoid pathogenic autoreactivity by clearing apoptotic cells (49). Human equivalent of B1 cells, which express CD5, are normally present in the naïve repertoire, but they are usually excluded from the germinal center reactions (50). Thus, germinal center exclusion of potentially autoreactive B1 subsets may be one essential checkpoint in mature B1 cells. The MZ B cells that mature rapidly into plasmablasts can produce autoantibodies (51). How tolerance is regulated in the MZ B cell subset is unclear. The follicular B cells, after they encounter antigen and receive T cell help, generate germinal centers to mount an affinity-matured antibody response and generate memory B cells. The germinal center serves as a major checkpoint in establishing follicular B cell tolerance, where a stringent balance of proliferative and apoptotic signals is required to prevent the survival of self-reactive B cells while ensuring expansion of the normal B cell repertoire. Mechanisms of positive and negative selection at the level of germinal center are not well understood. Receptor editing might contribute to negative selection at this stage (52) and inhibitory Fc receptor Fcγ2RIIB may regulate B cell survival in the germinal center (53), whereas T cells may serve to mediate positive selection of germinal center B cells.

Thus, tolerance of self-reactive B cells occurs in the bone marrow, and in the periphery at different stages of maturation of B cells as well as at the level of mature B cells (Fig. 20-2A). Peripheral tolerance of B cells generally results from contact with antigen in a way that does not promote full activation, either because the B cell is still immature and hence less responsive to B cell activation pathways or because the B cell contacts the antigen in absence of costimulatory molecules. If the antigen is a weak one, the B cell becomes anergic.

Thus, elaborate mechanisms of T and B cell tolerance act in concert to maintain normal lymphocyte homeostasis and avoid pathologic autoimmunity. Elucidating the nature of these mechanisms may lead to better approaches for sustaining a balanced response to self and promoting reactivity to non-self at the same time. How tolerance mechanisms fail resulting in pathologic autoimmunity will be discussed in the following section.

Immune Tolerance Defects in Lupus

While substantial progress has been made in understanding fundamental mechanisms of self-tolerance, how impairment in this process causes autoimmune disease remains largely unclear. A number of mechanisms have been proposed, and a few have been demonstrated in animal models of autoimmunity. As summarized in Table 20-2 , some of these mechanisms depend on alterations in autoantigen itself (54 ,55 ,56), some on changes in the processing and presentation of autoantigen at the level of APCs, some on changes in the T and B cells, and some on the aberrant immune regulation. Based on our current understanding, it appears that alterations at different levels may account for loss of self-tolerance in different animal models and probably in different subsets of SLE, and that multiple impairments could well account for the loss of self-tolerance in a single model or patient (54). This concept is certainly consistent with the complex multigenic nature of the genetic predisposition of SLE.

Abnormalities at the Level of Autoantigens in Tolerance Breakdown in Lupus

That only certain self proteins frequently elicit an autoimmune response has intrigued many investigators to speculate that autoimmunity might occur as a result of altered self or modified self serving as a potential source of autoantigen. Several mechanisms have been proposed to account for such modifications.

Mutations in a self-antigen creating a neo-epitope might trigger autoimmunity. For example, in a cDNA library made from peripheral blood lymphocytes of a patient with primary

Sjogren' syndrome, one study identified a deletion of an (A)-residue in a cDNA encoding for the nuclear autoantigen La (SS-B). This leads to a frame shift mutation and a premature stop codon within one of the protease sensitive regions of the La protein (57 ,58 ,59). The region where the deletion occurred, represents a hot spot region in the La gene(s), which is located in one of the major autoepitope regions of the La antigen. Translation of the patient's mutant La mRNA in transfected mouse cells resulted in a C-terminally truncated La peptide. Because of the lack of the nuclear location signal the La peptide remained in the cytoplasm. The modified La peptide shared homology with (1) La protein itself and (2) a series of DNA binding proteins including other autoantigens and viral proteins such as topoisomerase I, RNA dependent RNA polymerase of influenza virus and reverse transcriptase. The mutant La peptide represents a putative neo-epitope that could be involved in triggering of the autoimmune response.

Table 20-2: Potential Mechanisms for Loss of Self-tolerance in the Development of Lupus

	Site of Alteration	Alterations	Ref.
1.	Autoantigen	a) Altered self	
		i) Mutations in autoantigens	(57,58,59)
		ii) Excessive polymorphisms of autoantigens	(60)
		iii) Noncanonical alternative mRNA splicing at high frequency	(63)
		iv) Post-translational modifications of autoantigens	(68,70)
		v) Direct modification of host proteins by viruses	(89)
		b) Molecular mimicry	(76)
		c) Excess:	(87,88)
		– Altered proteolytic cleavage of autoantigens	(55,317)
		– Inducible autoantigen expression by cytokines such as IFN-α	(79)
		Reduced clearance	(82)
		d) Activation of TLR ligands (e.g., chromatin as an endogenous ligand for TLR9)	(89)
		e) Altered recognition in endomembrane traffic	
2.	APC	a) Altered antigen processing	(87,85,86)
		b) Altered MHC class II expression and presentation	(90,91,92)
		c) Altered migration of APCs to sites of tolerance induction	(90)
3.	T cells	a) Disturbed homeostasis	(10)
		b) Reduced apoptosis	(100)
		c) Loss of anergy	(107)
		d) Enhanced constitutive signaling	(125,137,138,155,318)
		e) Immune deviation	
4.	B cells	a) Impaired tolerance at the early immature stage in bone marrow	(40,142)
		b) Impaired tolerance during transition to mature stages in the periphery	(40,142)
		c) Impaired regulation at the level of mature B cells	(40)
		d) Defective follicular exclusion	(154)
		a) Impaired receptor editing	(142)
		b) Apoptosis	(319)
		c) Enhanced constitutive signaling	(158,160)
		d) Presentation of autoantigens by B cells	(166)
5.	Regulatory T cells and cytokines	a) Reduced induction or activation of $CD8^+$ inhibitory T (Ti) cells	(34,169,170,320)
		b) Reduced function of $CD4^+CD25^+$ Treg cells	(184,321)
		c) Insufficiency of natural killer T (NKT) cells	(322,323)
		d) Reduced production of immunoregulatory cytokines such as TGF-ß	(34,182)

Stadler et al asked whether alterations in self-proteins might actually be genetic polymorphisms. To address this question, they analyzed sequence variability in the known human autoantigens (*n* = 348) and compared it with other human genes (*n* = 14,881). Remarkably, the autoantigens contain significantly more single nucleotide polymorphisms (SNP) within coding regions than other human genes do. Autoantigens had 7.2 SNPs per gene as compared to 3.6 SNPs in the control gene. Such increase in polymorphisms in a protein may have important outcomes for the protein structure, function and antigenicity (60). Indeed, human Ro52, a major autoantigen in rheumatic diseases, contains two synonymous and three nonsynonymous SNP, and one of the nonsynonymous SNP is located in the central immunodominant region of the autoantigen (61). Further, an intronic SNP that leads to aberrant splicing of Ro52 mRNA resulting in the generation of a shortened version of the

Ro52 protein is strongly associated with anti-Ro52 autoantibodies in primary Sjogren syndrome (62).

Some autoantigens can be differentially immunogenic because of existence of alternatively spliced isoforms (63). A recent study used a bioinformatics approach to analyze the extent of alternative splicing within 45 randomly selected self-proteins associated with autoimmune diseases as compared to 9554 randomly selected proteins in the human genome. They found occurrence of alternative splicing in 100% of the autoantigen transcripts, which is significantly higher than the approximately 42% rate of alternative splicing observed in the randomly selected human gene transcripts. Within the isoform-specific regions of the autoantigens, 92% and 88% encoded MHC class I and class II-restricted T cell antigen epitopes, respectively, and 70% encoded antibody binding domains. Furthermore, 80% of the autoantigen transcripts underwent noncanonical alternative splicing, which is also significantly higher than the less than 1% rate in randomly selected gene transcripts.

Posttranslational modifications in a protein could also act a means to promote autoreactivity (56 ,64). The immune system normally utilizes posttranslational modifications to control its function. Essentially all immunoglobulin molecules undergo these modifications. Posttranslational modifications are also documented for TCR molecules. For example different glycosyl groups are present on TCR in different stages of development. Posttranslational modifications are also reported for cytokines like IL-2 and IL-6. When such modifications occur in self-antigens, they exert more subtle effects through altering the susceptibility of a protein to proteolytic cleavage during antigen processing. Since these modifications occur after the lymphocyte has undergone negative selection, the existing B and T lymphocytes can recognize the modified antigens causing their loss of tolerance. This process may spread to the unmodified self-protein through epitope spreading, and autoimmunity is sustained through the continuous supply of unmodified protein (64). Posttranslational modifications in several self-antigens as well as immune responses directed toward posttranslationally modified self-antigens have been noted in autoimmune diseases, such as SLE, rheumatoid arthritis, and multiple sclerosis (64 ,65 ,66 ,67). For example, the spontaneous conversion of an asparagine residue or aspartic acid residue to an isoaspartyl residue renders cytochrome c and snRNP D peptides immunogenic in murine models of SLE autoimmunity (68). Mice develop T cell responses to the isoaspartic acid peptides but not to the native aspartic acid containing peptides. However, mice also develop autoantibodies that recognize both the isoaspartic peptides and the native aspartic acid peptides. These autoantibodies can diversify to recognize other antigens such as dsDNA. Additionally, isoaspartic acid residues have been found in histone H2B, a common autoantigen in spontaneous and drug-induced lupus (69). Finally, patients with SLE have been shown to have autoantibodies that react to the C-terminus of snRNP, which contains symmetrical dimethyl arginines (70) as well as phosphorylated serine/arginine-rich residues of the SR protein (a family of pre-mRNA splicing factors). Interestingly, some autoantibodies were directed at dephosphorylated SR proteins that normally would exist in a phosphorylated state (71).

The above mechanisms, including somatic mutations, genetic polymorphisms, alternative splicing, and posttranslational modifications could generate epitopes for which the immune system is not tolerized (63). The modified antigens can be taken up, processed and presented by APCs and recognized by existing potentially self-reactive B and T cells resulting in breakage of tolerance and induction of autoimmunity.

Bacterial, viral and parasitic infections are known to induce and exacerbate autoimmune diseases, presumably via eliciting molecular mimicry. Association between the development of SLE and viruses such as Epstein Barr virus (EBV), coxsackie virus and retroviruses like HTLV have been described (72 ,73 ,74 ,75). For example, analysis of autoantibody responses in patients with SLE prior to the onset of clinical disease led to identification of an initial autoantigenic epitope that appears in some patients positive for antibodies to 60 kD Ro antigen. This initial epitope cross-reacts with a peptide from the latent viral protein EBV nuclear antigen-1 (EBNA-1). Animals immunized either with the initial epitope of 60 kDa Ro or with the cross-reactive EBNA-1 epitope progressively develop autoantibodies binding to multiple epitopes of Ro and spliceosomal autoantigens. The immunized animals eventually develop clinical symptoms of lupus such as leukopenia, thrombocytopenia, and renal dysfunction. This study provides a strong evidence for association of EBV infection and development of SLE (76).

Defective apoptosis can also result in the generation of neoepitopes. Proteolytic cleavage by caspases of lupus associated autoantigens, like poly (ADP-ribose) polymerase and catalytic subunit of DNA-dependent protein kinase (DNA-PKCs), has been shown to disturb homeostasis and cause increased apoptosis (77). As a result of nuclear fragmentation and membrane blebbing in apoptosis, autoantigens that are targeted in SLE are reorganized and transported to cell surface (78). A recent study suggested that secondary necrosis can be an additional source of proteolytically modified form of specific autoantigens (79). These investigators treated Jurkat cell lines with different apoptosis inducers. During the initial apoptotic stages, several autoantigens, including polyADDP ribose, polymerase, and topoisomerase were cleaved into apoptosis fragments. These apoptotic cells underwent secondary necrosis in the absence of phagocytosis with additional proteolytic modifications of autoantigens (79). This process may further contribute to increased presentation of altered self-peptides by DCs to autoreactive T cells.

Apoptotic defects and impaired removal of apoptotic cells could contribute to an overload of autoantigens (particularly nucleosomes) in circulation or in target tissues that could become available to initiate an autoimmune response. Nucleosomes are formed during apoptosis by organized cleavage of chromatin. These nucleosomes together with other autoantigens cluster in apoptotic bodies at the surface of apoptotic cells. Systemic release of these autoantigens is

normally prevented by swift removal of apoptotic cells. However, if excessive apoptosis exceeds the rate of removal of apoptotic bodies, nucleosomes are released. Furthermore, during apoptosis these autoantigens may undergo posttranslational or posttranscriptional modifications, which make them more immunogenic.

Activation of toll-like receptors (TLRs) by autoantigens can amplify the autoimmune response by activating the innate immune component. Chromatin-containing CpG motif-rich DNA or RNP antigens containing double-stranded RNA (dsRNA) can potentially trigger lupus-like autoimmune responses by providing accessory signals through TLR9 on DCs, macrophages or B cells, or through TLR3 on DCs (80 ,81). Further, immune complexes containing IgG bound to chromatin can activate murine DCs through both TLR9-dependent as well as TLR9-independent pathways (82), which may affect autoimmune responses. Indeed, TLR9-deficiency specifically reduces the generation of anti-dsDNA and antichromatin autoantibodies in MRL-lpr mice (83). The viral dsRNA can also activate DCs via TLR3 to induce the production of type I interferons and other cytokines associated with disease activity in SLE. Consistent with the role of TLR3 in lupus, TLR3 expression is increased in infiltrating antigen-presenting cells as well as in glomerular mesangial cells in kidney sections of MRL-lpr mice (84).

Altered processing and presentation of self epitopes can also lead to generation of new autoantigens for which the immune system is not tolerized. A recent study examined whether some epitopes in a native protein, mouse lysozyme-M, can be cryptic because of the unavailability of a proteolytic site, and whether it could be reversed to immunodominance by introduction of a novel cleavage site in the flanking region of the epitope. Using site-directed mutagenesis, they created the dibasic motif (RR or RK; R = arginine, K = lysine), a target of intracellular proteases, in the region adjoining one of the three cryptic epitopes (located at 46–61, 66–79, or 105–119) of mouse lysozyme-M. Interestingly, the mutated lysozyme proteins, but not unmutated mouse lysozyme-M, were immunogenic in mice. The T cell response to the altered lysozyme was attributable to the efficient processing and presentation of the previously cryptic epitope, and this response was both epitope- and MHC haplotype-specific (85). In xenobiotic models of lupus-like autoimmunity, cell death following exposure to autoimmunity–inducing agents leads to generation of novel protein fragments that may activate self-reactive T lymphocytes (86). During apoptosis, interaction of several autoantigens with granzyme B has been shown to generate unique protein fragments which are not observed during any other form of cell death. Interestingly, nonautoantigens are either not cleaved by granzyme B or are cleaved to generate fragments identical to those formed in other forms of apoptosis. Therefore the ability of granzyme B to generate unique fragments appears to be an exclusive property of autoantigens (87 ,88).

One study suggested that the altered recognition of autoantigens in endomembrane traffic might elicit autoimmunity (89). They showed that the RNA transcription termination factor La, a frequent target of Sjogren autoantibodies, appears in the acinar cell cytoplasm and plasma membranes during viral infection and during in vitro exposure to cytokines. The endomembrane compartments where proteolysis occurs contain La, galactosyltransferase, cathepsin B, and cathepsin D. MHC class II molecules cycle through this compartment. This traffic may permit trilateral interactions in which B cells recognize autoantigens at the surface membranes, and CD4 T cells recognize peptides presented by MHC II, the B cells provide accessory signals to CD4 T cells, and CD4 T cells provide cytokines that activate B cells.

Impaired APC Functions in Tolerance Breakdown in Lupus

We have recently found that Langerhans DCs in skin of lupus-prone MRL-lpr mice display reduced MHC class II molecule expression and impaired migration capacity to draining lymph nodes, when compared with normal strains (90). Importantly, correction of these defects results in improved lupus dermatitis, suggesting a possible role of reduced APC function in the induction of peripheral T cell tolerance (to skin-specific autoantigens in this case) and in the development of lupus (dermatitis in this case) (90). This point was more elegantly made in a recent study, which used a nucleosome-specific TCR transgenic mouse model to infer that thymic DCs from lupus mice are less efficient than those from normal mice in presenting naturally processed nucleosomal peptides in the steady state (91). This impairment is probably owing to lesser amounts of MHC class II and costimulatory molecules on thymic DC of lupus mice than on their normal counterparts. Thus, a relative deficiency in the natural display of self-epitopes by thymic DCs may account for the positive selection of autoreactive Th cells in lupus-prone mice, and/or absence of negative selection. Similarly, deficiency in the natural display of autoepitopes by Langerhans cells may account for the loss of peripheral tolerance to skin autoantigens.

Another study, however, has found increased maturation of DCs and macrophages in a mouse model, where introgression of the SLE3 lupus-susceptibility locus from lupus-prone mice onto a normal B6 background causes T cell hyperactivity (92). Such T cell hyperactivity was thought to be because of the presence of a more mature, less apoptotic, and more pro-inflammmatory APCs in the autoimmune congenic mouse B6.SLE3 (92). Monocytosis in lupus-prone BXSB mice (93 ,94) and increased macrophages in (NZB/NZW)F1 and MRL-lpr mice (95) have also been implicated in the development of lupus. Other studies have postulated imbalance in DC homeostasis as a contributing factor in SLE. According to this idea, cytokines such as IFN-α/β drive differential differentiation of distinct subsets of DCs in SLE. In fact, serum from SLE patients induces normal monocytes to differentiate into DCs, which is dependent on the actions of IFN-α. Thus, unabated induction of DCs by IFN-α may drive autoimmune

responses in SLE (96). In fact, plasmacytoid DCs that are primary producers of IFN-α are present in large numbers in most lupus-affected, but not in normal, skin tissue specimens. The role of increased plasmacytoid DCs in peripheral tissue sites (97) in breaking local T cell tolerance remains unclear.

B cells may also serve as important APC in breaking T cell tolerance. Using anti-snRNP Ig transgenic mice, Mamula and colleagues showed that whereas both normal and autoimmune (MRL-lpr) mice harbor autoreactive T cells, transgenic B cells can tolerize autoreactive T cells in the periphery of normal mice only (98). Thus, B cells (anti-snRNP transgenic B cells in this case) served as important APCs for T cell tolerance in normal mice and for T cell activation in MRL mice. The study further suggested that anti-snRNP B cell anergy in normal mice could be reversed by autoreactive T cells from autoimmune mice in a cognate manner, indicating an important role of T cells in the development of lupus (as described below).

T Cell Abnormalities Contributing to Tolerance Breakdown in Lupus

As described in the following subsections, different laboratories have used various animal models of lupus or T cells from patients with SLE to investigate tolerance abnormalities in T cells. These studies have detected different impairments occurring at almost every level of central or peripheral tolerance mechanisms (Fig. 20-1B).

Impaired Clonal Deletion of Lupus Autoreactive Th Cells

Several approaches have been used to determine if lupus T cells arise as a consequence of failed negative selection. Using transgenic mice expressing TCR of a pathogenic autoantibody–inducing Th cell that was specific for nucleosomes and its histone peptide H4 (71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94), one group reported that whereas introduction of the lupus TCR transgene causes marked deletion of transgenic thymocytes in the normal mouse backgrounds, such deletion is not detected in the lupus-prone (SWR × NZB)F1 (SNF1) thymus (91). Thus, impaired central tolerance may contribute to the positive selection of autoreactive pathogenic Th cells in lupus (Fig. 20-1B). This idea is further supported in studies where a drug procainamide-hydroxylamine that induces lupus in humans interferes with central tolerance mechanisms in the thymus, resulting in the emergence of chromatin-reactive T cells followed by humoral autoimmunity in C57BL/6 × DBA/2 F1 mice (99). To address this issue in humans, T cells from SLE patients were cultured with thymic stromal cells. In these experiments, T cells from SLE patients are more resistant to induction of apoptosis by thymic stromal cells than normal T cells. Thus, SLE T cells have intrinsically acquired a mechanism to evade central tolerance mechanisms in SLE, whereby interactions between thymic stromal and lymphoid cells leads to subsequent survival of autoimmune T cells (100). Other studies, however, suggest that central tolerance mechanisms are intact and that perturbation in peripheral tolerance mechanisms contributes to autoimmune response in lupus.

Neonatal and Adult Tolerance to Exogenously Administered Peptide Antigens in Lupus

To understand mechanisms and outcome of tolerance induction in lupus, we administered MHC class II-binding foreign or self peptides, namely hen egg lysozyme (HEL) 106–116 or self immunoglobulin A6.1 VH58–69, to newborn lupus (NZB/NZW F1) or normal (BALB/c) mice (101). A comparable level of tolerance, as measured by peptide-specific T cell proliferation and IL-2 production in response to subsequent peptide challenge, was induced in both lupus-prone and normal mice. Lupus-prone mice, however, had increased anti-DNA antibody production in response to a neonatally administered self VH peptide. Comparable levels of tolerance were also induced in adult lupus-prone and normal control mice, when peptide antigens were administered IV in high doses of soluble form or intraperitoneal (IP) in high doses of emulsified form (102 ,103 ,104). The older lupus-prone animals, however, tend to have relatively more leakiness in tolerance, particularly in T helper (Th) functions and peptide-specific antibody responses (Singh RR, unpublished data). These studies demonstrate lack of a major tolerance defect in the induction of experimental tolerance in lupus-prone mice.

Intact Central Tolerance, but Impaired Peripheral T Cell Control Mechanisms

Several groups have studied mechanisms and outcome of tolerance induction in lupus using transgenic mice expressing TCR of a T cell–specific for a conventional peptide antigen (for example, pigeon cytochrome C [PCC]). In the PCC peptide TCR transgenic model, the relevant antigen exposure results in intrathymic deletion of immature $CD4^+CD8^+$ DP thymocytes, TCR downregulation and thymocyte apoptosis, which are comparable between a nonautoimmune mouse strain (B10.BR) and an autoimmune–prone MRL-MpJ strain (105). Thus, central tolerance to a conventional antigen is intact in lupus-prone MRL mice. Using the NZB model, another study inferred that there is no generalized T cell tolerance defect in lupus-prone mice (106). Similar conclusions have been reached in many studies. Thus, autoreactive T cells in lupus mice may arise because of defects in peripheral control mechanisms. Further, lupus T cells may be abnormally activated in the setting of incomplete but normal tolerance.

Using elegant gene microarray profiling, functional, and biochemical studies, a recent study showed that activated T cells of patients with SLE resist anergy and apoptosis (Fig. 20-1B) by upregulating and sustaining cyclooxygenase-2 (COX-2) expression, along with the anti-apoptotic or survival molecule c-FLIP (cellular homolog of viral FLICE inhibitory protein)

(107). Inhibition of COX-2 causes apoptosis of the anergy-resistant lupus T cells by augmenting Fas signaling and reducing c-FLIP. Studies with COX-2 inhibitors and COX-2–deficient mice confirmed that anergy-resistant lupus T cells, and not cancer cells or other autoimmune T cells, selectively use this COX-2/FLIP antiapoptosis program. Thus, an imbalance in the proapoptotic/antiapoptotic mechanisms may contribute to the persistence of autoreactive clones (107).

Studies in mouse models also show that CD4 T cells from lupus mice are more resistant than nonautoimmune mice to anergy induction (Fig. 20-1B). Anergy avoidance in the periphery may be one of the causes for abnormal T cell activation in response to self-antigen in SLE (108). Indeed, T cells from SLE patients and lupus-prone mice display phenotypes of in vivo activation, such as CD25, HLA-DR, and high levels of CD40L (109 ,110). Furthermore, CD8 T cells from SLE patients have been found to have high expression of perforin and granzyme (111), which correlate with disease activity. We have also observed increased percentage of $CD44^{hi}CD62L^{lo}CD69^{+}$ T cells in the spleen of MRL-lpr mice (Dubey S and Singh RR, unpublished data). Therefore, there is ample evidence in both human and murine systems that T cell activation is a hallmark of disease development in SLE. However, the mechanisms that cause T cells to become hyperactivated or overexcitable have not been well defined.

Heightened response to peptide antigens, particularly those with low affinity for TCR, appears to drive the polyclonal T cell activation seen in lupus mice (112). Several studies have also demonstrated the presence and role of intrinsic T cell abnormalities, such as diminished activation thresholds, in T cells from patients and mice with SLE. The following sections narrate efforts of several laboratories trying to define such intrinsic T cell abnormalities in lupus.

T Cell Signaling Defects in SLE

T cells use a cell surface multisubunit structure, the TCR/CD3 complex, as an antigen specific recognition site. The TCR α/ß or γ/δ are the antigen-binding sites but because of having very short cytoplasmic domains they are not capable of any signal transduction that is carried out by the CD3 complex. Human and murine SLE T cells, when stimulated through the TCR/CD3 complex, exhibit several abnormalities in T cell signaling (Fig. 20-1B). These include aberrant tyrosine phosphorylation, altered calcium flux, and heightened mitochondrial potential. A major and well studied outcome of this aberrant signal transduction in SLE T cells is reduced IL-2 production, a phenotype of lupus T cells observed more than 20 years ago (113 ,114 ,115). Reduced response to IL-2 by T cells accompanied reduced IL-2 production in some SLE patients (113). Linker-Israeli and colleagues noted a severe defect in IL-2 production by mononuclear cells from all 19 SLE patients regardless of the stimulant used and irrespective of the patients' disease activity (116). Another study in 32 SLE patients and 27 healthy volunteers, however, found a correlation between degree of IL-2 depression in PHA stimulated cells and disease activity (117). This study also showed that IL-2 production was restored in SLE patients by addition of either phorbol myristate acetate (PMA) or ionomycin to cultures (118). Defective IL-2 production has also been reported in mouse models of lupus, including MRL-lpr, BXSB, and BWF1 mice (114 ,119 ,120 ,121). In MRL-lpr mice, reduced IL-2 production precedes the onset of clinical illness and becomes increasingly severe with age (121) (Dubey and Singh, unpublished data). Spleen cells from MRL-lpr mice also fail to respond normally to IL-2 (121). It is therefore important to focus on the IL-2 defect in SLE T cells, since it acts as an essential regulator of immune response by promoting activation of immune system and terminating it when required by inducing activation-induced cell death of autoreactive T cells. In fact, treatment of MRL-lpr mice with *Il2* gene delivered via vaccinia virus or attenuated salmonella vectors results in significant improvement in lupus disease (122 ,123). Consistent with reduced IL-2 production, proliferative responses of T cells from SLE patients when cultured with thymic stromal cells are lower than their normal counterparts (100).

Several mechanisms have been proposed to explain defective IL-2 production in SLE. Reduced phosphorylation and expression of the TCR/CD3ζ chain (124) is one such mechanism. Two SLE patients have been shown to harbor a 36bp exon 7 deletion in the TCR ζ mRNA. Many other mutations found in SLE patients have been mapped to the third immune coreceptor tyrosine-based activation motif (ITAM) motif or the GTP/GDP binding site in the TCR ζ molecule. These mutations have been implicated in the downregulation of TCR ζ chain and consequent reduced IL-2 production in SLE patients (125). Furthermore, transfection of SLE T cells with TCR ζ chain increases surface expression of TCR and normalizes TCR/CD3-induced free intracytoplasmic calcium (124 ,126).

Under physiologic conditions, the signal generated by the CD3 complex triggers phosphorylation of phospholipase C (PLC-γ) on Tyr and Ser residues, hydrolysis of phosphatidylinositol 4,5 biphosphate to phosphatidylinositol 1,4,5 biphosphate and a rapid rise in intracellular Ca^{2+}. The rise in intracellular calcium upon activation has been reported to be higher in SLE T cells than in control T cells (127). This increase in calcium flux in T cells, however, did not correlate with disease activity. The aberrant calcium flux is probably because of an IgG anti-TCR/CD3 complex antibody in human SLE serum (128). Tsokos et al. have recently shown that this anti-TCR/CD3 complex antibody stimulates translocation of Ca^{2+} Calmodulin kinase from the cytosol to the nucleus. This event induces upregulation of CREM (cAMP response element CRE modulaor) transcript and protein, phosphorylation of CREM and binding of pCREM homodimers to -180 site of IL-2 promoter, thus leading to decreased IL-2 production. Further studies from this group suggest that protein phosphatase2A (PP2A), the primary enzyme that dephosphorylates CREB in T lymphocytes, is involved in the suppression of IL-2 production. Thus, PP2A represents a negative regulator of IL-2 promoter activity. Consistent with

this idea, the mRNA, protein, and catalytic activity of PP2A are increased in patients with SLE regardless of disease activity and treatment (128).

Perl et al. have suggested another mechanism of altered calcium flux in lupus T cells. They have shown nitric oxide-dependent mitochondrial hyperpolarization to be responsible for higher calcium flux in lupus T cells (129). In contrast to above studies showing increased calcium flux in SLE T cells, Sierakowski et al. found lower calcium flux upon anti-CD3 stimulation in SLE patients than in controls. Ionomycin-induced calcium flux, however, is similar between SLE patients and controls. The reduced calcium flux upon TCR stimulation in T cells was also seen in patients with mild disease or in those whose T cells produced normal amounts of IL-2 (117). We have observed reduced calcium flux upon TCR signaling in T cells from autoimmune MRL-lpr mice at an age (≥8 weeks) when they begin to develop disease. At this time point, however, B cells when stimulated through their receptor exhibit higher calcium flux. Interestingly, T cells from these mice display a split activation phenotype, i.e., whereas these T cells show evidence of in vivo activation as exhibited by increased expression of activation markers and IFN-γ production, they have reduced IL-2 production and calcium flux upon TCR stimulation (Dubey and Singh, unpublished data).

Two cytoplasmic intracellular signaling pathways important in T cell activation, differentiation, and effector function are the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3c-kinase (PI3-K). There are three major groups of MAP-kinases in mammalian cells (130): extracellular signal regulated kinases (ERK), p38 MAP kinases, and c-Jun N-terminal kinases (JNK). Defects in MAPK signaling pathway in T cells have been shown to account for reduced IL-2 production by SLE T cells. For example, the activity of ERK-1 and ERK-2 is diminished in resting as well as TCR-stimulated peripheral blood T cells from SLE patients, which can lead to diminished translocation of nuclear factor AP-1, resulting in altered coordination of signals needed for normal IL-2 production and maintenance of tolerance in T cells (131). Studies using the graft versus host disease (GVHD) model of murine lupus have found increased activity of PI3-K and JNK, but not for raf-1, p38 MAPK, or ERK-1. Increased PI3-K activity in the chronic GVHD model is consistent with a role for persistent T cell activation in lupus-like disease, as evidenced by increased phosphorylation of TCR-associated Src-family kinases (Lck and Fyn) (132). Consistent with these data, treatment with a PI3-K inhibitor improves disease in MRL-lpr lupus mice (133).

T cell abnormalities in lupus can also be explained by the altered lipid raft composition and dynamics. The organization of signaling molecules into discrete membrane associated microdomains, called lipid rafts, is vital for regulation of T lymphocyte activation pathways (134 ,135). Lipid rafts play a central role in signal transduction, in the immune response and in many pathological conditions on the basis of two important raft properties, their capacity to incorporate or exclude proteins selectively, and their ability to coalesce into small domains. As reported recently, SLE T cells contain larger pools of lipid rafts compared with normal T cells and produce lipid rafts more robustly upon anti-CD3 treatment than normal T cells. These changes are accompanied by a qualitative alteration in the composition of lipid rafts in SLE: whereas CD3ζ and LAT (linker of activated T cells) are uniformly distributed on the surface of normal T cell membrane, these molecules are organized in discrete clusters on membrane of SLE T cells. Unlike normal T cells, lipid rafts from SLE T cells contain FcRγ and activate Syk kinase (136).

The localization of Lck to lipid rafts is essential for normal TCR-mediated signaling. Lck is significantly reduced in both lipid rafts and nonraft portions of T lymphocytes from SLE patients. Reduced expression of Lck in lupus T cells occurs because of increased ubiquitination and subsequent degradation of Lck, so that T cells become unresponsive to TCR-mediated signals (137). These findings imply chronic in vivo activation of T cells in SLE. However, the direct pathogenetic implications of reduced Lck in lupus T cells as well as factors that regulate Lck homeostasis in lipid raft domains and cause degradation of Lck in lupus T cells remain to be clarified. Further studies have shown increased expression of raft-associated ganglioside GM1 in SLE T cells. CD45, a tyrosine phosphatase that regulates Lck activity, is also differentially expressed and its localization into lipid rafts is increased in SLE T cells. Such altered association of CD45 with lipid raft domains may regulate Lck expression in SLE T cells. The altered lipid raft occupancy is not induced by serum factors from patients with SLE, but cell-to-cell contact is required to activate proximal signaling pathways (138).

While most studies have focused on identifying genes associated with altered T cell functions in SLE, epigenetic regulation of gene expression, such as histone acetylation and methylation, may also contribute to impaired SLE T cell function (139). In fact, treatment with histone deacetylase inhibitors, such as trichostatin A, which corrects these impairments and suppresses lupus in mice (140), holds promise for humans.

B Cell Abnormalities Contributing to Tolerance Breakdown in Lupus

Breaking the B Cell Tolerance Checkpoints

Appearance of self-reactive antibodies precedes the onset of clinical manifestations in humans and animals with SLE (141). Where in the B cell pathway tolerance is first broken and which mechanisms account for such breakdown remains to be determined, however (Fig. 20-2B). As described above, there are many B cell tolerance checkpoints that can be located at three broad steps of B cell pathway, namely immature state in the bone marrow, then in the periphery from the immature state to mature naïve state, and finally at the level of mature B cell subsets (Fig. 20-2A). At the immature B cell stage in the bone marrow, most polyautoreactive and antinuclear B cells in healthy individuals are silenced through clonal deletion, anergy, or receptor editing (38). Although there is evidence that

receptor editing occurs in SLE patients, whether it functions to eliminate autoreactivity appropriately is not known (40).

A recent study examined the B cell repertoire at the transitional and naïve stage in the peripheral blood of three newly diagnosed, untreated SLE patients (142). All three patients had increased numbers of naïve B cells expressing self-reactive antibodies as measured by reactivity to Hep-2 antigens. One of the three patients had a high frequency of self-reactive B cells among new B cell emigrants, implying a defect in early B cell tolerance in the bone marrow in some SLE patients (Fig. 20-2B).

As described above, self-reactive B cells that escape tolerance in the bone marrow are subject to removal in the peripheral lymphoid organs when they transition from the immature state in the bone marrow to the mature naïve state. B cell tolerance is impaired at this step in at least some patients with SLE, as in two of the three SLE patients studied autoreactive B cells failed to be removed during their maturation from the transitional to the mature naive stage (142) (Fig. 20-2B). Studies in mouse models suggest that negative selection mediated by BCR signaling confers B cell tolerance at the transitional stages in the periphery. In fact, evidence shows that clonal deletion of B cells at their T1 stage of development is defective in murine lupus (143). In NZB mice, IgM crosslinking in resting or isolated T1 B cells prevents mitochondrial membrane damage and apoptosis induction (143 ,144). Since T2/T3 cells can be rescued from negative selection by costimulatory signals, increased expression of costimulatory molecules in SLE patients (145) can rescue transitional B cells destined to undergo BCR-mediated apoptosis. BAFF that can also enhance the survival of autoreactive transitional B cells (146) is increased in the circulation of some patients with SLE (147). Extrinsic factors such as the sex hormone estradiol can also diminish the BCR signal and thereby potentially diminishes the negative selection of autoreactive B cells (148) (Fig. 20-2B). This might be one explanation for the predominance of SLE and several other autoimmune diseases in women.

SLE patients have high numbers of naïve B cells that can secrete polyreactive antibodies that react with ssDNA, dsDNA, insulin, and LPS (142). It is not clear whether the polyreactive autoreactive B cells reflect a defect in negative selection that correlates with development of disease or they represent precursors of the B cells that produce pathogenic autoantibodies (40). Although lupus patients have cross-reactive antibodies, the polyreactivity is usually restricted to a set of nuclear and nucleoprotein antigens. Thus, it will be important to know when the generalized polyreactivity is converted to restricted cross-reactivity in patients with SLE.

Finally, self-reactive B cells that escape tolerance processes throughout their transitional stages may mature to be autoantibody-secreting B cells. These mature autoantibody–secreting cells may assume the phenotypic characteristics of any B cell subset: B1, marginal zone (MZ) B cells, and follicular B cells (40) (Fig. 20-2B). However, it is unclear which of these B cell subsets contribute to disease pathogenesis in mice and which B cell subsets are responsible for autoantibody production in humans with SLE.

In mouse models, all three subsets can produce pathogenic autoantibodies. For example, B1 cells produce high-affinity IgM anti-dsDNA autoantibodies in the moth-eaten mouse strain that is deficient in the inhibitory phosphatase SHP-1 (148). The human equivalent of murine B1 cells, CD5-expressing B cells, generally produce polyreactive, low-affinity IgM autoantibodies using germline-encoded V genes. However, somatic mutation has been described in human autoreactive $CD5^+$ B cells (149), which can some times differentiate into cells with features of germinal center cells (150). Thus, impaired generation or regulation of B1 B cells may be involved in the pathogenesis of SLE. In fact, VH4–34-expressing, $CD5^+$ B cells that produce pathogenic IgM antilymphocyte antibodies are normally excluded from germinal center reactions, but these cells enter germinal centers in patients with SLE and contribute to the memory B cell pool (50).

The MZ B cells have several features required to break T cell tolerance. For example, they can act as APCs as they express costimulatory molecules and can activate T cells (151). These cells are easily activated by DC and mature rapidly into plasmablasts (51). MZ B cells can also generate T cell–independent autoimmune responses and undergo heavy chain class switching and somatic mutation in extrafollicular regions of the spleen in lupus-prone mice (152). MZ B cell can also initiate germinal center formation (51). In humans these cells are present in circulation as $IgD^{low}IgM^+CD27^+$ and can populate all secondary lymphoid organs. The factors that regulate differentiation and entry of MZ B cells to germinal center or extrafollicular foci of antibody production are not known. MZ B cell development depends on BAFF that is upregulated in SLE. In fact, MZ B cells can produce pathogenic autoantibodies in lupus-prone (NZB/NZW) F1 mice (153).

After antigen encounter and T cell help, follicular B cells normally generate germinal centers where they mount an affinity-matured antibody response and generate memory B cells. Since lupus autoantibodies are mostly somatically mutated and class-switched IgGs, they are likely to be produced by antigen-experienced B cells, implicating abnormalities in tolerance in late stage, germinal center-matured B cells. Mechanisms of negative and positive selection in germinal centers are not well understood. Presence of autoreactive T cells and lack of inhibitory mechanisms such as inhibitory Fc receptor Fcγ2RIIB on the B cells may contribute to positive selection and differentiation of autoreactive B cells at this stage.

To understand B cell tolerance in SLE, a recent study analyzed autoreactive B cells in tonsil biopsies from patients and normal individuals (154). They show that autoreactive B cells exist but do not secrete IgG in normal subjects, but these cells expand and secrete IgG in patients with SLE. In SLE, but not in RA, 9G4 B cells escape normal censoring and actively participate in productive germinal center reactions, leading to the generation of increased levels of IgG memory and plasma cells. The specific peripheral tolerance checkpoint

that is broken occurs at an early point in the germinal center reaction during the transition from the pregerminal center to the centroblast stage, thus implicating faulty germinal center exclusion of autoreactive B cells in the pathogenesis of SLE (Fig. 20-2B).

B Cell Receptor Signaling Defects, Hyperactivation, and Loss of Tolerance in SLE

The strength and the duration of B cell response largely depend on the integrity of BCR and availability of costimulatory (CD19, CD21) or inhibitory receptors. Patients with SLE manifest B cell abnormalities that include B cell proliferation, increased calcium flux, hyperresponsiveness to physiologic stimuli, and altered production and response to cytokines (155 ,156). One cause of the B cell overexcitability in lupus is supposed to be increased signaling through the BCR. In this context, expression of lyn protein, a key negative regulator of B cell signaling, is reduced in B cells from a majority of patients with SLE. SLE B cells also have altered translocation of Lyn to lipid rafts. This altered Lyn expression is associated with heightened spontaneous proliferation, anti-dsDNA autoantibodies, and elevated IL-10 production (157). Another study reported persistently reduced tyrosine phosphatase CD45 and elevated protein tyrosine phosphatase SHP-1 in the BCRs from SLE patients. Since Lyn and SHP-1 act in concert within a negative signaling pathway in which CD45 counteracts SHP-1 mediated regulation, altered expression of these molecules may contribute to defective feedback regulation in SLE B cells.

B cells preferentially express the FcγRIIb1 (CD32) isoform, cross-linking of which in normal B cells suppresses the B cell signal transduction. Defective inhibitory signaling via FcγRIIb1 is purported to cause increased calcium flux in SLE B cells (158). Furthermore, germline deficiency of Fcγ2RIIB causes an accumulation of plasma cells secreting anti-DNA antibodies in mice (53), suggesting a role of this Fc receptor in regulating B cell differentiation at the germinal center stage. In fact, Fcγ2RIIB polymorphisms have been associated with autoimmunity in mice and man. For example, a polymorphism in the FcγRIIb1 gene, FCGR2B c.695T > C that results in the non-conservative replacement of 232Ile at the transmembrane helix to Thr, is associated with susceptibility to SLE in Asians. This polymorphism (Fcγ2RIIB 232Thr) is less potent than wild-type molecule (Fcγ2RIIB 232Ile) in inhibiting BCR-mediated phosphatidylinositol-3,4,5-trisphosphate accumulation, Akt and PLCγ2 activation, and calcium mobilization after IgG Fc-mediated coligation with BCR. Further, the Fcγ2RIIB 232Thr is less effective than wild-type Fcγ2RIIB 232Ile in its localization to detergent-insoluble lipid rafts (159). Thus, altered balance between positive and negative signaling molecules may modify the BCR signaling thresholds and contribute to disruption of B cell tolerance (158 ,160).

B cell activating factor (BAFF) and BAFF receptors appear to affect many stages of B cell differentiation, ranging from the development, selection, and homeostasis of naïve B cells to antibody producing plasma cells. Excessive BAFF rescues self-reactive B cells from anergy, thereby breaking B cell tolerance (161). Mice overexpressing BAFF exhibit increased B cell numbers in spleen and lymph node and autoimmune phenotype similar to that observed in patients with SLE (146 ,162). Inhibition of BAFF by TACI-Ig and BAFFR-Ig has been proven to be beneficial in murine model of SLE (163). Elevated serum levels of BAFF have been detected in some SLE patients (164). BAFF-R is consistently occupied on blood B cells in SLE patients. Dysregulation in expression of these molecules may contribute to breakage of B cell tolerance in SLE. To delineate the mechanism of B cell tolerance by BAFF, one study used anti-HEL–specific BAFF transgenic mice and showed that self-reactive B cells upon which deletion is stringently enforced in early stages of development are mostly unaffected by BAFF. However, self-reactive B cells that are destined for deletion during later stages of maturation are rescued by BAFF from undergoing apoptosis (146). Thus, increased BAFF expression in SLE-prone individuals may precipitate autoimmunity through positive selection of B cells at their late stages of maturation.

B Cell Role in Breaking T Cell Tolerance

Role of B cells has been recently explored beyond their traditional function of autoantibody production. B cells process and present self-antigens to naïve T cells (165). Mamula et al. reported that mice are normally unresponsive to immunization with native mouse snRNP (a lupus autoantigen), suggesting that the tolerance to this self-antigen is intact in these animals. Such tolerance can be broken, however, if the animals are immunized with native foreign (human) snRNPs, as evident by presence of T cells and crossreactive anti-snRNP antibodies. When mice are immunized with human and mouse snRNPs together in adjuvant, T cells specific for mouse snRNPs can be elicited. Furthermore, B cells purified from mice immunized with foreign antigen (human A protein), when transferred into naive mice, can present self-antigen (mouse snRNP) and activate self-reactive $CD4^+$ T cells specific for mouse antigen (166). Similarly, B cells induced to make autoantibody by immunization of mice with the nonself-protein human cytochrome c can present self-antigen mouse cytochrome c to activate autoreactive T cells. This mechanism of breaking T cell self tolerance can account for the role of foreign antigens in breaking not only B cell but also T cell self tolerance, leading to sustained autoantibody production in the absence of the foreign antigen. In consonance with these observations, B cell–deficient MRL-lpr mice have fewer numbers of CD4 and CD8 memory cells than their B cell-intact counterparts (167), whereas secreted Ig-deficient, but B cell–intact MRL-lpr mice continue to have spontaneous T cell activation and expansion (168). Thus, B cells serve as an important role as APCs in inducing spontaneous activation of T cells in autoimmunity.

Impairments of Regulatory T Cells and Factors as Mechanisms of Loss of Tolerance

T and B cells are normally self-tolerant. We have reported that this tolerant state can be broken in otherwise healthy, nonautoimmune mice by in vivo stimulation of the Th cells that are capable of promoting autoantibody production, e.g., by immunization with autoantigenic peptides (such as anti-DNA VH peptides). This state of loss of tolerance (or autoimmunity) is short-lived, however. Recovery from autoimmunity in these mice correlates temporally with the appearance of certain $CD8^+$ and $CD4^+$ T cells that are capable of suppressing autoantibody production (34 ,169 ,170). In fact, $CD8^+$ regulatory T cell lines derived from nonautoimmune mice can suppress in vivo autoantibody production and nephritis when implanted into lupus-prone (NZB/NZW) F1 mice (171). Thus, self-reactive B and Th cells exist in the normal immune repertoire but are kept in control to avoid pathologic autoimmunity. These control mechanisms are defective in lupus mice, which have impaired activation of such inhibitory, suppressor and Treg cells (34 ,169). Impairments in $CD8^+$ T cell suppressor functions have also been described in human SLE (172). These observations might have therapeutic implications because we can correct this impairment in lupus mice by modifying the delivery of peptide antigens, for example, by DNA vaccination with minigenes that encode certain T cell epitopes derived from anti-DNA variable regions (173). Vaccination with nucleosome-derived peptides can also induce suppressor $CD8^+$ and $CD4^+CD25^+$ T cells, as can administration of an Ig-derived peptide, which suppress disease in lupus mice (174). Because similar T cell epitopes exist in humans, strategies described here might be useful in therapy of the human disease.

The VH peptide-reactive $CD8^+$ inhibitory T (Ti) cells described above produce TGF-ß (34 ,169 ,171 ,173). Recent studies have further demonstrated a role of TGF-ß in Ig peptide-mediated suppression of lupus-like autoimmunity in (NZB/NZW) F1 and other models of lupus (175 ,176 ,177). TGF-ß and other cytokines play an important role in maintaining self-tolerance (178 ,179), as TGF-ß1 knockout mice develop lethal systemic inflammation and autoantibodies (180 ,181). This TGF-ß–mediated self-tolerance appears to act via controlling T cell activation, as the deletion of the TGF-ß receptor type II gene in T cells alone can cause severe autoinflammatory disease (Adams and Singh, unpublished). TGF-ß is also required for the normal functioning of Treg and Ti cells. The regulatory role of TGF-ß in the development of lupus-like autoimmunity is further supported in studies which have shown reduced production of TGF-ß by immune cells from patients with SLE; addition of TGF-ß to the lupus PBMC cultures reduces production of autoantibodies (182). However, TGF-ß production is increased in SLE patients having advanced disease with tissue fibrosis. For example, SLE patients who have congenital complete heart block that is believed to occur as a result of fibrosis of cardiac conduction system have a TGF-ß1 genotype that is associated with increased fibrosis. Further, their PBMCs produce increased spontaneous and mitogen-stimulated TGF-ß1 secretion (183). Thus, TGF-ß may play dual, seemingly paradoxical, roles during the development and progression of lupus disease (2): In early stages of disease development TGF-ß deficiency may predispose to immune dysregulation, breakdown of immune tolerance, and development of autoimmunity, whereas in late stages of disease increased TGF-ß production in local tissues may predispose to impaired tissue repair and remodeling and development of tissue fibrosis.

The VH peptide-reactive $CD4^+CD25^+$ Treg cells can also inhibit autoantibody production in vitro in (NZB/NZW) F1 mice (184). To test the role of such Treg cells on lupus disease in vivo, Bagavant et al. performed thymectomy on day 3 of life, which is known to cause deficiency of $CD4^+CD25^+$ Treg cells and generalized autoimmune disease in normal background, in the NZM2328 model of lupus (185). Indeed, the day 3-thymectomy accelerated anti-dsDNA antibody production and proliferative glomerulonephritis and sialoadenitis and induced severe prostatitis, thyroiditis, and dacryoadenitis. To test whether “refilling” these thymectomized mice with Treg cells will obviate lupus exacerbation, they transferred $CD25^+$ T cells from young, healthy NZM2328 mice into syngeneic mice 4 to 7 days after thymectomy. Such transfer prevented the development of prostatitis, thyroiditis, and dacryoadenitis, abolished the accelerated dsDNA antibody response, but had little or no influence on the accelerated development of lupus nephritis and sialoadenitis (185). Thus, a deficiency of $CD4^+CD25^+$ Treg cells may contribute to abnormal immunoregulation in NZM2328 mice, but it may not be sufficient to cause lupus nephritis.

That regulatory mechanisms constitute an important checkpoint against the development of pathologic autoimmunity was also demonstrated in a recent study, where introduction of a transgenic TCR specific for a pathogenic nucleosome-specific T cell in a lupus-prone strain caused suppression of autoimmune disease despite positive selection of autoreactive Th cells. The autoimmune disease suppression in TCR transgenic lupus mice was associated with a marked downregulation of the transgenic TCR, upregulation of endogenous TCRs in the periphery, and induction of potent regulatory T cells. Thus, presence of autoreactive Th cells in large numbers since birth may elicit regulatory mechanisms to preempt pathologic autoimmunity (91).

Autoantigens in SLE

Common Autoantigens in Lupus

Autoimmunity in SLE is directed to some highly conserved intracellular molecules particularly against nuclear and cell membrane phospholipid components (186). Most studies have focused on autoantibody responses to functionally related nucleic acid–containing macromolecules such as chromatin or ribonucleoprotein (RNP) particles, since autoantibodies to double-stranded DNA (dsDNA) and Sm antigens of the U-1 small nuclear RNP (snRNP) complex

are considered pathognomic of SLE (187). These and other autoantibodies and characteristics of the relevant autoantigens are described in detail in other chapters.

In brief, high-affinity antibodies to dsDNA are hallmarks of SLE. Some subsets of these autoantibodies cause renal and vascular injury (1 ,188). The common features of such pathogenic autoantibodies, such as class-switched IgGs and somatic mutation, indicate that anti-dsDNA antibodies arise as a result of an antigen-driven process. The antigenic stimuli driving the production of anti-dsDNA autoantibodies remain elusive, but recent studies provide some possible candidates. Since nucleic acids are poor or not immunogenic, DNA-binding protein in complex with DNA is purported to break tolerance to DNA (189). One possible explanation is that some peptides can serve as surrogate anti-dsDNA epitopes thus activating T cell help for the production of anti-dsDNA antibodies (190 ,191). Another possibility supports a hapten-carrier like mechanism, where T cells specific for peptides derived from the DNA-binding proteins (such as histones) provides help to DNA-specific B cells. For example, immunization of animals with DNA-protein complexes, rather than with protein-free DNA, induces robust anti-dsDNA antibody response (189). A third possibility is that anti-dsDNA antibody response could occur during the autoantibody response toward the protein constituent of the ribonucleoprotein autoantigens such as nucleosomes or snRNPs (186).

Autoantibody against the Sm autoantigens of the snRNP complex is also pathognomic of SLE. The snRNPs are ubiquitous self-antigens that are components of the spliceosome complex, which normally functions to excise intervening introns and generate mature messenger RNA transcripts. In most snRNP particles, seven core proteins, B, D1, D2, D3, E, F, and G, form a heptamer ring, with the snRNA passing through the centre. The Sm epitopes are distributed on the outside surface of the ring. James et al. used overlapping octapeptides spanning the full length of the B/B' protein to identify an epitope, PPPGMRPP, within the C-terminus of SmB'/B, that is recognized very early in animal models and in some SLE patients (192). Over time, the immune response spreads beyond this initial epitope to other snRNP autoantigens including U1-specific RNP epitopes frequently targeted by antibodies present in patients with mixed connective tissue disease (192). Most Sm-precipitin–positive lupus sera, however, recognize certain SmD polypeptides, such as the glycine–arginine (GR)-rich carboxyl region of SmD1 (193 ,194). The levels of the anti-SmD83–119 strongly correlate (as does antinucleosome) to disease activity. Levels of anti-dsDNA and anti-SmD183–119 strongly correlate with lupus nephritis (195 ,196).

Potential mechanisms that make an antigen an autoantigen are described above and have been recently reviewed by several authors (54 ,56 ,186 ,197 ,198). Table 20-3 summarizes briefly some common features. Not all features described in this table are applicable to all autoantigens. In addition to their being evolutionary conserved and ubiquitously expressed, lupus autoantigens are highly diverse, yet this diversity is restricted to certain set of autoantigens causing a “restricted polyclonality” of autoimmune response in lupus. Several mechanisms have been proposed to explain this phenomenon, which have been reviewed in several publications (199 ,200 ,201 ,202 ,203). According to a unique mechanism that we have suggested (204), T cell epitopes (amino acid sequences that can serve as T cell determinants) are shared among variable regions of different lupus-related autoantibodies but not among other antibodies. Thus, a T cell epitope present in an anti-DNA Ig may activate T cells that can deliver help to B cells specific for antiphospholipid or anti-red blood cells or other related autoantigens, but not to B cells specific for an unrelated antigen. This is one explanation why lupus autoantibodies are polyclonal, yet restricted to a recurring set of autoantigens. The shared T cell epitopes in autoantibodies may originate as a result of replacement mutations in mutationally “cold” framework regions, which do not affect the binding of antibody to its antigen but create T cell epitopes (205 ,206). In fact, while mutations in normal Ig involve hot spot areas, mutations in lupus Ig occur in nonhot spot areas, which might be responsible for creating T cell epitopes (207).

Table 20-3: Some Common Features of Autoantigens Described in Lupus

		Ref.
1.	Ubiquitously present	(54,186)
2.	Evolutionary conserved	(56,186)
3.	Genetically polymorphic	(54,60)
4.	Expressed in apoptotic blebs	(319)
5.	Restricted polyclonality, i.e., against autoantigens that are structurally or functionally related	(292)
6.	Restricted polyclonality through shared T-cell determinants among variable region of different autoantibodies	(200,204)
7.	Posttraslationally modified	(68,71)
8.	Substrates of apoptotic enzymes (caspases)	(86,88)
9.	Mutated somatically	(57,58,59)
10.	Charged or coil-coil structure	(54)
11.	Molecular mimics of infectious agents	(72,76)
12.	Able to interact with TLR or other receptors	(80,81,324)

The lupus autoantigens are presumed to initiate and perpetuate the autoimmune response in T and B cells, but the exact mechanisms as to how and when this occurs is still not understood. These mechanisms are discussed in other sections of this and other chapters. In brief, autoreactive T cells such as nucleosome-specific T cells have been identified in SLE patients, which drive the formation of anti-dsDNA and antihistone antibodies (208 ,209).

Identification of Autoantigenic Epitopes in Lupus: Studies in Animal Models

Work in the late 1980s suggested that autoantibody production in humans and mice with SLE is antigen-driven and is dependent on Th cells that are mostly $CD4^+$ (210 ,211 ,212 ,213). To identify the nature and specificity of such autoreactive Th cells, several laboratories have used diverse approaches, including T cell pepscanning of candidate autoantigens, isolating autoreactive T cell clones and deducing potential autoantigens, screening phage display libraries, and eluting naturally processed self-peptides from MHC class II molecules. These approaches have led to identification of epitopes that activate autoreactive Th cells in humans and mice with lupus and modulate disease in lupus mice (Table 20-4).

T Cell Epitope Mapping by Pepscan of Potential Autoantigens

Ig VH Peptides as Autoantigenic Th Epitopes

Early work in late 1980s and early 1990s suggested that human or murine B cells can process Ig molecules and present Ig-derived peptides in the context of their surface MHC class I and class II molecules (206 ,214 ,215 ,216 ,217 ,218 ,219). Moreover, Ig-derived peptides are eluted from MHC class II molecules, suggesting that they are naturally processed (220 ,221). An evidence for functional role of Ig peptide-reactive T cells came from studies in a transgenic mouse: T cells from mice expressing a transgene encoding a TCR specific for an Ig-derived peptide provided help for B cell production of antibodies (222). Furthermore, Ig peptide-reactive T cells follow rules of conventional T cell tolerance and activation. This was elegantly shown using a TCR transgenic mouse strain that expresses a TCR specific for a κ variable region peptide in mAb 36–71, and another double transgenic mouse strain that expresses both the TCR transgene and the κ chain of mAb 36–71 (223). The TCR transgenic T cells undergo central deletion in thymus in double transgenic mice that have been exposed to the antigen (κ chain of mAb 36–71) since birth. In contrast, adoptive transfer of transgenic T cells into κ (Ig) transgenic recipients results in T and B cell activation, lymphadenopathy, splenomegaly, antichromatin antibody production, and lupus nephritis. It is believed that normal as well as lupus-prone mice generally attain T cell tolerance to germline-encoded antibody sequences (206 ,224 ,225), whereas somatically mutated antibody sequences can activate T cell help because they arise in rare B cells at a late stage of T and B differentiation thus creating neoepitopes (206). Interestingly, this group found that clonal expansion of a spontaneous autoreactive B cell lineage from a lupus mouse correlated with VH somatic mutations in mutationally “cold” framework region. While these mutations did not affect the affinity of the antibody product for chromatin, one of them created a MHC class II–restricted T cell epitope. This mutation was shared by all seven members of the lineage, thus making a point of massive clonal expansion (205).

The above observations led us to postulate that SLE B cells process their endogenous or surface Ig into peptides that are presented on MHC class II molecules. These peptide-MHC complexes then activate autoreactive Th cells, which, in turn, stimulate B cells for the increased production of autoantibodies (102 ,204 ,226 ,227). To map the T cell epitopes in anti-DNA mAb VH regions, over 400 overlapping peptides from anti-dsDNA monoclonal antibodies were cultured with naïve splenocytes from young (NZB/NZW)F1 mice; T cell proliferation and anti-DNA antibody forming cells (Elispots) were estimated. Several peptides consistently induced T cell proliferation and/or help for anti-DNA antibody production (102 ,227). These peptides bound different MHC class II molecules; some peptides bound more than one MHC molecule (226). Some epitopes were dominant; others were subdominant or cryptic in the context of whole Ig molecule or its heavy chain (226).

Several lines of evidence support the role of these peptides in autoantibody production and lupus. First, many peptides increased anti-dsDNA antibody production in vitro when cultured with syngeneic splenocytes (204). Secondly, adoptive transfer of peptide-specific T cell lines or immunizations with peptide/adjuvant emulsions increased serum IgG anti-dsDNA antibody levels, accelerated nephritis, and decreased survival (226). More importantly, IV treatment with high doses of a combination of three peptides significantly decreased anti-DNA antibody levels, serum creatinine, and proteinuria, and improved survival (102). The treatment was effective when initiated in the prenephritic age group (102). Minimal or no diminution of anti-DNA antibodies and clinical disease was observed when treatment was initiated in older lupus mice that already have large numbers of highly activated T and B cells (Singh, unpublished observations) (Table 20-5). Apparently, antigen-primed memory T cells, which are abundant in lupus mice that have established disease, are relatively resistant to tolerance induction (228).

The limitations may be overcome by using a peptide that can target a broad repertoire of T cells responding to early and late appearing T cell epitopes. To design such a “consensus” peptide that would contain optimal or best-fit residues in a T cell epitope, we statistically analyzed more than 400 overlapping peptides for the presence of residues that were associated with T cell activation at each of 15 amino acid positions in a peptide. A peptide based on this analysis (Table 20-4) more strongly stimulated T cell help for anti-DNA antibodies than most wild type peptides (229). A tolerogenic regimen of this peptide in young (NZB/NZW)F1 mice was highly effective in delaying the development of anti-DNA antibodies and nephritis. This peptide was also effective when injected into 20-week-old (NZB/NZW) F1 mice that already had high levels of circulating anti-DNA antibodies. Strikingly, treatment with this peptide decreased anti-DNA antibody as well as antibodies that bind nucleosome and cardiolipin (230).

Findings supporting the role of Ig-derived peptides in the pathogenesis of (NZB/NZW) F1 disease have been recently reported from two other laboratories. Jouanne et al. (231)

reported that injection of a peptide corresponding to the VH complementarity determinant region (CDR) 3 region of natural polyreactive autoantibody to young pre-autoimmune (NZB/NZW)F1 mice delayed development of proteinuria and 50% survival rate (231). In another study, Brosh et al. (232) showed that splenocytes from the naïve or immunized (NZB/NZW) F1 mice proliferated in response to a CDR3-based peptide derived from a mAb anti-DNA, 5G12. This peptide also modulated lupus in (NZB/NZW) F1 mice when administered in young age (233). In the SWR/NZB F1 mice, however, treatment with peptides corresponding to CDR1 or CDR2 of anti-DNA monoclonal antibodies did not have any significant effect on the incidence of nephritis (234).

Table 20-4: Potential T-cell Epitopes That Are Implicated in SLE

References	Model	Method of Identification	Peptide Source	Peptides	Peptide Sequence/Comment
(A) Studies in mouse models					
Singh, et al. 1995 and 1998 (102,204,226) and unpublished	NZB/NZW F1	T-cell pepscan using >400 overlapping peptides	VH regions of 4 anti-dsDNA mAbs	A6 p34 A6 p58 A6 p84 ds3 p33 Others	MNWVKQSHGKSL FYNQKFKGKATL SEDSALYYCARD FITWVKQRTGQGLEW
Kaliyaperumal, et al. 1996, 1999 (234,243)	SWR/NZB F1	T-cell cloning, and deducing peptides that activate T-cell clones	Core histones of nucleosomes	H2B10–33 H416–39 H471–94	PKKGSKKAVTKAQKK DGKKRKRSR KRHRKVLRDNIQGI TKPAIRRLAR TYTEHAKRKTVTAMD VVYALKRQG
Waisman, et al. 1997; Brosh, et al 2000 (245,246)	Anti-DNA mAb-induced SLE in mice	Selected CDR-based peptides	Anti-DNA mAb	pCDR1 pCDR3	TGYYMQWVKQSPE KSLEWIGYYCARFL WEPYAMDYWGQGS
Singh, et al. 1998; Hahn et al., 2001 (229, 230)	NZB/NZW F1	Statistical analysis of 439 peptides from anti-DNA VH	Artificial	Consensus	FIEWNKLRFRQGLEW
Brosh, et al. 2000 (232)	NZB/NZW F1	Selected CDR-based peptide	Anti-DNA mAb	pCDR3	YYCARFLWEPYAMD YWGQGS
Freed, et al. 2000 (251)	MRL-lpr	Eluting MHC class II-bound peptides from lymph nodes	Histones (H2A.2), ribosomal proteins (60S, 40S), RNA splicing factor (Srp 20), 26S proteasome, Ig γ1-chain, Ig γ2b-chain, RNA editase-1, C1r, ferritin, axin, lysozyme c, saposin D, nucleoporin NUP155, 14-3-3 protein [see ref. (231) for sequences]		
Monneaux, et al. 2000–2004 (238,239,325)	MRL-lpr, NZB/NZW F1		U1-70K snRNP	p131-151 P140	RIHMVYSKRSGK PRGYAFIEY RIHMVYSKRS(P) GKPRGYAFIEY
Fan and Singh, 2002 (173)	NZB/NZW F1	Bio-informatics and cell binding assays	Identified multiple epitopes that have high proteolytic cleavage scores, dissociation half-time scores and MHC class I-binding		
Kaliyaperumal, et al. 2002 (253)	SWR/NZB F1	Eluting MHC class II-bound peptides from an APC line fed with crude chromatin	H1' 22–42 Brain transcription factor BRN-3		
Suen, et al. 2001, 2004 (254,255)	NZB/NZW F1	Pulsing bone marrow-derived DCs with the protein and detecting T-cell responses to epitopes	T-cell epitope located at the C-terminus of U1A protein; Several epitopes in H2A, H2B, H3, and H4		
Fournel S, et al. 2003 (241)	NZB/NZW F1	T-cell proliferation and cytokine responses upon ex vivo stimulation	Histone H4 Histone H3	Overlapping 11 peptides Peptides 53-70, 64-78, and 68-85 Peptide 56-73 Peptide 61-78	No response Proliferation, IL-2, IL-10, and IFN-γ IFN-γ, but no proliferation IL-10, but no proliferation
(B) Studies in human SLE					
Williams, et al. 1995 (260)	In vitro culture with PBMCs	Selected V region peptides	Human anti-DNA mAbs, B3 and 9G4	16-mer peptides	See ref. (260)
Linker-Israeli, et al. 1996 (261)	PBMCs	Epitope mapping	Human anti-DNA mAb	12-mer overlapping	Sequence not published
Lu, et al. 1999 (259)	T-cell clones, lines and fresh PBMCs	Epitope mapping	Histones	H2B10–33 H416–39 H471–94 H2A34–48 H391–105 H449–63	Same as in mice (see ref (243)) LRKGNYAERVGAGAP QSSAVMALQEASEAY LIYEETRGVLKVFLE
Talken, et al. 1999 (268)	T-cell clones	Epitope mapping	Sm-B	Sm-B248–96	See ref. (268)
Davies, et al. 2002 (270)	PBMC stimulation	T-cell proliferation by overlapping 15-mer peptides	Human La antigen	La 49–63	Similar T-cell response in HLA-DR3+ patients and controls
Dayan, et al. 2000 (263); Sthoeger, et al. 2003 (264)	PBL	T-cell proliferation and/or IL-2 production	Human or murine anti-DNA peptides	Fewer patients than controls show proliferative response; Peptides inhibit 16/6 Id-induced proliferation and IL-2 production; increase TGF-β production	
Kalsi, et al. 2004 (262)	PBMC stimulation	Cytokine release in response to 7 peptides	Human anti-DNA mAb	7 VH region peptides	IFN-γ/IL-10 release frequent in SLE; HLA-DQB1*0201/DRB1*0301 among responders
Monneaux, et al. 2005 (271)	PBMC stimulation	T-cell proliferation and cytokine release	U1-70K snRNP	p131-151 P140	RIHMVYSKRSGKPRGYAFIEY RIHMVYSKRS(P)GKPRGYAFIEY

Table 20-5: Peptide-Based Vaccination and Therapies for SLE

References	Model	Stage of Disease	Peptides	Method of Delivery	Effect of Treatment
Singh, et al. 1995 (102) and unpublished	NZB/NZW F1	Prenephritic Diseased	Combination of 3 VH peptides (A6.1 VH p34, p58 and p84)	IV, soluble	Decreased anti-DNA and nephritis, and prolonged survival No effect
Singh, et al. 1996 (101) unpublished	NZB/NZW F1	Neonatal	A6.1 VH p58–69	IP, IFA	'Split' T cell tolerance; increased anti-DNA Ab
Waisman, et al. 1997 (245)	Induced SLE in normal mice	Neonatal	pCDR1, pCDR3	IP, soluble	Decreased anti-DNA Ab
Gaynor, et al. 1997 (248)	SCID mice		A decapeptide that bound anti-DNA	IP, soluble	Decreased renal Ig deposition
Kaliyaperumal, et al. 1999 (234)	SWR/NZB F1	Prenephritic Diseased	H2B10–33, H416–39, H471–94	IV, soluble	Delayed onset of nephritis Prolonged survival; halted progression of nephritis
Jouanne, et al. 1999 (231)	NZB/NZW F1	Prenephritic	VH CDR3 of a natural polyreactive autoAb	Soluble	Delayed proteinuria and improved survival
Eilat, et al. 2000 (233)	NZB/NZW F1	Prenephritic	pCDR3 from a mAb anti-DNA	IV, soluble	Decreased disease
Hahn, et al. 2001 (230)	NZB/NZW F1	Prenephritic Diseased	Consensus VH	IV, soluble	Decreased anti-DNA and nephritis Dramatically prolonged survival
Monneaux, et al. 2003 (240)	MRL-lpr	Predisease	Phosphorylated analog of U1–70K snRNP131–151 (P140)	IV, soluble	Reduced IgG anti-dsDNA Ab and proteinuria and enhanced survival
Fan and Singh, 2002 (173)	NZB/NZW F1	Prenephritic and diseased	MHC class I-binding VH epitopes	Minigenes (plasmid DNA vectors)	Killed B cells, and reduced anti-DNA and nephritis, and prolonged survival
Wu, et al. 2002, 2004 (279,280)	SWR/NZB F1	Prenephritic	Histone peptide H471	Intranasal soluble	Suppressed autoantibody production and reduced the severity of nephritis
Fan, et al. 2002 (305)	NZB/NZW F1	Prenephritic	MHC class I-binding VH epitopes	CpG-ODN-peptide conjugate	Killed B cells, and reduced anti-DNA and nephritis, and prolonged survival
Shen, et al. 2003 (281)	NZB (Autoimmune hemolytic anemia model)	Preautoimmune	Anion channel protein band 3 peptide 861–874	Insoluble Intranasal, soluble analog	Increased T-cell responses and anemia Th2 deviation, and reduced severity of anemia
Suen, et al. 2004 (255)	NZB/NZW F1	Prenephritic	H3111–130	ID	Suppressed anti-DNA and delayed nephritis
Riemekasten, et al. 2004 (278)	NZB/NZW F1	Prenephritic	SmD1 83–119	600–1000 μg IV	Delayed autoantibody production, postponed the onset of lupus nephritis, and prolonged survival
Fujio, et al. 2004 (306)	NZB/NZW F1	Prenephritic	Engineered nucleosome-specific Treg cells	Multiple gene transfer	Suppressed autoantibody production and nephritis
Amital, et al. 2005 (282)	MRL-lpr	Prenephritic	Competitive agonists of a laminin peptide		Prevented renal Ab deposition and reduced renal disease
Human studies					
Mauermann, et al. 2004 (265)	SCID mice engrafted with human PBLs		Human anti-DNA VH peptide	IP	Suppressed anti-DNA, but not antitetanus, Ab; reduced proteinuria and renal deposition of human IgG
Monneaux, et al. 2005 (271)	Human PBMCs and/or CD4+ T cells		Phosphorylated analog of U1–70K snRNP131–151 (P140)	In vitro	Prevents CD4+ T-cell proliferation, but not regulatory cytokines
Zhang and Reichlin, 2005 (250)	In vitro binding	15-mer peptide DNA surrogate	In vitro	Inhibited binding of human anti-dsDNA Ab to dsDNA	
Mozes, et al.	Human clinical trial	SLE patients	Edratide (TV-4710), a human anti-DNA VH peptide		Phase I studies completed by Teva Pharmaceuticals in 2004. Phase II trial is in progress (January 2006).

T Cell Pepscan of U1–70K snRNP Autoantigen

Muller et al. tested a series of overlapping peptides to identify an epitope present in residues 131–151 of the spliceosomal U1–70K small nuclear ribonucleoprotein (snRNP) (235 ,236 ,237). This peptide is recognized very early by IgG antibodies and $CD4^+$ lymph node T cells in lupus-prone MRL-lpr and (NZB/NZW) F1 mice (238 ,239). The ability of this peptide to stimulate T cells from mice bearing different MHC haplotypes (H-2^k of MRL-lpr and H-$2^{d/z}$ of (NZB/NZW) F1) correlated with its binding to I-A^k, I-E^k, I-A^d, and I-E^d murine MHC molecules.

Interestingly, an analog of peptide 131–151 sequence phosphorylated on Ser140 (named peptide P140) is strongly recognized by lymph node and peripheral $CD4^+$ T cells and by IgG antibodies from MRL-lpr mice (210 ,239). Whereas IV treatment of young MRL-lpr mice with the phosphorylated analog P140 peptide but not with the parent peptide 131–151 in saline reduces proteinuria and dsDNA IgG antibody levels and enhances the survival (240), subcutaneous administration of P140 in Freund adjuvant accelerates lupus nephritis. The therapeutic effect of IV administered P140 correlates with transient abolition of T cell intramolecular spreading to other regions of the U1–70K protein (203), which is important because the conserved T cell epitope sequence contains an RNA-binding motif called RNP1 that is also present in other sn/hnRNPs and often targeted by antibodies from lupus patients and mice (203 ,237). Thus, modifying responses to this promiscuous and conserved epitope may target a broad autoreactive Th cell repertoire in different models and patients.

T cell Pepscan of Histone Autoantigen in (NZB/NZW) F1 Mice

A panel of overlapping peptides spanning the whole sequences of H4 and H3 were cultured with $CD4^+$ T cells from unprimed (NZB/NZW) F1 lupus mice (241). None of the 11 H4 peptides stimulated $CD4^+$ T cells in these mice, whereas several H3 peptides representing sequences 53–70, 64–78, and 68–85 elicited proliferation and induced secretion of IL-2, IL-10, and IFN-γ. The H3 peptides 56–73 and 61–78 induced the production of IFN-gamma and IL-10, respectively, without detectable proliferation, suggesting that they may act as partial agonist of the TCR. Moreover, they found that this conserved region of H3, which is accessible at the surface of nucleosomes, is targeted by antibodies from (NZB/NZW) F1 mice and lupus patients, and contains motifs recognized by several distinct HLA-DR molecules. It might thus be important in the self-tolerance breakdown in lupus.

Cloning Autoreactive Th cells That Induce Nephritis, and Deducing Autoantigens That Can Activate These Autoreactive T Cells

Nucleosome Core Histone Peptides as Th Auto-Epitopes

Datta et al. cloned Th cells from the SWR/NZB F1 mouse model of lupus. Some of these Th cells initiate and sustain the production of pathogenic autoantibodies, induce lupus nephritis, and recognize nucleosomes (242). In subsequent work they stimulated these autoreactive lupus Th cells with 145 overlapping peptides spanning the four core histones H2A, H2B, H3, and H4. From these studies, they localized the critical lupus epitopes in the core histones of nucleosomes at amino acid positions 10–33 of H2B and 16–39 and 71–94 of H4 (243) (Table 20-4). Autoimmune T cells of SWR/NZB F1 mice are spontaneously primed to these epitopes from early life. Moreover, immunization of preautoimmune SWR/NZB F1 mice with these peptides precipitates lupus nephritis (243).

More importantly, a brief therapy with the nucleosomal core histone peptides, administered IV to 3-month-old prenephritic mice already producing pathogenic autoantibodies, markedly delayed the onset of severe lupus nephritis. Chronic therapy with the peptides injected into 18-month-old mice with established glomerulonephritis prolonged survival and halted the progression of renal disease (Table 20-5) (234).

Selecting Candidate Peptides in an Immunogen Used to Induce Lupus in Normal Strains

SLE-like disease can be induced in normal mice by injecting human or murine anti-DNA monoclonal antibodies that bear a 16/6 Id that is frequently present on Ig of mice and humans with SLE (244). Using this model, Mozes et al. found that two peptides representing regions of FR1/CDR1/FR2 (termed as “pCDR1”) and FR3/CDR3 (termed as “pCDR3”) of the VH of a mAb, 5G12, stimulated T cell proliferation in BALB/c and SJL mice, and induced proteinuria, leukopenia, and glomerular Ig deposits (245) (Table 20-4). A T cell line reactive with pCDR3 also induces experimental lupus in naïve mice (246 ,247). Neonatal administration of these peptides prevented anti-DNA antibody production in this monoclonal antibody-induced model of SLE (Table 20-5). Further studies from this group have continued to demonstrate the immunomodulatory effects of these peptides in other models of lupus as well as on human cells, as described below. These findings along

with results described above (102 ,204 ,226 ,227) further indicate an important role of Ig-derived peptides in murine models of lupus.

Screening Phage Display Library to Identify Peptides That Bind Anti-DNA Antibody

An entirely different approach was used by Gaynor et al. (248) to identify nephritogenic peptides. They screened a peptide display phage library with mouse monoclonal antibodies that bind dsDNA and cause nephritis, and identified a decapeptide DWEYSVWLSN that specifically binds an anti-dsDNA monoclonal antibody, R4A. Immunization with this peptide induced IgG Antibodies that bind DNA, cardiolipin, and Sm/RNP, and caused Ig deposition in glomeruli (191 ,249). Administration of this peptide in soluble form protected mice from renal deposition of the anti-DNA antibody in vivo (248) (Table 20-5).

Another study demonstrated potential utility of a peptide DNA surrogate in inhibiting human anti-DNA antibodies. Using the anti-dsDNA antibodies to screen a phage peptide display library, Zhang and Reichlin demonstrated that purified polyclonal anti-dsDNA antibodies and a monoclonal anti-dsDNA antibody specifically bind a 15 mer peptide ASPVTARVLWKASHV (250). This 15-mer peptide can inhibit anti-dsDNA antibodies binding to dsDNA antigen in immunoassays and in the *Crithidia luciliae* assay.

Eluting “Naturally Processed” MHC Class II–Bound Self Peptides

All four approaches mentioned in the preceding sections have examined the T cell activation or mAb binding by peptides in vitro or ex vivo. These approaches, however, do not directly address if these peptides are naturally processed in vivo during the development of autoimmune response. To address this issue, efforts have been made to elute self peptides bound to MHC class II molecules present on APCs. In one study, a number of peptides from diverse source of antigens, including histones, small nuclear RNP, RNA processing enzymes, Ig γ1, IgG γ2b, lysozyme, and 26S proteasome, were identified in the eluates of MHC class II molecules I-A^k and I-E^k isolated from the lymph nodes of MRL-lpr mice, but not from non-autoimmune C3H animals (251) (Table 20-4). Identification of multiple peptides from several different protein donors in the MRL-lpr but not in C3H mice raises a possibility that the autoimmunity in lupus may not be limited to a few autoantigens. It is possible that one or a few epitopes initiate the autoimmunity, which later spreads to other epitopes in the same or the other antigens. Isolation of MHC-bound peptides at an early versus late stage of disease in lupus mice should be able to address this issue.

Further studies revealed that 11 of these peptides identified in these eluates could stimulate proliferation of T cells in both lupus and nonautoimmune mice. However, only four of them (peptides 4–23 of SRp20, 84–103 of H2A, 42–59 of $ß_2$-microglobulin and 110 of I-A^k induced proliferation of T cells in lupus mice (252). It remains to be determined whether these eluted peptides serve as natural autoantigens in lupus.

In another study, a murine lupus APC line was fed with crude chromatin, and the processed peptides were eluted from MHC class II molecules isolated from these APCs. The eluted peptides were then tested for their functional ability to stimulate autoimmune Th clones (253). Of the three major eluted peptides, peptide homologous to histone H122–42 stimulated autoimmune Th cells to augment the production of antinuclear autoantibodies. This peptide potently accelerated lupus disease in SNF1 mice and stimulated T cells from young unprimed SNF1 mice. T cells reactive with this peptide cross-reacted with other core histone epitopes, but not with other lupus autoantigens.

Employing Professional APCs to Assist in Mapping of Th Auto-Epitopes

One group pulsed bone marrow-derived DCs with lupus autoantigens U1A protein (254) or nucleosome (255) and tested in vitro recall T cell responses to individual epitopes. The authors found this approach to be highly efficient for detecting in vitro responses of freshly isolated T cells from unprimed lupus ((NZB/NZW)F1) mice. Several potential auto–T cell epitopes of core histone proteins (H2A, H2B, H3, and H4) were identified using this approach. Intradermal immunization with one of these peptides, H3(111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129 ,130), suppressed anti-dsDNA and anti-ssDNA IgG levels and delayed the progression of glomerulonephritis in lupus-prone (NZB/NZW)F1 mice. These investigators are also using bone marrow-derived DCs to modulate in vivo T cell responses in lupus mice. Thus, nucleosome-pulsed bone marrow-derived DCs elicit release of IL-4 and IFN-γ, representing a Th0 (i.e., mixed Th1 and Th2) pattern of cytokine production. Such modulation of T cell responses to autoantigens might be of therapeutic benefit.

Identification of Self-Epitopes in Human SLE

Human T cells reactive with several lupus autoantigens including DNA-histones, the snRNP antigenic proteins Sm-B, Sm-D, U1–70kD, and U1-A, and heterogeneous RNP (hnRNP) A2 have been isolated from the peripheral blood of SLE patients, as summarized in a recent review (256). Datta et al. first described T cell lines from patients with SLE, which augmented the production of IgG anti-DNA antibodies ex vivo (257). Subsequent characterization of lupus autoantibody-promoting T cells by this group showed that these cells are usually $CD4^+$, can provide help to anti-DNA and anti-histone B cells, and use restricted CDR3 characteristic of antigen selection (258). In a further study (259), they tested 154 peptides spanning the entire length of core histones of nucleosomes for the ability to stimulate an anti-DNA autoAb-inducing Th clone, as well as $CD4^+$ T cell lines and freshly isolated T cells in PBMCs from 23 patients with SLE. In contrast to normal T cells, lupus T cells responded vigorously

to certain histone peptides, irrespective of the patient's disease status (Table 20-4). Interestingly, most of the peptides that activated human T cells from lupus patients were previously identified as T cell epitopes in lupus-prone mice (Table 20-4) (243 ,259). Several additional epitopes, including peptides 34–48 of H2A, 91–105 and 100–114 of H3, and 49–63 of H4, were also found to activate human T cells from lupus patients. Most of these sequences are located in the regions of histones that are accessible at the surface of nucleosome and that contain B cell epitopes targeted by lupus autoantibodies. Importantly, most T cell epitopes have multiple HLA-DR binding motifs, i.e., they are promiscuous with regard to their binding to HLA molecules. Thus, peptides containing these epitopes could potentially be used to treat many patients obviating the need for the development of individualized therapy.

To determine if Ig-derived peptides activate T cells from SLE patients, Williams et al. cultured PBMCs from 28 lupus patients and 13 healthy individuals with selected 16-mer peptides from two anti-DNA autoantibodies, B3 and 9G4 (260). Three of 13 healthy individuals (23%) versus 17 of 28 SLE patients (61%) had T cells that proliferated in response to at least one V region peptide. In another study, Linker-Israeli et al. (261) cultured 12-mer overlapping peptides from the VH of two anti-dsDNA antibodies, B3 and F51, with PBMCs from patients with SLE, their first-degree relatives or unrelated healthy individuals. The expression of early T cell activation markers, CD25 and CD69, and cytokines were determined by flow cytometry. SLE patients had significantly increased T cell activation markers and IL-4 secreting cells than either first-degree relatives or unrelated controls. A subsequent study was done by these investigators in a larger cohort (31 patients and 20 matched healthy controls) to analyze cytokine release by PBMCs in response to seven peptides from the CDR1/FR2 to CDR2/FR3 VH regions of human anti-DNA monoclonal antibodies (262) PBMCs from significantly higher proportions of SLE patients than controls responded to VH peptides by generating IFNγ and IL-10. Three peptides were more stimulatory in the SLE patients than controls. There was a skewing of the immune response to Th2 bias as the disease progressed from early to later stage. Although none of the peptides was restricted by any particular MHC class II allele, among responders there was increased prevalence of HLA-DQB1*0201 and/or DRB1*0301, alleles known to predispose to SLE. Thus, responses to some VH peptides are more frequent in SLE and vary with disease duration. Increased peptide presentation by SLE predisposing HLA molecules might permit brisker increased T cell responses to autoantibody peptides, thus increasing risk for disease.

Guided by their findings in the 16/6Id murine model (see above), Mozes et al. examined immune responses of SLE patients to peptides encompassing CDRs of a monoclonal anti-DNA antibody with a 16/6 Id. In contrast to the above data showing increased responses to anti-DNA derived peptides (260 ,261 ,262), peripheral blood lymphocytes (PBL) from significantly fewer patients (37%) than controls (59%) proliferated in response to one of the anti-DNA peptides (263). A subsequent study by the same group reported in vitro proliferation of PBLs from 24 of the 62 SLE patients tested following stimulation with the human 16/6 Id (264). Interestingly, peptides from both the human and murine anti-DNA autoantibodies specifically inhibited the 16/6 Id-induced proliferation and IL-2 production. The latter inhibitions correlated with increased production of TGF-ß. This study suggested that certain anti-DNA peptides may downregulate autoreactive T cell responses in SLE patients. Indeed, treatment of severe combined immunodeficient (SCID) mice engrafted with PBLs of patients with SLE by repeated incisoproximal administration of a human monoclonal anti-DNA autoantibody peptide (hCDR1) suppressed human anti-dsDNA antibodies but not antitetanus toxoid antibodies (265). Such treatment also reduced proteinuria and renal deposits of human IgG and murine complement C3 in the engrafted SCID mice.

Human T cells reactive with various snRNP antigens, including Sm-B, Sm-D, U1–70kD, and U1-A, have been identified and characterized (256). Subsequent studies on snRNP-reactive human T cell clones showed that they typically exhibit TCRαß$^+$ CD4$^+$ CD45RO$^+$ phenotype, recognize antigen in the context of HLA-DR, and produce substantial amounts of IFN-γ, moderate quantities of IL-2, and variable amounts of IL-4 and IL-10 (266). Further, these cells can also provide help for relevant autoantibody production in vitro (267). Talken et al. (268 ,269) established two sets of T cell clones from patients with connective tissue diseases: one set reacted with Sm-B autoantigen and the others recognized U1–70kD polypeptide. Both sets of T cell clones had a highly restricted TCR CDR3 ß or α chain gene usage, respectively. Further analysis revealed that the Sm-B-reactive T cell clones recognized a peptide, Sm-B248–96 in the context of HLA-DR. Subsequent T cell epitope mapping studies of human T cell clones reactive with the snRNPs U1–70kD, Sm-B, and Sm-D revealed that there are limited T cell epitopes on these proteins and that almost all reside within functional regions of the protein; either within the Sm motifs for Sm-B and Sm-D or within the RNA binding domain for U1–70kD.

In another study, synthetic 15-mer overlapping peptides spanning the entire La sequence were cultured with PBMCs from patients with SLE and controls with a goal to identify T cell epitopes in the La antigen. They found significant, albeit a low-level, T cell proliferative response to a peptide (La 49–63) in HLA DR3$^+$ patients or healthy controls (270). This study highlights difficulties in identifying relevant pathogenic T cell epitopes using PBMC-based T cell proliferation read-out experiments. The study further suggests that the presence of self peptide-reactive T cells in the peripheral blood of healthy individuals is not uncommon. Thus, mechanisms other than loss of central tolerance might be involved in avoiding pathologic autoimmunity.

As described above, Muller et al. identified a CD4$^+$ T cell epitope in peptide sequence encompassing residues 131–151 of the spliceosomal U1–70K snRNP protein (RIHMVYSKRSGKPRGYAFIEY) and its analog phosphorylated at Ser140 (called P140; RIHMVYSKRS(P)GKPRGYAFIEY) in

the MRL-lpr and (NZB/NZW)F1 lupus models (238 ,271). Importantly, administration of the phosphorylated peptide P140 ameliorates the clinical manifestations of treated MRL-lpr mice (238 ,239). Since this peptide sequence that is completely conserved in the mouse and human U1–70K protein contains an RNA-binding motif often targeted by antibodies from lupus patients and mice, they investigated these peptides as potential candidates for the treatment of patients with lupus (271). Binding assays with soluble HLA class II molecules and molecular modeling experiments indicate that both peptides behave as promiscuous epitopes and bind to a large panel of human DR molecules. In contrast to normal T cells and T cells from non-SLE autoimmune patients, PBMCs, and/or $CD4^+$ T cells from 40% of SLE patients proliferate in response to peptide 131–151. Interestingly, the phosphorylated analog peptide P140 prevents $CD4^+$ T cell proliferation but not secretion of regulatory cytokine IL-10. Thus, P140 can serve as a "universal" immunomodulatory T cell epitope.

Thus, patients with SLE have circulating T cells that recognize diverse sets of autoantigenic peptides, which include core histone peptides, Sm-B, U1–70kD, and peptides derived from the V region of autoantibodies. The significance of these T cells in the disease pathogenesis remains to be fully understood. Such studies, particularly those identifying immunomodulatory epitopes in autoantigens, have potential to lead to antigen-specific therapies for SLE in humans.

Autoantigen-Based Vaccination and Peptide Therapies in Lupus

Current therapeutic modalities for lupus include immunosuppressive regimens, such as corticosteroids and cyclophosphamide, which are often administered at high doses. Although these treatments have been successful to a substantial degree in reducing mortality, they are associated with the development of numerous side effects and long-term complications. To avoid such drawbacks associated with immunosuppressive therapy several groups have focused on antigen-specific therapies.

Tolerizing DNA-Specific B Cells

Because many pathogenic autoantibodies bind DNA, there have been attempts to tolerize DNA-specific B cells. For example, more than 30 years ago, Borel et al. showed that it is possible to prevent lupus in an animal model by inducing tolerance to denatured DNA (272). About 15 years later, this finding was translated into human disease by showing that a DNA–IgG conjugate inhibits the formation of anti-dsDNA antibodies in vitro by lymphoid cells from SLE patients (273). Such studies eventually led to a clinical trial to evaluate a dsDNA-directed B cell tolerogen, a synthetic molecule with the ability to bind dsDNA antibodies, thus leading to anergy or apoptosis of B cells, which has shown delayed renal flares and reduction of anti-dsDNA antibodies in a subgroup of patients (274).

Tolerizing Lupus Th Cells

The above DNA-based tolerogen, however, has had a limited success in human SLE to date. Since strong evidence favors the requirement of T cell help for pathogenic autoantibody production (211 ,275), several groups have focused on strategies based on peptides that will specifically target autoreactive T and B cells. These approaches have mostly focused on identification of peptide sequences which can modulate autoimmune response, as summarized in Table 20-4 . Treatment with many of these peptides tolerizes pathogenic Th cells and/or induces regulatory or inhibitory T cells that suppress pathogenic Th or B cells. Such peptide-specific tolerance results in suppressed autoantibody production and lupus manifestations in murine models (102 ,173 ,275). Ongoing studies are trying to map such epitopes in humans (259 ,262). One clinical trial using an analogous peptide is in progress (264 ,265 ,275). Most studies using peptides to modulate lupus are described above and some are described in the following paragraphs. Table 20-4 provides a summary of these peptide and related therapies.

Degenerate recognition or cross-reactivity based on seemingly different peptides can occur in lupus. Thus, a single T cell hybridoma established from a (NZB/NZW)F1 mouse immunized with one self-Ig peptide recognizes several Ig-derived determinants, which had little sequence homology with the immunizing peptide (204). Such T cell recognition was not completely degenerate, as foreign peptides did not stimulate the self-reactive hybridoma. Such degenerate cross-recognition has also been described in humans with SLE. Thus, a single TCR on a human snRNP-reactive T cell clone can recognize two distinct snRNP autoantigenic peptides that have no apparent sequence homology (276). Similarly, a peptide SmD183–119 of SmD1 protein (D1 protein of the Smith [Sm] proteins, part of snRNP) activates T cell help for anti-dsDNA antibody production in (NZB/NZW) F1 mice (277). Importance of these studies is that tolerogenic treatment with one peptide should lead to tolerance in all cross-reactive T and B cells. Indeed, induction of high dose tolerance to the SmD183–119 peptide by IV injections of 600–1000 μg per month delays the production of autoantibodies including anti-dsDNA antibodies, postpones the onset of lupus nephritis, and prolongs survival (278). Tolerance to this peptide can be adoptively transferred by $CD90^+$ T cells, which also reduce T cell help for autoreactive B cells in vitro. The treatment was associated with increased frequencies of $IL\text{-}10^+/IFN\text{-}\gamma^+$ $CD4^+$ type 1 regulatory T cells, which can also prevent autoantibody generation and anti-CD3-induced proliferation of naive T cells.

Above studies on antigen-based therapies have mostly used parenteral routes of peptide administration. Mucosal delivery of peptides by oral feeding or nasal instillation can also induce strong peptide-specific tolerance in Th cells and suppress autoimmune diseases. The efficacy of mucosal tolerance has also been tested in lupus-prone mice (279). Nasal instillation of a histone peptide H471 that expresses a dominant T cell epitope in the histone protein H4 of mononucleosome induces a dose-dependent tolerance to the peptide H471 as well as to the whole mononucleosomes

in lupus-prone SNF1 mice. This is accompanied by an increase in IL-10 and suppression of IFN-γ production by lymph node cells. Furthermore, chronic nasal instillation of mice with the H471 peptide suppresses the development of autoantibodies and reduces the severity of glomerulonephritis in lupus-prone SNF1 mice. Such nasal tolerance restores the numbers of $CD4^+CD25^+$ Treg cells, which these authors found to be reduced in lupus-prone (NZB/NZW) F1 and SNF1 mice (280).

In preceding sections, we have described induction of tolerance in Th cells that mostly augment production of autoantibodies against nuclear and nucleoprotein antigens. Humans and mice with lupus also develop autoantibodies and T cells against cell- or tissue-specific protein antigens. For example, $CD4^+$ T cells from NZB mice respond to the anion channel protein band 3, a major target of the pathogenic red blood cell (RBC) autoantibodies in these mice. A band 3 peptide 861–875 is a dominant T cell epitope recognized by NZB T cells. Injection of NZB mice with the peptide 861–874, which is insoluble, accelerates the development of RBC-bound autoantibodies and autoimmune hemolytic anemia. Inhalation of this peptide also primes T cells for both peptide-specific and whole band 3 responses. By contrast, inhalation of a soluble analog (Glu861, Lys875) of this peptide deviated the autoimmune response toward a Th2 profile with increased IgG1 RBC-bound IgG, and reduced severity of anemia (281).

Other Non-T–Cell Based Peptide Therapies

Laminin Peptide Competitive Analogs to Inhibit Autoantibody Binding and Deposition in Tissues

Naparstek et al. have shown that murine pathogenic lupus autoantibodies bind to the laminin component of the extracellular matrix. Further analysis showed reactivity of these autoantibodies with a 21-mer peptide located in the globular part of the α-chain of laminin. Immunization of young lupus-prone mice with this peptide accelerated renal disease. They further found that the binding of lupus autoantibodies to the extracellular matrix could be inhibited in vitro by some competitive peptides that cross-react with the antilaminin antibodies. Treatment of MRL-lpr mice with these peptides prevented antibody deposition in the kidneys, ameliorated renal disease, and prolonged survival of the peptide-treated mice (282).

Mechanisms of Peptide-Based Therapies in Lupus

Neonatal Peptide Tolerance

Can peptide vaccines worsen lupus? Our initial attempts to tolerize lupus-prone mice met with difficulty. IP injections of a peptide emulsified in incomplete Freund adjuvant (IFA) were given to newborn mice. Most mice had excellent tolerance of type 1 T cell responses, but had activation of type 2 responses; peptide-specific T cell proliferation, and IL-2 and IFN-γ secretion was suppressed, but peptide-specific IgG antibodies, and secretion of IL-4, IL-5, and IL-10 was increased. This type of split T cell tolerance was associated with increased anti-DNA autoantibody production in (NZB/NZW) F1 mice (101). Induction of a similar split tolerance in adult mice was also associated with increased peptide-specific antibodies and type 2 cytokine production (Singh, unpublished observations).

Induction of "Direct" Tolerance in Th Cells

Subsequently, we found that IV administration of high doses of soluble peptides tolerizes both type 1 and type 2 T cell responses, and strongly suppresses peptide-specific T cell proliferation and antipeptide antibody responses, presumably through induction of apoptosis (102 ,283) (Singh, unpublished observations). This strategy successfully suppressed anti-dsDNA antibody production, delayed the onset of nephritis, and prolonged survival in young (NZB/NZW)F1 mice.

Inhibiting Determinant Spreading

Kaliyaperumal et al. (234) recently reported that IV administration of histone peptides in 18-month-old SWR/NZB F1 mice strongly suppressed autoantibody response to several antigens, a phenomenon they termed as "tolerance spreading." The anergy, deletion, suppression, or immune deviation that are classical mechanisms of tolerance induction did not appear to be operative in their system. Instead, they suggested that competition for MHC loading or modulation of some unknown signals involved in T-B cell interactions might have been responsible for tolerance induction in their model (234). In another study, Hahn et al. showed that treatment with a consensus anti-DNA variable region-based peptide not only reduced anti-DNA antibody levels, but also decreased the production of autoantibodies that bind nucleosome and cardiolipin (230), suggesting the spreading of tolerance to structurally unrelated lupus autoantigens.

There are also reports where treatment with autoantigenic peptides can accelerate disease spreading of pathogenic T cell responses to other epitopes or determinants. As a reminder, epitope or determinant spreading has been proposed as an important process, whereby the T cell responses spontaneously broaden from one part of an autoantigen to other parts of the same autoantigen as well as to other autoantigens during the progression of autoimmune diseases (201). In the NOD model of autoimmune diabetes, Tian J et al. found that treatment of newborn mice with an autoantigenic ß-cell peptide (in adjuvant) results in spreading of T cell response to other ß-cell autoantigen determinants, far in advance of when autoimmunity would have naturally arisen to these determinants. Thus, rather than limiting the loss of self-tolerance, immunotherapy caused the natural spreading hierarchy to be bypassed and autoreactivities to develop precociously (284). This study further underscores the need for caution in the clinical application of antigen-based immunotherapeutics in autoimmune disorders.

Induction of Regulatory T Cells and Cytokines

As discussed above, the nonautoimmune mouse strains can curtail pathologic autoimmunity by generation of autoantigenic peptide-reactive $CD8^+$ inhibitory T cells, which produce TGF-ß (34 ,169 ,172 ,174). TGF-ß produced by these cells appears to be important in their ability to inhibit autoantibody production, as the addition of an anti–TGF-ß antibody to cultures abrogates the inhibitory effect. Recent studies have further demonstrated an important role of TGF-ß in autoantigenic peptide-mediated suppression of lupus-like autoimmunity in (NZB/NZW) F1 and other murine models of lupus. Further studies have shown that such peptide-induced $CD8^+$ inhibitory T cells express regulatory T cell molecule Foxp3 and are more resistant to apoptosis than $CD8^+$ T cells from unprimed (NZB/NZW) F1 mice (177).

Most above studies have used high-dose peptide treatment regimens, which might induce allergic/anaphylactic reactions (285). To address this issue, a recent study showed that repeated low-dose treatment of lupus-prone (SWR × NZB)F1 (SNF1) mice by subcutaneous injections of 1 µg of highly conserved histone auto-epitopes suppresses IFN-γ responses of pathogenic lupus T cells, diminishes autoantibody production, delays nephritis and prolongs life span, without causing allergic/anaphylactic reactions or generalized immunosuppression (173). The protective effect is mediated through the generation of long-lasting, TGF-ß–producing $CD8^+$ and $CD4^+CD25^+$ Treg cells, which can suppress autoimmunity.

Induction of Cytotoxic T lymphocytes (CTL) That Ablate Autoreactive B Cells

Fan and Singh identified MHC class I–binding epitopes in the VH regions of anti-DNA monoclonal antibodies. The $CD8^+$ T cells reactive with these peptides elicit CTL responses against anti-DNA B cell hybridomas as well as B cells from diseased (NZB/NZW) F1 mice in vitro. This ablation of anti-DNA B cells occurs in a peptide-specific manner, as B cells that do not express Ig containing the relevant VH epitope are not subjected to killing. Induction of such CTLs in vivo is associated with reduced IgG anti-DNA antibody production (172).

Inhibiting T Cell Chemotaxis

In an experimental model of SLE induced in mice by immunization with a human anti-DNA mAb that expresses 16/6Id, treatment with a peptide located in the CDR1 of an anti-DNA mAb ameliorates the clinical manifestations of SLE and downregulates, ex vivo, the 16/6Id-induced T cell proliferation. The beneficial effects of the treatment with this peptide are associated with down-regulation of IFN-γ, IL-10, and TNF-α secretion and upregulation of the immunosuppressive cytokine TGF-ß in serum and in splenic cells cultured ex vivo (175 ,176 ,286). Further studies in this model suggested that TGF-ß–induced suppression may be mediated by downregulation of ERK phosphorylation, stromal cell-derived factor-1 α (SDF-1α; CXCL12)-induced T cell adhesion and migration, and expression and function of cell adhesion receptors LFA-1 (αLß2) and CD44 (287). The peptide-treated mice have reduced SDF-1α–induced T cell chemotaxis through fibronectin and collagen type I. SDF-1α is a pleiotropic CXC chemokine that affects the function of various cell types, including T cells, via its interactions with the CXCR4 receptor. The SDF-1α also regulates leukocyte proliferation, survival, and entry into sites of inflammation, and activation of T cells within blood vessels and in extravascular sites, where it can act either in its matrix-bound or soluble forms (288 ,289). Therefore in some models, the beneficial effects of peptide treatment may be mediated by affecting the chemotaxis and interaction with the extracellular matrix of autoreactive T cells.

Inhibiting Autoantibody Binding to Extracellular Matrix

As described above, treatment with certain laminin peptide analogs that cross-react with the antilaminin antibodies can suppress lupus disease in MRL-lpr mice. The authors suggested that the beneficial treatment correlates with the ability of these peptides to directly inhibit the binding of lupus autoantibodies to the extracellular matrix (282).

In summary, it appears that the mechanism of tolerance and peptide therapy depends on the nature of individual autoantigen or peptide, its form, dose, and route of delivery, and the state of T cell activation in the host. One peptide derived from anti-DNA IgG VH is in clinical trial at the time of this writing (January 2006). Generally, however, the variables listed would have to be individually worked out for different disease states and for autoantigens before other such therapy would be applicable to humans.

Will Peptide-Specific Treatment Ever Be a Reality, Since Patients with SLE Have a Highly Diversified and Polyclonal T and B Cell Repertoire?

Recent work from several laboratories suggest that, in contrast to the widely held notion that one autoimmune disease is caused by one or a few related autoantigenic epitopes, autoimmunity is fundamentally a continuously evolving process. The autoimmune responses shift, drift, and diversify with time not only to other epitopes in the original antigen but also to other antigens (101 ,290). Compared to many organ-specific autoimmune diseases, humans and mice with SLE develop a widespread polyclonal T and B cell activation (109 ,291). It is, therefore, not surprising that self-peptides of several different specificities are eluted from MHC class II molecules of lupus mice (251). Interestingly, the T cell activation although polyclonal is restricted to a set of autoantigens (204).

Several different mechanisms have been suggested to account for such “restricted polyclonality” in lupus. First, Craft et al. reported that it is the intrastructural organization of the autoantigenic complex that determines the restricted polyclonality in lupus mice (292). Secondly, Shi et al. (293) demonstrated a remarkable “promiscuity” in the recognition of histone peptides by T cells that induce pathogenic autoantibody production. Thirdly, we reported that Ig-derived T cell

epitope sequences are recurrent among lupus-associated autoantibodies of different specificities but are uncommon in the normal antibody repertoire (170 ,204). Based on this observation, we hypothesize that such sharing of T cell epitopes among various autoantibody V region sequences is responsible for restricted polyclonality in lupus. Thus, activation of T cells reactive with one or a few epitopes initially would drive activation of several B cells that display the shared peptide motif. These mechanisms are summarized in a recent review article (200).

Further complicating the issue of antigen therapies is the presence of polymorphisms in the HLA regions, thus requiring formulation and testing of expensive individualized therapy. Anticipating this problem, investigators are attempting to develop consensus and/or promiscuous autoantigenic epitopes that can bind many HLA molecules and modulate a broad repertoire of self-reactive T cells (170 ,200 ,230 ,293). For example, Kaliyaperumal et al. (234) used a set of highly promiscuous, nucleosomal epitopes that bind many MHC molecules across the species barrier and Hahn et al. (230) designed an artificial “consensus” Ig-based peptide that could suppress autoreactivities to several different autoantigens. Similarly, a $CD4^+$ T cell epitope in the spliceosomal U1–70K snRNP131–151 and its phosphorylated analog P140 can bind multiple HLA-DR molecules and suppress human T cell proliferation, thus serving as a “universal” T cell epitope (271). Parallel efforts in this area in humans and mice offer hopes for developing antigen-specific therapies for SLE. In fact, Teva Pharmaceuticals has already completed a phase I clinical trial in SLE patients using human anti-DNA antibody-derived peptides and a phase II trial is in progress in 2006. It is important to emphasize that a rigorous characterization of the exquisite T cell epitopes that activate regulatory and suppressor T cells versus those that stimulate pathogenic Th cells, in humans is critical to ensure that we do not run into premature disappointment, as seen in some other antigen therapy trials. Complicating the selection of peptides for treatment, our murine studies suggest that suppressor and Th cell epitopes might colocalize or overlap (170), as if nature has done a fine balancing act by putting together the “protective” and “pathogenic” epitopes.

Peptide-Specific Therapy: Lessons from Other Autoimmune Diseases

A large body of work in animal models of organ-specific autoimmune diseases suggests that tolerance-inducing treatments with autoantigenic peptides can be successfully used to suppress autoimmunity in experimental myasthenia gravis, autoimmune neuritis, EAE, and collagen-induced arthritis (285 ,294 ,295 ,296 ,297 ,298 ,299). Such treatments have also been attempted in human diseases such as rheumatoid arthritis with modest success (300). It may be important, however, to remind here that tolerance strategies and mode of peptide delivery that provide beneficial response in organ-specific diseases might not be applicable to SLE. For example, administration of peptides emulsified in IFA that induces peptide-specific immune deviation toward type 2 T cell response prevents subsequent induction of EAE and other organ-specific diseases (301). Similar treatment using an Ig-derived peptide, however, enhanced anti-DNA antibody levels in one study (101). This differential effect might be a result of differences in the nature of autoimmune pathology between the two types of diseases: inflammation in organ-specific autoimmune diseases is mostly mediated by type 1 T cells, whereas both type 1 and type 2 cytokines appear to be important for tissue damage in SLE.

In autoimmune diabetes that results from autoreactive T cell responses directed against multiple pancreatic ß-cell antigens, a phase II clinical trial with glutamic acid decarboxylase (GAD65) antigen in patients having GAD65 autoantibodies was associated with an increase in potentially regulatory $CD4^+CD25^+$ T cells (302). Generally, however, some antigen-specific therapies that readily inhibited autoimmune diabetes in animal models have been problematic or ineffectual when moved to human clinical trials. Studies in mouse models of diabetes indicate that the kinetics and frequency at which ß-cell autoreactive T cell responses are generated against major ß-cell autoantigens varies greatly in individual diabetic mice (303). If ß-cell autoreactive T cells with various specificities also develop in such a stochastic fashion during the course of diabetes development in humans, it would be very difficult to determine what antigen-based immunotherapy would be most efficacious for any given individual. This suggests that the stochastic development of autoreactive T cell responses may indeed be a hurdle that must be overcome in developing autoimmune disease prevention protocols for use in humans. Thus, we must adopt cues from such successes and failures in animal models and clinical studies in other autoimmune diseases to formulate the research agenda for successful antigen-specific therapies for lupus.

Gene Vaccination Approaches for SLE

Peptide “Minigene” Vaccines to Suppress Lupus

We have recently demonstrated that the VH of anti-DNA antibodies contain epitopes that can be processed efficiently owing to their high cleavage probability score to bind MHC class I molecules (172). We hypothesized that $CD8^+$ CTLs reactive with such Ig VH epitope will recognize and lyse B cells that can process and display the relevant VH epitope on their surface class I molecule. We found, however, that it is generally difficult to induce $CD8^+$ CTLs in lupus mice. Since antigen delivery via a plasmid DNA or viral vectors generally elicits strong peptide-specific $CD8^+$ T cell response (295 ,304), we surmised that delivery of Ig VH epitopes via plasmid DNA vectors as minigenes might elicit CTL responses in lupus mice. Indeed, vaccination of (NZB/NZW) F1 mice with plasmid DNA vectors that encode such epitopes activates CTL responses against anti-DNA antibody producing B cells, inhibits anti-DNA antibody production, retards the development of lupus nephritis, and prolongs survival (172). We have been able to induce similar anti-VH

CTL responses in lupus-prone mice by delivering MHC class I-binding VH epitopes conjugated to CpG-oligodeoxynucleotides (305). Thus, minigene mediated induction of anti-VH CTLs that can ablate autoreactive B cells represents a novel approach to treat autoantibody-mediated diseases.

A recent study used autoantigen-specific T cells for the local delivery of therapeutic molecules (306). They engineered nucleosome-specific regulatory T cells by multiple gene transfer (nucleosome-specific TCR-α, TCR-β, and CTLA4Ig). Treatment with these engineered cells suppressed pathogenic autoantibody production and nephritis in (NZB/NZW)F1 mice without impairing the T cell–dependent humoral immune responses. Thus, autoantigen-specific regulatory T cells engineered by multiple gene transfer is a promising strategy for treating autoimmune diseases.

Establishing Immune Tolerance in SLE by Approaches Other Than Peptide-Specific Therapies

Studies from animal models of other autoimmune diseases such as diabetes suggest that some “nonspecific” immunomodulations that inhibit autoimmune disease, and because of their general ability to improve tolerance induction mechanisms, may prove more successful in preventing disease in high risk humans than antigen-based therapies (303). For example, as described above, a recent study has identified one mechanism by which T cells from patients with SLE avoid peripheral tolerance. The study found that lupus T cells selectively use the COX-2/FLIP antiapoptosis program to resist anergy (107). Interestingly, such defect in lupus T cells can be reversed in vitro by some, but not all, COX-2 inhibitors, which cause apoptosis of the anergy-resistant T cells and suppress the production of pathogenic autoantibodies to DNA. Thus, further understanding of the mechanisms of peripheral T cell tolerance in lupus and translating those findings to clinical trials can open new avenues for treatment.

Aberrant expression and regulation of costimulatory signals have been implicated in the breakdown of T and B cell tolerance in SLE. These studies have prompted human SLE trials including soluble CTLA4-Ig fusion protein that blocks T cell–dependent B cell functions (307). Treatment of lupus-prone mice with CTLA4-Ig in combination with TACI-Ig (that blocks BAFF) results in the delayed onset of proteinuria, which is associated with prolonged depletion of B cells past the T1 stage, and a decrease in the absolute number of activated and memory $CD4^+$ T cells (308). Thus, blockade of T cell–mediated costimulation in human SLE might repair tolerance defects in autoreactive cells.

Studies of B cells from SLE patients undergoing B cell depletion therapy using rituximab suggest that apparently nonspecific therapies may repair the specific tolerance defects in SLE (309). Thus, CD20-targeted B cell depletion effectively normalizes the significant disturbances in peripheral B cell homeostasis that typically occur in SLE, including naive lymphopenia, expansion of a novel population of IgD/CD27 double-negative cells, the presence of plasma cell precursors, and expansion of autoreactive memory B cell populations (310). Stem cell transplant could also be another method of resetting normal tolerance in all immune cells. Finally, repairing epigenetic defects, such as by treatment with histone deacetylase inhibitors, such as trichostatin A, which correct these impairments and suppresses lupus in mice (140) holds promise for human SLE.

It is important to mention here that most studies have focused on the prevailing notion that T cell–mediated production of autoantibodies that cause tissue damage underlies the pathogenesis of lupus. Challenging this notion are some recent studies, where lupus-associated organ damage can be “uncoupled” from the production of antinuclear autoantibodies (2 ,185 ,311 ,312). Consistent with this idea, some individuals have high levels of antinuclear autoantibodies but no SLE-associated organ damage, whereas many SLE patients with end organ damage have no antinuclear autoantibodies. In some cases, autoantibodies deposit in tissues, but do not cause any local inflammation and damage. Studies in other autoimmune diseases also provide credence to this idea. For example, the presence of autoantibodies to β-cell antigens is not always related to the clinical onset of hyperglycemia and diabetes (302). These studies, however, do not exclude the possibility that the more relevant pathogenic autoantibodies may differ in antigen specificity, binding affinity and Ig isotype and/or subclass, making their detection more difficult. It is also possible that autoantibodies may contribute to tissue lesions in some individuals, but not in others. Additionally, autoimmunity may play a role in the initiation, but not in perpetuation, of tissue lesions.

Synthesis

Immunologists have been fascinated with the idea of identifying the disease-specific autoantigen(s) and using them in antigen-specific therapies (275 ,285 ,313 ,314 ,315 ,316). This task becomes particularly difficult in SLE, where, in contrast to organ-specific autoimmune diseases, there is no organ-specific autoantigen target. Painstaking efforts by several laboratories have led to identification of peptides that activate potentially pathogenic Th cells. A diverse group of self-antigens appear to be the source of these peptides. It is likely that peptides from several different antigens activate autoreactive Th cells that promote autoantibody production and disease in SLE. Probably, an interconnected circuitry of reciprocal T-B–cell recognition drives the spreading of response from one T cell to another until a massive expansion of diverse arrays of T and B cells has occurred. Such T-B–cell diversification might pose difficulty for designing antigen-specific therapies. The good news, however, is that tolerogenic treatment with one or just a few peptides appear to quell autoimmune responses against a variety of autoantigens. Such peptide treatments appear to retard the progression of nephritis and prolong the survival in lupus-prone mice. While we have

a long way to go to understand these processes in mice and humans with lupus, the initial studies in lupus mice offer new hope for similar treatment approaches in patients with SLE. Remarkably, striking similarities exist between peptides that appear to activate T cells in patients with SLE and peptides that activate Th cells that induce autoantibodies that cause disease in lupus mice. However, experience in other autoimmune diseases such as type 1 diabetes has taught us that rush to clinical trials using autoantigenic peptides must not occur without full realization that the biologic basis by which a tolerogenic therapy may suppress disease in an animal model may not be directly translatable to humans. Further, we must fully understand the mechanisms and pathways of loss of tolerance in self-reactive T cells in both animals and humans. In that regard, different models, including different animal strains and human peripheral blood cells, tonsils, and spleen, and diverse methodologies are being used to uncover tolerance defects in SLE. These studies reveal that whereas breakdown of central tolerance involving positive and negative selection in bone marrow or thymus might explain lupus-like autoimmunity in some instances, impaired peripheral tolerance appears to confer self-reactivity in many cases. In the periphery, these impairments in SLE involve breakdown of anergy or deletion of autoreactive cells or loss of normal censoring at different checkpoints during the development of immune responses. Whereas impairments at the level of APCs, adhesion, constimulation and interactions between different immune cells, and lymphoid organization such as faulty germinal center exclusion of autoreactive B cells might explain the loss of normal censoring in some cases, intrinsic ability of lupus T and B cells to become easily overexcitable might be responsible for lupus-like autoimmunity in other cases. Full understanding of these mechanisms might open new therapeutic avenues that do not depend on rigorous antigen specificity but will be based on repairing the alterations in specific immune mechanisms. Finally, some SLE patients may come to clinic at a late stage when their disease cannot be tackled by repairing faults in immune tolerance. Recent studies have also begun to unravel that faulty immune tolerance might not be the cause of lupus disease development in some cases. For such cases, we must understand the mechanisms of end organ damage.

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Section III
Autoantibodies

Chapter 21

The Structure and Derivation of Antibodies and Autoantibodies

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The humoral immune response protects an organism from environmental pathogens by producing antibodies (immunoglobulins) that mediate the destruction or inactivation of microbial organisms and their toxins. To perform this function, the immune system generates antibodies to a diverse and changing array of foreign antigens, yet it must do so without generating pathogenic antibodies to self. The production of high-affinity antibodies that bind to self-determinants is a prominent feature of systemic lupus erythematosus (SLE) (1). Some autoantibodies in SLE are considered markers for disease (anti-Sm/ribonucleoprotein [RNP], antinuclear antibody), because they have no established pathogenicity; others play a role in disease pathogenesis and cause tissue damage (anti-DNA, anticardiolipin, anti-Ro) (2 ,3 ,4 ,5 ,6).

There have been extensive investigations of autoantibodies in SLE, which address a number of specific questions:

- Do polymorphisms of immunoglobulin variable region genes contribute to disease susceptibility?
- Do B cells producing autoantibodies arise from an antigen-triggered and -selected response? If so, are these triggering and selecting antigens self or foreign?
- Are particular B cell lineages or differentiation pathways responsible for autoantibody production?
- What are the characteristics of pathogenic autoantibodies, and how do they mediate pathology?
- What defects in immune regulation permit the sustained expression of pathogenic autoantibodies?

This chapter discusses autoantibody structure, assembly, and regulation. Based on new advances in our knowledge of autoantibody structure and regulation, novel potential therapeutic strategies are also briefly addressed.

Structure of the Antibody Molecule

Antibodies are glycoproteins produced by B lymphocytes in both membrane-bound and secreted forms. They are composed of two heavy chains and two light chains. Generally, the two heavy chains are linked by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. The intact molecule has two functional regions: a constant region that determines its effector functions, and a variable region that is involved in antigen binding and is unique to a given B cell clone (7) (Fig. 21-1). The light chains appear to contribute solely to antigen binding and are not known to mediate any other antibody function. In contrast, the heavy chains possess a constant region that determines the isotype (i.e., class: immunoglobulin M [IgM], IgD, IgG, IgA, or IgE) of the antibody molecule (Fig. 21-2). Rarely, the same variable region associated with a different constant region may display an altered binding to antigen and the constant region may infrequently help determine the antigenic specificity of the antibody (8 ,9). IgM is the first isotype produced by a B cell and the first to appear in the serum response to a newly encountered antigen. IgM antibodies normally polymerize into pentamers known as macroglobulin, thus conferring higher functional binding strength, or avidity. A 15-kd glycoprotein called the J chain is covalently associated with the pentameric IgM, and mediates the polymerization process (10 ,11). IgM antibodies can activate complement via the classic pathway and therefore cause lysis of cells expressing target antigens. Under the appropriate conditions, B cells producing IgM can switch to the production of the other isotypes. IgG is the predominant isotype of the secondary (also called memory) immune response. In humans, the IgG isotype is divided into four subclasses, IgG1, IgG2, IgG3, and IgG4, all of which possess different functional attributes. IgG1 is the most abundant in the serum. Antinuclear antibodies in SLE are mainly of IgG1 and IgG3 subclasses (12). In addition to activating complement, IgG antibodies can promote Fc-receptor (FcR)-mediated phagocytosis of antigen-antibody complexes. High concentrations of antigen-IgG complexes can downregulate an immune response by cross-linking membrane immunoglobulin and FcRII receptors on antigen-specific B cells. This may be an important mechanism for turning off antibody production after all the available antigen is bound to antibody, and there is some evidence for defective FcRII function in some lupus patients. The IgA constant region allows antibody translocation across epithelial cells into mucosal sites such as saliva, lung, intestine, and the genitourinary tract; IgA antibodies can be found as monomers in serum and as dimers in the mucous secretions. The J chain, implicated in IgM polymerization, is not required for IgA dimerization, but does have a role in

maintaining IgA dimer stability and is essential for transport of IgA by the hepatic polymeric Ig receptor (13). IgE antibodies can trigger mast cells and eosinophils, which are important cellular mediators of the immune response to extracellular parasites and cause allergic reactions.

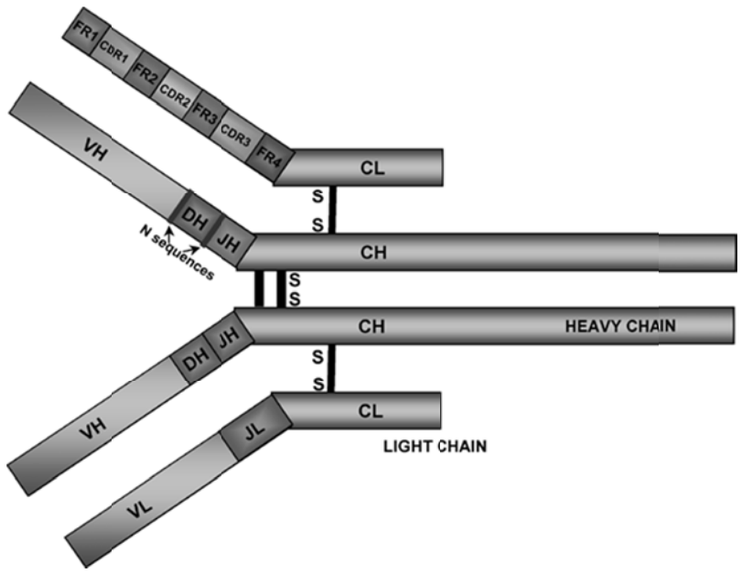

Figure 21-1. A prototypic antibody molecule. C, constant region; CDR, complementarily-determining region; D, diversity region; FW, framework region; J, joining region; V, variable region.

Every complete antibody has two identical antigen-binding sites, each of which is composed of the variable regions of a heavy and a light chain. When the variable regions from the light and heavy chain pair, the hypervariable segments or complementarily-determining regions (CDRs) come together and generate a unique antigen-binding site (Fig. 21-1). The antigen-binding site is divided into the highly polymorphic CDRs, and the more conserved framework regions (FRs). There are three distinct CDRs in both the heavy and light chain, and the most variable portion of the antibody molecule is the CDR3 (14 ,15). There are four FRs. Radiograph crystallographic studies have shown that the amino acids of the CDRs are arranged in flexible loops, while the FRs have more rigid structure that maintains the spatial orientation of the antigen-binding pocket (16), consistent with the fact that CDRs contain the contact amino acids for antigen binding and thus contribute more than the FRs to antigenic specificity.

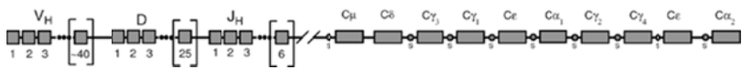

Figure 21-2. The heavy-chain immunoglobulin gene locus on chromosome 14. C, constant region; D, diversity gene locus; J, joining gene locus; S, switch region; V, variable gene locus.

Antibody molecules are Y-shaped structures that can be cleaved into functionally distinct fragments by proteases like papain and pepsin (17 ,18). Limited digestion with papain cleaves the antibody into three fragments: two identical Fab (fragment antigen binding) fragments and an Fc (fragment crystallizable) fragment. The Fab fragment consists of the entire light chain and the heavy chain variable region with the CH1 domain. It contains the antigen-binding site, which is formed by the variable regions of the light and heavy chain at the tip of each Fab fragment. The Fc fragment is composed of the two carboxy-terminal domains from the heavy chains, the hinge region and CH2 and CH3, and interacts with soluble and cell membrane bound effector molecules. The Fc fragment does not have antigen-binding activity. The Fab portions are linked to the Fc fragment at the hinge region, which allows independent movement of the two Fabs (19). Another protease, pepsin, cleaves the antibody molecule on the carboxy-terminal side of the heavy chain disulfide bridges producing several small fragments and an F(ab)2 fragment, that contains both Fabs linked to each other with an intact hinge region. F(ab)2 cannot be obtained from IgG2 by pepsin. However, lysyl endopeptidase digestion will generate F(ab)2

from IgG2 (20). Based on the fact that the F(ab)2 fragment has the same avidity for antigen as the intact antibody, but does not possess any effector functions, this cleavage product may have therapeutic applications.

The variable region of an antibody may itself serve as an antigen, called an idiotype. Anti-idiotypes are antibodies that bind to specific determinants in the CDRs or FRs of other antibodies (21 ,22) (Chapter 15). Antibodies that share the same idiotype presumably have a high degree of structural homology and may be encoded by related variable region genes (23). Idiotypes have been postulated to be important in the regulation of the immune response because they can be recognized by both T and B cells (24 ,25 ,26 ,27) (Chapter 15). Anti-idiotypic antibodies may therefore be useful reagents for tolerizing pathogenic autoantibody-producing B cells (see below).

Antibody Assembly

The immunoglobulin light and heavy chain variable region genes are formed by a process of rearrangement of distinct gene segments, permanently separated in the genome in all cells except B lymphocytes. In B cells these genes are rearranged by a process called somatic recombination. During this process, V (variable), D (diversity), and J (joining) segments are brought together to form a heavy chain variable region gene, and V and J segments to form a light chain variable region gene (28 ,29 ,30 ,31 ,32 ,33).

In humans, heavy chain V, D, and J gene segments each come from gene clusters that are randomly arrayed on chromosome 14 (33 ,34) (Fig. 21-2). The 50 to 100 functional heavy chain V segment genes are divided into seven families, which share 80% homology by DNA sequence primarily in FRs (35 ,36 ,37 ,38). V gene family members are interspersed randomly along the V locus. There are approximately 30 functional D gene segments and six known J gene segments for the human immunoglobulin heavy chain (38).

Each V, D, and J gene segment is flanked by conserved heptamer/nonamer consensus sequences that are crucial for the rearrangement process. The conserved heptamer is always most proximal to the coding sequence, followed by a 12- or 23-base pair (bp) spacer sequence, and then by the conserved nanomer. The length of the spacer corresponds to either one or two turns of the DNA double helix, which allows the heptamer and nanomer to be brought together on one side of the DNA helix and enables them to interact with the proteins that catalyze the recombination process. The heptamer-spacer-nanomer sequence is known as recombination signal sequence (39).

Assembly of the complete heavy chain gene begins with the joining of a D segment from the D cluster to a J segment in the J cluster, mediated by DNA cleavage and deletion of the intervening DNA. In a similar manner, a V gene segment is next rearranged to the DJ unit to form a complete VDJ variable region (28 ,37). This process of variable region recombination is very elaborate and requires a complex of enzymes called V(D)J recombinase (40). Most of these enzymes are also necessary for the maintenance of double-stranded DNA (dsDNA) and are present in all cells. However, for the first cleavage step, specialized enzyme products of the recombination activating-genes RAG-1 and RAG-2 are required (41). The proteins encoded by these genes are active in the early stages of lymphoid development. Signals from both stromal cells and the cytokines interleukin-3 (IL-3), IL-6, and IL-7 are necessary for induction of RAG genes in lymphoid progenitors (42). RAGs bind to the nanomer sequence and initiate VDJ recombination by generating dsDNA breaks at the end of the recombination signal sequence. Joining of the coding segments is mediated by several enzymes involved in repair of dsDNA breaks: Ku 70, Ku 80, DNA-PKs, XRCC4, ligase, and Mre 11 (43). Members of the high-mobility group family of proteins HMGl and HMG2 (44) also play a significant role in the formation and stabilization of the precleavage and postcleavage synaptic complex (45 ,46).

Antibody diversification can be further generated by the addition of P and N nucleotides at the VD and DJ junctions. If the single-stranded DNA (ssDNA) that is present after the break can form a hairpin loop, the resulting double-stranded (palindromic, P) sequences are added at the junction. Alternatively, N-nucleotides or nontemplate-encoded nucleotides, are randomly inserted at the VD and DJ junctions by the enzyme terminal deoxynucleoridyl transferase (TdT) (47). Such N sequences are common in antibodies of the adult immunoglobulin repertoire but are less frequent early in the ontogeny of the B cell repertoire (48). These random modifications create unique junctions and increase the diversity of the antibody repertoire. Because VDJ joining is imprecise and includes P and N sequences, CDRs of variable length and sequence are generated.

After generation of a functional heavy chain, the light chain gene segments can rearrange from either of two loci, κ or λ. The ratio of the two types of light chains varies in different species. For example, in mice the κ/λ ratio is 20:1, whereas in humans it is 2:1. Although the light chain isotype has in general not been found to influence main properties of the antibody molecule, it has been reported that λ light chain can contribute to a faster clearance of some IgG isotypes than the κ chain (49). The reason for this difference is unclear considering that light chain isotype is not expected to alter conformation of heavy chain second and third constant regions which are important for binding to FcR and subsequent clearance. The light chain variable region is composed of only two gene segments: V and J. Genes for the V and J segments of κ light chains are located on chromosome 2. The κ locus contains approximately 40 functional V gene segments, which are grouped into seven families, and five J segments (50 ,51 ,52 ,53 ,54). The λ light chain locus is on chromosome 22, and contains at least seven V gene families, with up to 70 members (55 ,56 ,57 ,58 ,59). As with the heavy chain, V and J elements of the light chain loci also rearrange by recombination at heptamer/nonamer consensus sites. Only rarely are N sequences inserted at the VJ junction of the light chain (60).

RAG gene expression is high in pro-B and pre-B cells. Once a heavy chain rearrangement successfully occurs, the heavy chain associates with a nonpolymorphic light chain,

termed surrogate light chain. The appearance of an Ig heavy chain with surrogate light chain on the cell surface of large pre-B cells coincides with inactivation of the RAG-2 protein. Degradation of both RAG-1 and RAG-2 messenger RNA (mRNA) occurs and there is no further heavy chain rearrangement. Later, RAG-1 and RAG-2 are again expressed and light chain gene rearrangement takes place. When a complete Ig molecule is expressed at the cell surface, RAG expression ceases (61).

The importance of the V(D)J recombination process has been demonstrated in animal studies as well as in some hereditary immune disorders. Mutations that abolish V(D)J recombination cause an early block in lymphoid development resulting in severe combined immune deficiency (SCID) with a complete lack of circulating B and T lymphocytes. Mice missing either RAG-1 or RAG-2 are unable to rearrange immunoglobulin genes or T cell receptor genes (62). In humans, a loss or marked reduction of V(D)J recombination activity can cause a T-B-SCID (63 ,64) or B-SCID phenotype (65). Mutations that impair but do not completely abolish the function of RAG-1 or 2 in humans result in Omenn syndrome, a form of combined immune deficiency characterized by lack of B cells and oligoclonal, activated T lymphocytes with a skewed T-helper-2 (Th2) profile (66). It is clear, however, from studies of immunodeficient mouse strains that additional gene products also are needed for successful rearrangement to occur. Defects in any of the components of the dsDNA break repair machinery such as Ku70, Ku 80, DNA PKs, DNA ligase IV, and XRCC4 lead to an immunodeficient phenotype with increased radiation sensitivity as a common feature (67).

While the rearranged heavy chain VDJ segment is initially associated with an IgM constant region gene, it can undergo a second kind of gene rearrangement during the secondary response to associate with the other downstream constant region genes (68 ,69 ,70) (Fig. 21-2). Switch sequences located upstream of each constant region gene mediate heavy chain class switching (71).

Although all somatic cells are endowed with two of each chromosome, only one rearranged heavy chain gene and one rearranged light chain gene normally are expressed by a B cell. This phenomenon is known as allelic exclusion. A productive rearrangement on one chromosome inhibits assembly of variable region genes on another chromosome. Rearrangement of the first chromosome is often unproductive because of DNA reading frame shifts or because nonfunctional variable region gene segments called pseudogenes are used. If rearrangement on the first chromosome does not lead to the formation of a functional polypeptide chain, then the immunoglobulin genes on the other chromosome will undergo rearrangement. Mono-allelic expression avoids potential expression of immunoglobulins or B cell receptors (BCRs) with two different specificities on the same B cell, which could interfere with normal selection processes (see below). Regulation of allelic exclusion seems to occur at the recombination level suggested by the observation that transgenic mice allow the expression of two pre-arranged alleles at either the heavy or light chain locus (72). Single-cell analysis of germline transcription in pro-B cells have shown transcription of Vk genes on both chromosomes (73); however, the earlier expressed alleles are almost always the first to undergo rearrangement (74). Methylation of DNA mediates gene repression and decreases the probability of recombination when methylation is found in the proximity of recombination signal sequences (75).

While the heavy chain has a single locus of V, D, and J segments on each chromosome, the light chain has two. The κ locus is the first set of light chain gene segments to rearrange. If these rearrangements are nonproductive on both chromosomes, however, then the V and J segments of the λ locus rearrange to produce an intact light chain (76 ,77). Thus, while the heavy chain has two loci from which to form a functional gene, the light chain may rearrange at four loci. Moreover, additional or secondary rearrangements can occur in B cells already expressing an intact antibody molecule if that antibody has a forbidden autospecificity. These additional rearrangements, which are termed receptor editing, are important in allowing B cells to regulate autoreactivity.

Immune tolerance mediated by receptor editing occurs frequently in developing B cells (78). High-affinity receptor binding to self antigen induces a new gene recombination (79) and the replacement of the gene encoding a self-reactive receptor by a gene encoding a nonself-reactive receptor (80 ,81). Receptor editing occurs at both light and heavy chain loci, but at a much lower frequency at the heavy chain locus (82). There is some debate about whether Ig gene rearrangement can occur also in mature B cells or only in immature B cells (83). RAG protein expression in germinal centers, as well as after immunization (84 ,85) has suggested that antibody genes may undergo modification not only in developing but also in mature B cells (85 ,86 ,87). Immunization of BALB/c mice with a multimeric form of a peptide mimetope of dsDNA induces the generation of dsDNA-reactive B cells. Mature B cells that respond to peptide re-express RAG for a short period of time only, suggesting that receptor editing can also participate in peripheral tolerance (88). The regulation and function of secondary rearrangements of Ig genes in mature B cells remains incompletely understood, however as some data suggest rearrangement events can be initiated in germinal center B cells that fail to bind antigen.

Generation of Antibody Diversity

The immune system has several mechanisms to ensure a large antibody repertoire. Before exposure to antigen, B cell diversity results from (a) combinations of V, D, and J gene segments and V and J segments into heavy and light chain genes, respectively; (b) junctional diversity produced by N or P sequence insertion and/or imprecise joining of gene segments; and (c) the random pairing of heavy and light chains. These three mechanisms are consequences of the process of recombination used to create complete Ig variable regions. The fourth mechanism, called somatic hypermutation (SHM), occurs later on rearranged DNA. This mechanism introduces point mutations

into rearranged variable region genes (Table 21-1). These mechanisms are potentially capable of producing a repertoire of 1011 different antibodies (89).

Table 21-1: Mechanisms of Antibody Diversity

Combinatorial diversity of V, D and J gene segments for heavy-chain variable region and V and J gene segments for light-chain variable region
Junctional diversity of rearranged heavy- and light-chain variable regions
N-terminal addition
Imprecise joining
Random association of heavy and light chains
Somatic point mutation

Cross-linking of surface immunoglobulin on the B cell by a multivalent antigen is the first in a series of critical steps that eventually can lead to B cell activation and antibody production. Following cross-linking of membrane immunoglobulin, the antigen-antibody complexes are internalized, and the antigen is cleaved and processed in the cell. Peptide fragments of protein antigen bound to the major histocompatibility complex (MHC) class II molecules are then expressed on the cell surface, where they can be recognized by antigen-specific helper T cells (Fig. 21-3). These T cells provide the co-stimulation and cytokines that are necessary for full B cell activation.

On initial exposure to an antigen, naive B cells recognizing the antigen enter secondary lymphoid organs such as the spleen or lymph nodes, where they proliferate and begin to secrete IgM. The antibodies of this primary immune response generally are polyreactive and display low affinity to a multitude of antigens, even to antigens without obvious structural homology (Table 21-2). The amplification of antigen-specific B cells occurs in specific regions of the lymphoid tissue called germinal centers. SHM (discussed later), leads to the selection of high-avidity B cell clones. Within the germinal center heavy chain isotype switching, and further differentiation to plasma and memory B cells also occur.

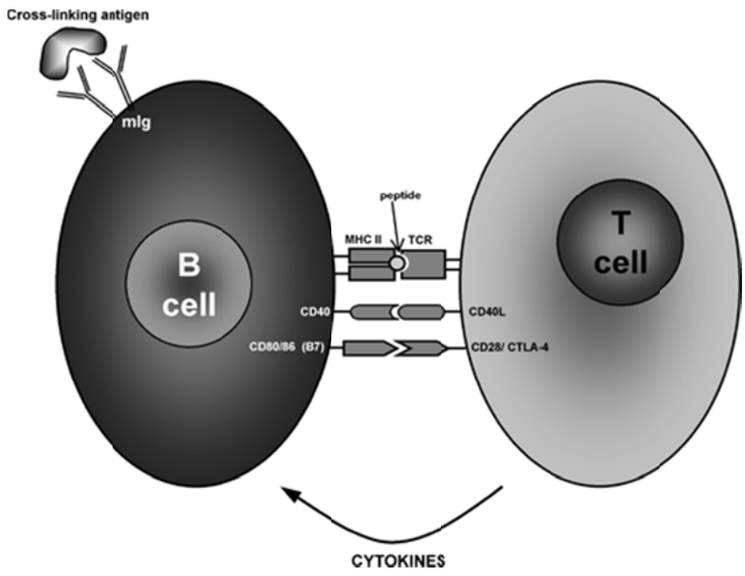

Figure 21-3. B cell-T cell cognate interactions. MHC II, class II major histocompatibility complex; mIg, membrane immunoglobulin; TCR, T cell receptor.

Table 21-2: Distinguishing Features of the Naïve and Antigen-Activated Antibody Repertoire

Feature	Naïve	Antigen Activated
Isotype	Primarily IgM	Primarily IgG
Specificity	Polyreactive	Monospecific
Affinity	Low affinity	High affinity
Sequence	Germline gene encoded	Somatically mutated (high R-to-S ratio)
Titer	Low titer	High titer

Studies using mice with targeted disruptions of particular genes have shown that in addition to a cognate interaction between the T cell receptor and an MHC class II molecule, other pairs of B cell–T cell contacts are also necessary for germinal center formation and function (Fig. 21-3). One important interaction is between the CD40 receptor on the B cell and CD40 ligand (CD40L, gp39) expressed on activated CD4 T cells.

Activation of the CD40 receptor is thought to be necessary for the formation of germinal centers and germinal center reactions (90 ,91). Defective CD40 ligand on T cells in humans and mice causes X-linked hyper-IgM type I syndrome, which is characterized by a defect in isotype switching and severe humoral immunodeficiency, leading to increased susceptibility to infections with extracellular bacteria (92). Since the proliferation of autoreactive B cells in SLE is T cell dependent, current therapeutic approaches include blockade of the co-stimulatory signals important in the activation of T cells by antigen presenting cells (B7-CD28) and in the activation of B cells by antigen-specific T cells.

After the primary immune response is complete, specific antibody secretion decreases. Reexposure to the antigen and activated T cells, however, can activate memory B cells that arose in the germinal center response to initiate the secondary immune response. The secondary serum response is characterized by rapidly produced high titers of IgG antibodies that have greater specificity and increased affinity for antigen (93 ,94 ,95). The increase in both affinity and specificity is a consequence of SHM and selection process within the germinal center. Anti-dsDNA antibodies, which are the most well-characterized pathogenic autoantibodies to date, possess all the features of secondary response antibodies (96 ,97 ,98 ,99) (Table 21-2) (Chapter 23).

Somatic point mutations are single nucleotide substitutions that can occur throughout the heavy and light chain variable region genes (100 ,101 ,102) and represent a site-specific, differentiation stage-specific, and lineage-specific phenomenon (103). Somatic mutation takes place in dividing centroblasts, in which rearranged Ig variable region genes undergo a mutation rate of 1 bp per 10^3 bp/cell divisions compared to 1 bp per 10^{10} bp/cell divisions in all other somatic cells. The DNA mismatch repair system has been implicated in Ig gene mutation because it functions generally to correct point mutations in DNA. A genetic deficiency in a component of the mismatch repair system, PMS2, has been shown to enhance the rate of mutation, suggesting that the DNA mismatch repair system may be altered in hypermutating B cells (104). Similarly, mice deficient in Msh6, a component of the mismatch repair system, have altered nucleotide targeting for mutations (105). Because somatic mutation occurs concurrent with heavy chain class switching, although by a different mechanism, mutation is more common in IgG than in IgM antibodies.

The generation of high affinity antibodies through B cell maturation with SHM and class switch recombination (CSR) critically depends on the action of activation-induced cytidine deaminase (AID) (106). AID is a member of a family of Apobec cytidine deaminases that causes DNA conversions of cytosine to uracil, generating mutations in the immunoglobulin gene that can increase antibody affinity for the antigen (107). AID deficiency in humans causes a disorder called Hyper-IgM type 2 syndrome characterized by elevated serum levels of IgM and undetectable IgG, IgA and IgE (108). Mice with a homozygous deletion of AID display normal B cell maturation but are deficient in SHM and CSR while overexpression of AID is sufficient to induce SHM and CSR in B cell lines or fibroblasts (109 ,110). AID expression is tightly regulated and appears to be restricted to GC, although clearly CSR can occur outside GC. Genomic instability and higher mutation rates are likely to occur in the presence of poorly regulated AID expression, which can lead to malignancies.

Genealogies of B cells with serial mutations in their immunoglobulin gene sequences demonstrate how point mutations can lead to antibodies with altered affinity for antigen (111 ,112 ,113 ,114) (Fig. 21-4). While B cells producing antibodies with decreased affinity appear within the germinal center, progression of these cells to the plasma or memory

cell compartment is rare, as they fail to be amplified further in the immune response. In contrast, B cells producing antibodies of higher affinity continue to be amplified. SHM is an important process in the generation of high-affinity antibodies, and a suboptimal frequency of Ig V gene mutation leads to common variable immunodeficiency (CVID) (115). Mutated antibodies also can acquire novel antigenic specificities. In one in vitro system, a single amino acid change in a protective anti-pneumococcal antibody results in reduced binding to pneumococci and a newly acquired affinity for dsDNA (116). Abundant evidence suggests that antibodies to foreign antigen also can acquire autospecificity in vivo through somatic point mutation (117 ,118).

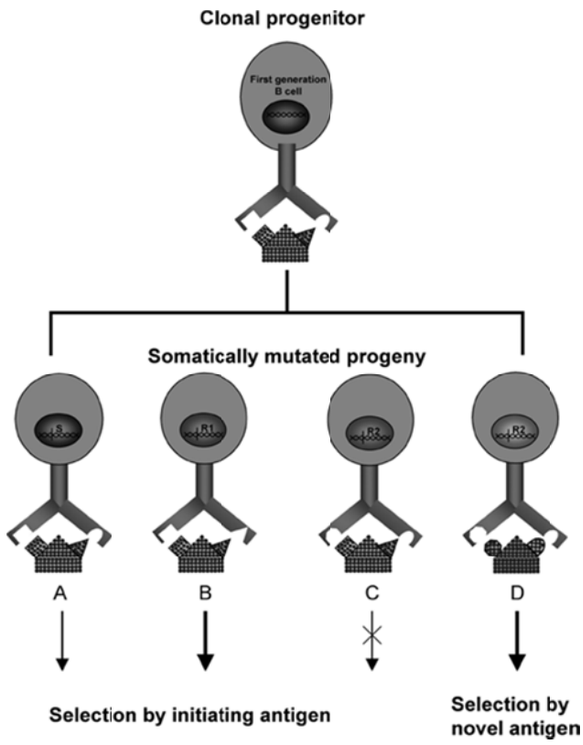

Figure 21-4. B cell genealogy. The progenitor B cell depicted at the top expresses an antibody that is encoded by germline immunoglobulin genes and has a low affinity for antigen. When antigen and T cell factors trigger B cell proliferation, class switching, and somatic mutation, numerous B cell progeny are possible. Three examples are schematized here. A: a B cell with a silent (S) point mutation. This nucleotide substitution does not encode a new amino acid. Therefore, the antibody molecule is unaffected, and affinity for antigen does not change. B: a B cell whose point mutation encodes an amino acid replacement (R), leading to increased affinity for antigen. This mutated antibody exemplifies affinity maturation. C: a B cell with a replacement mutation that alters antigenic specificity. This antibody can no longer bind to the initial triggering antigen. D: the same antibody as in C, despite no longer being able to bind to the initial triggering antigen, can acquire specificity for a novel (perhaps self) antigen.

Because a given amino acid can be encoded by more than one DNA triplet, not every point mutation causes an amino acid substitution that can change antibody affinity for an antigen. It is possible to indirectly analyze antigen selection during the course of the germinal center response by calculating the ratio of replacement (R) to silent (S) mutations in rearranged antibody genes (i.e., mutations that lead to amino acid changes vs. those that do not). Purely random point mutations within a DNA sequence containing equal numbers of each possible codon would result in a predicted random R-to-S ratio of approximately 3:1 (119 ,120). The random R-to-S ratio for a particular DNA sequence, however, might be lower or higher than this depending on the actual codon usage (121 ,122).

In an antigen-selected response, one might expect a higher than random R-to-S ratio, because B cells containing mutations leading to higher affinity for antigen would be favored to proliferate. Further, antigen selection would predict a higher frequency of R mutations in the CDRs, because these regions include the contact amino acids for antigen binding. This type of analysis has been performed to assess whether certain autoantibodies arise from antigen selected responses (96 ,97 ,98). There are two concerns, however, with this analysis. First, the assumption of purely random mutation is incorrect; recent studies have shown that bias for particular kinds of mutations occurs and that hot spots of mutation exist (123). Second, while antibodies with a higher-than-random R-to-S ratio probably are part of an antigen-selected repertoire, the converse clearly is not true: a single amino acid substitution is capable of conferring a tenfold increase in affinity (124 ,125). Thus, antigen selection may occur in the absence of a high R-to-S ratio.

Marginal Zone and B-1 B Cells in SLE: Role of “Natural” Autoantibodies

B-1 cells (also CD5 or Ly-1) represent a distinct population of B cells (126 ,127). B-1 cells are the only subset of B lymphocytes that constitutively express the pan-T cell surface antigen CD 5. The significance of this marker has not yet been elucidated. Data showing that CD5 is implicated in the maintenance of tolerance in anergic B cells (128), along with data demonstrating that CD5 mediates negative regulation of B cell receptor (BCR) signaling in B-1 cells (129), support the hypothesis that the expression of CD5 may help inhibit autoimmune responses.

At present, it is not clear whether the B-1 population is a separate lineage or merely an alternate differentiation pathway of conventional (i.e., B-2) B cells. B-1 cells are unique among mature B lymphocytes in that they appear to be a self-replenishing population that arises in the fetal liver (130). Although rare in the spleen and lymph nodes, B-1 cells comprise approximately 10% to 40% of B cells found in the peritoneum. Being a major source of natural autoantibodies (131 ,132 ,133), the B-1 lineage is of particular interest to those studying autoimmunity. Elevated numbers of B-1 cells are present in the autoimmune New Zealand black (NZB) mouse strain (130), and prevention of the autoimmune symptoms has been reported with their elimination (134). B-1 cell expansion is found in some patients with rheumatoid arthritis and Sjogren's syndrome (135), but an association with SLE is weaker (136 ,137).

B-1 cells generally express germline-encoded, polyreacrive IgM antibodies with limited V gene segment usage (130 ,131 ,132). Much controversy exists over the physiologic function of the B-1 lymphocytes, although there is now growing evidence that many of the low affinity autoantibodies made by this B cell subset are important in the clearance of apoptotic debris. Adoptive transfer experiments have shown that B-1 cells are poor at forming germinal centers (138), which are characteristic of a T-dependent B cell response and are thought to be necessary for antigen selection and class switching; however, class-switched, somatically mutated B-1 antibodies that appear to show evidence of antigen selection have been isolated from humans (139).

MZ B cells share many features with B1 cells. They are phenotypically characterized by cell surface expression of $IgM^{hi}IgD^{lo}CD21^{hi}CD22^{hi}CD23^{lo}CD1^{hi}$ and reside in the marginal zones that girdle the follicles in spleen and tonsils (140). Their hallmark functional characteristic is represented by early activation and rapid immunoglobulin secretion in response to T-independent (TI) antigens that arrive via a hematogenous route in the spleen. Like B1 cells, they are key players of innate immunity as they respond rapidly to antigen and do not generate a memory response. Although it has been generally accepted that this is a self-renewing and mostly non-recirculating population (141 ,142) recent studies suggest that a large population of IgM-positive peripheral B cells correspond to circulating splenic MZ B cells (143 ,144).

Both MZ and B-1 B cells have a high antigen presentation capacity and are strategically located to encounter and process foreign antigens. Both cells secrete polyreactive “natural” antibodies, including self-reactive ones that are generally germline-encoded (145). Low titers of low-affinity autoantibodies are part of the normal B cell repertoire (146 ,147 ,148 ,149). They are not unique to any autoimmune disease, nor is there any evidence that they are pathogenic. These natural autoantibodies resemble the antibodies of a primary immune response in that they are mainly IgM and polyreactive, and bind to a wide variety of both autoantigens

and foreign antigens that often have no apparent structural homology (150 ,151 ,152). “Natural” antibodies have also been shown to bind to altered phospholipids expressed on the surface of cells undergoing apoptosis. The opsonization of apoptotic cells increases their clearance and routes them to nonimmunogenic pathways (153). While sequence analysis shows that the antibodies made by MZ B cells are mainly encoded by germline (i.e., unmutated) genes (154 ,155 ,156 ,157 ,158), numerous exceptions exist (159). Analysis of the variable regions of natural autoantibodies suggests that they may contain more flexible hydrophilic amino acid residues in their CDRs than somatically mutated, affinity-matured antibodies, as well as longer CDRs (159), which may explain their polyreactivity. It is thought that they present a shallow groove for antigen binding that can accommodate more diverse structures.

There are some indications that the B cells producing natural antibodies may be clonally related to pathogenic B cells. Idiotypic analyses of natural anti-DNA antibodies from normal individuals and of potentially pathogenic anti-DNA antibodies from patients with SLE demonstrate that cross-reactive idiotypes are present in both populations (160 ,161). Some investigators have speculated that natural autoantibodies can be the precursors to pathogenic autoantibodies (162 ,163), while other data suggest that the two classes of autoantibodies arise from distinct B cell populations and that the SLE autoantibodies arise by the somatic mutation of genes that encode protective antibodies (99 ,164 ,165 ,166 ,167 ,168 ,169). Adoptive transfer experiments of MZ B cells, unlike B1 cells, have demonstrated T-dependent class-switching and SHM occurring resulting in the production of high-affinity antibodies (170 ,171). Assuming MZ B cells can undergo affinity-maturation, it is conceivable that an enhanced differentiation of MZ B cells along this pathway could contribute to autoimmunity.

Our understanding of innate immune B cells in humans has been further advanced through the study of a population of B cells that can be identified using a monoclonal antibody (9G4) that binds to a unique epitope encoded by the human heavy chain variable region gene V4–34 (172). These 9G4 positive B cells represent 5% to 10% of the mature naïve B cell repertoire and recognize autoantigens and pathogens. Additionally, these cells are present in the MZ B cell compartment and are normally excluded from the T-dependent IgG memory repertoire. However, in SLE patients, 9G4 positive B cells are expanded in the IgG memory population supporting the hypothesis that inappropriate positive selection of innate B cells into an adaptive immune phenotype is a feature of autoimmunity. Although 9G4 positive antibodies have not been demonstrated to have a direct pathogenic effect, they are elevated in up to 75% of patients with active SLE.

Further studies on the role of B cells in innate immunity have revealed the expression of Toll-like receptors (TLR) by these cells that recognize specific molecular determinants common to many pathogens. While in mouse B cells, co-engagement of TLRs and the BCR acts synergistically to induce activation, in humans, TLR9 and TLR10 expression appears to be induced following BCR activation (173). TLRs have been shown to bind exogenous ligands such as lipopolysaccharides (LPS), single- and double-stranded RNA and bacterial DNA (174 ,175 ,176). Potential endogenous ligands include DNA containing CpG motifs released from apoptotic cells (174). Therefore, inducible TLR expression and B cell activation from a wide range of self and foreign ligands could provide the link between innate immune dysregulation and autoimmunity.

Another potential role of MZ B cells in autoimmunity is as antigen presenting cells for self antigens, resulting in the activation of autoreactive CD4 T cells. This interaction can induce T cells to secrete IFN-γ, IL-4 and IL-10 (177). B cells themselves can secrete large amounts of IL-10 and upregulate the expression of cell surface costimulatory molecules that can lead to amplification of the T dependent immune response inducing GC formation and affinity maturation of self reactive B cells. Additionally, CpG binding to TLR9 in B cells from several lupus mouse strains increases the secretion of IL-10 and results in the suppression of IL-12 production (178). IL-10 has been shown to be elevated in patients with SLE and its serum level can correlate with disease activity (179 ,180). In an uncontrolled study, a small number of SLE patients with active disease were given anti-IL-10 antibody and experienced an improvement of disease activity (181). Similarly, anti-IL-10 treatment of NZB/W mice resulted in delay onset of lupus-like disease (182). However, MRL-lpr/lpr IL-10-/- mice showed an increased severity of lupus and higher concentrations of anti-dsDNA antibodies, suggesting that IL-10 can play different regulatory roles in SLE, whether it is a different stage of the disease or a different genetic background (183).

Pathogenic Autoantibodies

Indirect evidence for the pathogenicity of several autoantibodies present in SLE includes their association with clinical manifestations in SLE and their presence in affected tissue. In recent years there is increasing evidence to directly support the pathogenic potential of several lupus-associated autoantibodies. A transgenic mouse expressing the heavy and light chain of the secreted form of an anti-DNA antibody has been shown to develop glomerulonephriris, thereby confirming that anti-DNA antibodies cause renal disease (184). Support for the pathogenic role of anti-DNA antibodies in nephritis can also be found in recent autoimmune disease models displaying high titers of anti-DNA antibodies together with immunoglobulin deposition in the kidney and histologic nephritis (185 ,186 ,187 ,188). Perfusion of monoclonal mouse and polyclonal human IgG anti-DNA antibodies through isolated rat kidney induces significant proteinuria and decreased clearance of inulin (189). Addition of plasma as a source of complement markedly increases proteinuria, while preincubation of the antibodies with DNA can abolish binding to renal tissue (189). It is still unknown, however, whether pathogenic anti-DNA antibodies form immune

complexes with antigen in situ, or if the antibodies bind to a target antigen that is actually some component of glomerular tissue and/or tubular components. A decrease in binding of anti-DNA antibodies to glomerular elements with DNAse treatment in some experimental models (190) but not in others (191) suggests that both models pertain; some anti-DNA antibodies directly cross-react with glomerular antigens, while other anti-DNA antibodies may bind via a DNA-containing bridge. A number of investigators have administered monoclonal anti-DNA antibodies to nonautoimmune mice, either intraperitoneally in the form of ascites-producing hybridomas or intravenously as purified immunoglobulins (192 ,193). In these models, it is possible to demonstrate that anti-DNA antibodies differ with respect to pathogenicity (193 ,194), with some antibodies depositing in the kidney and others not. Moreover, those antibodies that deposit in the kidney may differ with respect to the localization of deposition. Studies performed with the congenic mouse strain NZM2328.C57Lc4 showed that these mice develop chronic glomerulonephritis and severe proteinuria despite the fact that they do not generate autoantibodies to dsDNA or other nuclear antigens (195), consistent with the clinical observation that kidney disease can arise in individuals with no DNA-reactive antibodies.

Recent studies have elegantly demonstrated the arrhythmogenic potential of anti-Ro antibodies. Affinity-purified anti-Ro antibodies from lupus mothers of babies with congenital heart block inhibit calcium currents and induce complete heart block in an ex vivo perfused human fetal heart system (196). Immunization of female BALB/c mice with recombinant La and Ro particles leads to first-degree atrioventricular block in 6% to 20% of pups born to immunized mothers and rarely to more advanced conduction defects (197). Finally, passive transfer of purified human IgG containing anti-Ro and anti-La antibodies to pregnant BALB/c mice results in fetal bradycardia and first-degree atrioventricular block (198).

Experimental evidence also supports the close epidemiologic association between antiphospholipid antibodies and thrombosis. Following experimental induction of vascular injury in mice, injection of affinity-purified immunoglobulin from patients with antiphospholipid syndrome results in a significant increase in thrombus size and a delay in disappearance of the thrombus (199). Injecting human monoclonal anticardiolipin antibodies into pregnant BALB/c mice results in fetal resorption, and a significant decrease in placental and fetal weight (200). Similar results have been obtained with passive transfer of monoclonal murine and polyclonal human anticardiolipin antibodies (201).

Looking at the epidemiologic and experimental data in combination, it seems clear that the importance of several lupus-associated autoantibodies lies not only in their diagnostic significance as markers for the disease, but also in a pathogenic role in tissue damage in affected target organs in SLE. Treating disease with the end point of lowering the titer of specific autoantibodies then becomes a therapeutic goal with a clear pathophysiologic rationale.

Heavy-chain isotype appears to be important in determining the pathogenicity of autoantibodies. For example, marked differences in the severity of induced hemolysis exist among IgG isotype switch variants of an antierythrocytic antibody, related to the capacity of each isotype to bind to Fc receptors (202). In murine lupus, the switch from serum IgM anti-DNA activity to IgG anti-DNA activity heralds the onset of renal disease (203). Similarly, human IgG antibodies present in the immune complex deposits within the kidneys of patients with SLE appear to trigger mesangial cell proliferation and subsequent tissue damage to a greater extent than IgM antibodies, perhaps because mesangial cells or infiltrating mononuclear cells have Fc receptors for IgG (204). The importance of isotype for anticardiolipin antibodies is intriguing (2); several groups have noted that IgG antiphospholipid and ß2-glycoprotein antibodies correlate better with clinical thrombosis than other isotypes do (Chapter 27). Nevertheless, pathogenicity has been shown also for IgM and IgA antibodies (199). IgM and IgA anticardiolipin antibodies also correlate with specific disease phenotypes. For example, IgM antiphospholipid antibodies are associated with hemolytic anemia (205).

It was formerly widely believed that antibodies could not penetrate live cells, and that nuclear staining of sectioned tissues was an artifact of tissue preparation. There is now evidence that some anti-DNA and antiribosomal P autoantibodies bind to the cell surface, traverse the cytoplasm, and reach the nucleus. Furthermore, there are data to demonstrate a pathogenic effect from cellular penetration by autoantibodies (206 ,207 ,208). While antigen translocation to the cell membrane may explain the accessibility of normally intranuclear antigens to interaction with autoantibodies (209 ,210), the capability to penetrate live cells and interact with cytoplasmic or nuclear components may be an additional pathogenic characteristic of some autoantibodies.

This chapter discusses aspects of autoantibody production, but it is increasingly evident that autoantibody–mediated tissue damage requires not just the presence of autoantibodies with particular pathogenic features but also the display of a specific antigen in the target organ (211). Differential display of antigen at the level of the target organ may contribute to genetic susceptibility to autoimmune disease. Evidence for such a hypothesis comes, in part, from a murine model of autoimmune myocarditis, where differential susceptibility to antimyosin antibody-induced disease in different mouse strains is dependent on differences in the composition of cardiac extracellular matrix (212). Similarly, in a rat model for tubular nephritis, antibody-mediated disease depends on genetically determined antigen display in the renal tubules (213).

Genetic and Molecular Analysis of Anti-DNA Antibodies

Genetic analyses of anti-DNA antibodies in both human and murine lupus have provided important information regarding the production of autoantibodies. There is currently no

evidence that a distinct set of disease-associated, autoreactive V region genes is present only in individuals with a familial susceptibility to autoimmunity and is used to encode the autoantibodies of autoimmune disease. It is also clear that no particular immunoglobulin V region genes are absolutely required for the production of autoantibodies (214). Immunoglobulin genes that are present in a nonautoimmune animal clearly are capable of forming pathogenic autoantibodies. The offspring of a nonautoimmune SWR mouse and an NZB mouse (SNF1 mice) spontaneously produce autoantibodies (215), with a large percentage of the anti-DNA antibodies deposited in the kidneys of (NZB × SWR) F1 mice encoded by Ig genes derived from the nonautoimmune SWR parent (215). In fact, both idiotypic and molecular studies show that the V region genes used to produce autoantibodies in lupus are also used in a protective antibody response in nonautoimmune individuals (216 ,217). Autoantibodies bear cross-reactive idiotypes that also are present on the antibodies that are made in response to foreign antigens, and V region genes used to encode autoantibodies also encode antibodies to foreign antigen (218 ,219 ,220 ,221). Indeed, a number of autoantibodies cross-react with foreign antigens, demonstrating that the same V region gene segments can be used in both protective and potentially pathogenic responses (222 ,223 ,224). These cross-reactive antibodies are capable of binding to bacterial antigen with high affinity, but they also possess specificity for a self antigen. Patients with *Klebsiella* infections and individuals vaccinated with pneumococcal polysaccharide develop antibacterial antibodies expressing anti-DNA cross-reactive idiotypes (216 ,225). In vivo, cross-reactive antibodies with specificity to both pneumococcus and dsDNA are protective in mice against an otherwise lethal bacterial infection, yet they also can deposit in the kidney and cause glomerular damage (226). It appears that cross-reactive antibodies are routinely generated during the course of the normal immune response in the nonautoimmune individual. Ordinarily, however, autoreactive B cells expressing a self specificity are actively downregulated and contribute little to the expressed antibody repertoire (117).

Although there is no evidence that specific genes encode only autoantibodies, there are data to suggest that autoantibodies are encoded by a somewhat restricted number of immunoglobulin V region genes (227 ,228 ,229). In murine lupus, extensive analyses of anti-DNA–producing B cells show that 15 to 20 heavy-chain V region genes encode most anti-DNA antibodies (165 ,230 ,231 ,232). There is a dramatic increase in the frequency of use of a particular J558 heavy chain gene in autoimmune as compared to normal mice, while nonautoimmune mice, immunized with an immunogenic DNA/DNA-binding peptide complex displayed intermediate usage (229). This supports the concept that differences in V gene usage that may be seen between autoimmune and nonautoimmune mice are quantitative, rather than reflecting a true qualitative difference. While molecular studies of human antibodies are more limited, idiotypic analyses also suggest restricted V gene usage. This observation is important as it suggests that antiidiotypes can play a role in therapeutic strategies. Furthermore, analysis of restriction fragment length polymorphisms, which is a tool used to identify the similarities and differences among particular genes in a population, has been used to examine whether distinct Ig gene polymorphisms associate with SLE (233 ,234 ,235). A deletion of a specific heavy-chain V gene, hv-3, was reported to be more frequent in individuals with SLE or rheumatoid arthritis (236 ,237). A specific germline VK gene, A30, was found to increase the cationicity (and therefore the pathogenicity) of human anti-DNA antibodies. A defective A30 gene was found in eight of nine lupus patients without nephritis, while this gene was normal in all nine lupus patients with nephritis (238). Polymorphism at the VK gene locus may then contribute to susceptibility to lupus nephritis. While these studies look at small numbers of patients, they suggest that polymorphisms in immunoglobulin genes may make some contribution to the generation of autoantibodies and expression of human lupus. Nevertheless, the anti-DNA response is no more restricted than are many responses to foreign antigen and the restricted V region gene usage does not appear to be skewed toward particular gene families.

SHM is a possible mechanism by which protective, antiforeign antibodies evolve into pathogenic autoantibodies (Fig. 21-4) (239 ,240). The characteristics and mechanics of SHM in SLE are, therefore, of interest. Examining ten human antibodies positive for a specific, lupus-associated idiotype (F4), Manheimer-Lory et al. (241) found no change in the frequency of somatic mutations or the distributions of such mutations in CDRs. While the normal process of somatic mutation is generally random, there is some bias for mutation at specific sequence motifs, termed mutational “hot spots.” Surprisingly, F4 positive antibodies displayed abnormal somatic mutation as shown by a decrease in hot-spot targeting. As mice transgenic for the anti-apoptotic gene bcl-2 also display this same decreased targeting of mutations to hot spots (242), the decreased targeting in F4 positive antibodies derived from lupus patients may reflect an abnormal state of B cell activation rather than defective machinery for somatic mutation. Studies have been performed (243) on the mutational process in the V gene repertoire in individual B cells from a small number of lupus patients. The frequency of mutations was increased in both productive and unproductive VK rearrangements, with evidence of increased targeting to mutational hot spots in framework regions, consistent with altered selection. A single study in mice found essentially no differences in somatic mutation between B cells of an autoreactive and normal strain (244). Abnormal somatic mutation may be due to important alterations in B cell activation in SLE; however, conflicting data prevent drawing firm conclusions as yet.

Autoantibody Induction

For the most part, autoantibodies that are present in SLE do not exist in an unstimulated B cell repertoire. Rather, autoantibodies in SLE usually reflect the process of SHM

and apparently are made by B cells following exposure to antigen and T cell help. For some autoantibodies, mutation of the germline sequences clearly is crucial in generating the autoantigenic specificity (97). These antibodies have a high R-to-S ratio, primarily in CDRs; however, the pitfalls of R-to-S ratio calculations have been discussed and should be considered in the analysis of anti-DNA antibodies (123 ,124). There also are lupus autoantibodies that have a high R-to-S ratio in framework regions (245). As these framework region mutations are less likely to alter antigenic specificity, it is tempting to speculate that they instead may facilitate escape from a putative regulatory mechanism. High-affinity anti-dsDNA antibodies also can be encoded by germline genes (246), but these are rarely found in disease.

There are various hypotheses regarding the nature of the eliciting antigen or antigens in SLE (Table 21-3). Several lines of evidence support the role of foreign, microbial antigens in the generation of autoantibodies (247). Lupus-prone strains of mice carrying the xid mutation, which impairs production of the antipolysaccharide antibodies that are required for antibacterial immunity, develop much lower titers of anti-DNA antibodies and decreased renal disease (248). Similarly, autoimmune–prone NZB mice raised in a germ-free environment produce reduced titers of anti-DNA antibodies and show delayed onset of autoimmune manifestations (249). It has been shown that raising lupus-prone lymphoprolifertive (MRL/lpr/lpr) mice in a germ-free environment and feeding them a filtered, antigen-free diet significantly decreases the severity of renal disease (250). Evidence that an antipneumococcal antibody can spontaneously mutate to become an anti-DNA antibody in an in vitro system (116), as well as in response to immunization with a pneumococcal antigen in vivo (117), also supports a close structural relationship between the autoantibody response and a protective antibacterial response. Finally, to further demonstrate the close relationship between a protective antibacterial and autoantibody response in lupus, Kowal et al. (251) generated a combinatorial immunoglobulin expression library in phage from splenocytes of a lupus patient immunized with pneumococcal polysaccharide. Four of eight (53) of the monovalent Fab fragments selected for expression of a SLE associated idiotype bound both pneumococcal polysaccharide and dsDNA, indicating that a significant portion of the human antipneumococcal response in SLE is cross-reactive with self antigen.

Table 21-3: Antigenic Triggers for Anti-dsDNA Antibodies

Foreing antigen
Molecular mimics
Bacterial DNA
Complexes of DNA and DNA-binding proteins
Self-antigen
RNP auto-epitopes
Histone peptides
Peptides derived from anti-dsDNA antibodies
Cryptic autoepitopes (sequestered autoantigens, altered processing/presentation)
Idiotypic network (antiidiotypic antibody—autoantigen)

dsDNA, double-stranded DNA; RNP, ribonucleoprotein

Molecular mimicry as well as SHM might be important mechanisms by which exposure to foreign, bacterial antigen can elicit autoantibodies. Molecular mimicry refers to a sufficient structural homology between foreign and self antigen that both antigens are recognized by a single, cross-reactive B cell. The best-known example of this mechanism in autoimmunity is rheumatic fever, in which the antibodies arising in the antistreptococcal response cross-react with cardiac myosin, leading to antibody deposition in cardiac muscle and carditis. A molecular mimic induces an autoantibody response by activating cross-reactive B cells specific for both foreign and self antigen. These B cells receive T cell help for autoantibody production from T cells activated by microbial proteins. In support of a possible role of molecular mimicry in inducing anti-DNA antibodies is the rise in autoantibodies seen even in nonautoimmune hosts following infection (252). Furthermore, nonautoimmune individuals vaccinated with pneumococcal polysaccharide generate antipneumococcal antibodies idiotypically related to anti-DNA antibodies (216). Infection does not usually lead to self-perpetuating autoimmunity, as the T cell help available for cross-reactive B cells dissipates following the clearing of the infectious agent. Failure to resolve the autoimmune process induced by a molecular mimic may be a result of a defect in reinduction of tolerance or to the persistence of the foreign antigen. Some possible causes for a lack of return to a tolerant state include activation of T cells specific for antigenic epitopes to which T cell tolerance had never been established (cryptic epitopes) (253), upregulation of costimulatory molecules, the presence of immunomodulatory cytokines, and abnormally enhanced intracellular signaling. It is also possible that regulatory cells are critical in the maintenance of peripheral tolerance following antigen activation and may be dysfunctional in SLE (254 ,255). Finally, it may be that lupus specific immune complexes containing RNA or DNA activate dendritic cells (DC) to create an immunogenic environment (256 ,257).

Peptide antigens that structurally mimic DNA can also elicit an autoantibody response (258 ,259). Screening a phage peptide display library with a pathogenic IgG2b anti-dsDNA antibody, revealed the D/E WD/E Y S/G consensus motif that is recognized by both murine and human anti-DNA antibodies (259). DWEYS inhibits the binding of a high percentage of anti-DNA antibodies to dsDNA in vitro and to glomeruli in vivo. Immunization of nonautoimmune BALB/c mice with a multimeric peptide containing the consensus motif induces significant serum titers of IgG anti-dsDNA antibodies, as well as antihistone, anti-Sm/RNP, and anticardiolipin antibodies. Monoclonal antibodies from peptide-immunized BALB/c mice resemble anti-dsDNA antibodies present in spontaneous murine

lupus, using similar VH and VL gene usage, and exhibiting arginines in heavy chain CDR3 regions (260).

There is some evidence to suggest that nucleic acids can induce anti-dsDNA antibodies (see below). While investigators have long known that mammalian dsDNA is poorly immunogenic, recent studies have focused on bacterial DNA as a potential trigger for induction of anti-dsDNA antibodies. Bacterial DNA contains unmethylated CpG motifs, which can bind to and activate TLR9 and may be an important adjuvant in the immune system (261). Preautoimmune lupus-prone mice immunized with bacterial DNA produce antibodies that not only bind to the immunizing antigen, but are also cross-reactive with mammalian DNA (262). However, the response of nonautoimmune mice to bacterial DNA was noncross-reactive, indicating that bacterial DNA alone is not sufficient to induce anti-dsDNA antibodies in a nonlupus-prone host. While mammalian DNA contains fewer CpG-motifs, these motifs are present and can activate TLR9 and perhaps other TLRs or scavenger receptors that are involved in DC activation. Failure to clear DNA properly and degrade it to nonimmunogenic fragments may contribute to anti-DNA antibody production.

Another possible model for induction of anti-DNA antibodies is by a hapten-carrier–like mechanism, in which T cells recognize epitopes of a protein carrier associated with DNA and provide help for autoreactive B cells specific for hapten (DNA). Novel peptide determinants of the protein component of the complex may then be presented by DNA-specific B cells to recruit autoreactive T cells, and further perpetuate an immune response. Immunization of nonautoimmune animals with DNA together with DNA-binding proteins such as DNAse I (263), Fus 1 (derived from *Trypanosoma cruzi*) (264), and the polyomavirus transcription factor T antigen (265) results in the generation of anti-dsDNA antibodies with structural similarity to anti-dsDNA antibodies present in spontaneous murine lupus.

Because anti-idiotypic antibodies can function like antigen to induce an antibody response, some investigators have emphasized a potential role for antiidiotype in activating autoantibody production. For example, the Ku antigen is a DNA-binding protein (266). Studies of anti-DNA and anti-Ku antibodies suggest that the anti-Ku antibodies are anti-idiotypic to anti-DNA antibodies (267). Several studies have found that mice immunized with an anti-DNA antibody and mice immunized with an anti-idiotypic antibody to an anti-DNA antibody each develop autoantibodies (268 ,269). This has also been shown for other autoantigen–autoantibody systems important in lupus, such as anticardiolipin antibodies (270). Interestingly, immunization with antibodies recognizing a DNA-binding protein (anti-p53 antibodies) can generate anti-DNA antibodies (271). While such studies suggest that the idiotypic network may contribute to the production of autoantibodies, others have suggested that antiidiotypes may function to induce or maintain clinical remissions and that the failure to generate an antiidiotypic response may perpetuate autoantibody production (272).

Autoantibody responses to DNA associated antigens are often simultaneously present in established SLE (Ro/La, Sm/RNP). Longitudinal studies begun early in the disease course, demonstrate that a particular response may be initially limited to a particular peptide epitope, and be followed by intramolecular (other epitopes in the same polypeptide) and intermolecular (epitopes in distinct, but structurally linked molecules) spread of the response (273). This process is termed epitope spreading, and is the result of processing by antigen-presenting cells (including B cells) of the multimolecular complex, and presentation of novel epitopes to nontolerized T cells. The initial target for epitope spreading may be a molecular mimic derived from a microorganism, or a self-antigen. Recent data have suggested that apoptosis can generate novel nuclear autoantigen fragments (274) that may become accessible to interaction with antibody molecules by translocation to the cell surface (210 ,275). Neoepitopes of particular antigens generated by specific forms of apoptosis, for example, granzyme induced rather than caspase involved might also explain defined autoantibody profiles that are associated with SLE.

The potential role for epitope spreading in diversification of the autoantibody response in SLE has been clearly demonstrated for the anti-Sm response. James and Harley (276) identified two B/B' octapeptides that were early targets of an anti-Sm response in lupus patients. Rabbits (277) and some inbred mouse strains (276) immunized with one of these octapeptides, PPPGMRPP, develop over time an immune response against other regions of Sm B/B' and Sm D. Furthermore, in some animals antinuclear antibodies and anti-dsDNA antibodies also arise. B cell epitope spreading has also been demonstrated in the Ro/La autoantigen system (278).

Investigators have (279) identified T cells in SNF1 lupus-prone mice that were pathogenic in vivo, and accelerated the development of an immune complex glomerulonephritis in preautoimmune mice. Many of these pathogenic T cell clones were found to respond to nucleosomal antigens, specifically histone peptides. Stimulating these T cell clones with the histone peptides leads to increased anti-DNA antibody secretion in a B-T cell coculture system, and peptide immunization in vivo induces severe glomerulonephritis (280). Other investigators have focused on the immunogenicity of peptides derived from the VH regions of anti-DNA antibodies themselves (281 ,282). They (281) reported that three VH derived 12-mer peptides induce a class II restricted proliferation of unprimed T cells from preautoimmune NZB × New Zealand white (NZW) Fl mice. Immunization of NZB × NZW F1 mice with one peptide, or transfer of a T cell line reactive with this peptide, increased their titer of anti-dsDNA antibodies and the severity of the nephritis. Further support for a possible role of self peptide in induction of anti-dsDNA antibodies can be found in studies showing that tolerization with self peptides can downregulate anti-dsDNA antibody production and nephritis in murine lupus (282 ,283 ,284). This observation suggests a potential therapeutic strategy in SLE.

B Cell Tolerance

Several transgenic mouse models have been described in which immunoglobulin V regions encoding anti-DNA or other autoantibodies have been introduced into the germline. The importance of these models is multifold: (a) they afford perhaps the best direct evidence that certain anti-DNA antibodies are pathogenic, (b) they have contributed significantly to understanding the tolerizing mechanisms that regulate anti-DNA–producing B cells and the defects that allow the survival and activation of these cells, and (c) they provide models in which to test novel therapies designed to block tissue injury or inactivate pathogenic B cells.

B cells expressing autoreactive immunoglobulin receptors arise in all hosts at times of B cell receptor diversification, both during formation of the naive B cell repertoire and again during the germinal center response. Regulation of these autoreactive receptors occurs through inactivation or deletion (285) (Table 21-4). These mechanisms appear to operate when membrane immunoglobulins are cross-linked by antigen in the absence of T cell help or costimulatory influences. Whether anergy or deletion occurs depends in part on the extent of membrane immunoglobulin cross-linking (286). Normally, the serum of nonautoimmune mice does not contain high-affinity IgG autoantibodies, illustrating that the normal immune system can efficiently regulate autoantibody–producing B cells. Initial studies of anti-DNA transgenic nonautoimmune mice showed that anti-DNA antibodies are eliminated from the immune repertoire through functional inactivation (i.e., anergy) or deletion (287 ,288). In lupus-prone mice, there appears to be a defect in some aspect of regulation, allowing the autoreactive B cells to survive and contribute to the expressed antibody repertoire. A more recent study demonstrated that “ignorance” is an additional possible fate of DNA-binding B cells (289). Bynoe et al. (289) isolated low-affinity, DNA-binding B cells from a nonautoimmune mouse transgenic for an anti-DNA heavy chain. These B cells were in a resting state and produced germline-encoded, nonpathogenic antibodies. These cells may be a potential source of pathogenic autoantibodies; they may be recruited into an ongoing immune response, and then become high-affinity (and pathogenic) antibodies via somatic mutation.

In humans it has been suggested that B cells expressing ANAs and polyreactive antibodies represent 55% to 75% of the repertoire expressed in the bone marrow. The majority of these autoreactive B cells are efficiently removed from the naïve repertoire at an immature stage before exiting the bone marrow (290). Analysis of the B cell repertoire from three patients newly diagnosed with SLE showed that autoreactive B cells comprised 25% to 50% of the total mature naïve B cells compared to the 5% to 20% observed in control subjects (291). While the study showed a deficiency in removal of autoreactive B cells from the immature and transitional stages, implying a defect in negative selection, the autoantibodies that remained were mostly polyreactive against cytoplasmic antigens, insulin or single-stranded DNA and rarely against double-stranded DNA. Hence, they may be precursors of lupus B cells, but they are not themselves pathogenic B cells.

Table 21-4: Mechanisms of B Cell Tolerance

Clonal anergy
Clonal deletion
Clonal ignorance
Receptor editing

Receptor editing (see antibody assembly) is another phenomenon that can be used by B cells to maintain tolerance (292). A second immunoglobulin rearrangement occurs, so that the transgenic heavy chain is paired with an endogenous light chain to generate a VH-VL combination that is no longer autoreactive (293).

Recent transgenic studies have bred anti-DNA transgenes onto autoimmune genetic backgrounds to better understand the differential regulation of the anti-dsDNA specificity in lupus-prone mice (294). An additional innovation has been the application of “knock-in” technology (in which the immunoglobulin transgene is inserted into its proper genetic locus), which provides a more physiologic system in that somatic mutation and isotype switching of the inserted V region may occur (295 ,296 ,297). No one single defect could be identified in tolerance mechanisms (deletion, anergy, receptor editing) to account for the selective expansion of anti-DNA specific B cells in lupus mice. In fact, it has been reported that autoimmune MRL/lpr/lpr mice can efficiently delete B lymphocytes with a transgenic autoreactive receptor (298).

It is important to understand that the various thresholds for tolerance induction in autoreactive B cells (deletion, anergy, indifference) are not static, but rather may be dynamically altered by immune modulators such as cytokines, hormones, or costimulatory molecules. Studies of transgenic and knockout mice, engineered to overexpress or be deficient in molecules of interest, have begun to unravel genes and pathways involved in B cell regulation and in B cell tolerance.

The B cell receptor (BCR) is a complex of surface immunoglobulin with the accessory molecules Igα and Igβ. Following receptor cross-linking by binding of antigen to the BCR, a complex cascade of signaling molecules becomes involved in transducing the signal from the BCR to eventually result in B cell activation and proliferation, or anergy and death. The potential involvement of enhanced signaling or decreases in negative regulatory signals as possible contributors to autoimmunity is discussed below (Table 21-5).

The finding that expression of a lupus-like syndrome in MRL/lpr/lpr and C3H gld/gld mice is due to a single defect in the apoptosis genes Fas and Fas ligand, respectively (299 ,300 ,301), has generated a large amount of interest in examining the role of dysregulated apoptosis in human autoimmunity (Table 21-5). Alterations in Fas and Fas ligand have been described in patients with systemic lupus,

with some studies describing a correlation with manifestations of disease and clinical activity (302 ,303 ,304 ,305 ,306). Interestingly, humans with a variety of defects in the Fas receptor have been described, some of which manifest as significant lymphadenopathy (Canale-Smith syndrome) reminiscent of the lymphoproliferative phenotype of lpr mice with defective Fas (307). While only a single lupus patient has been described with a Fas receptor defect, Fas mutations are clearly associated with dysregulated lymphocytes and even rarely defective apoptosis (308).

Table 21-5: Single Gene Defects Causing Autoimmunity

Molecules involved in apoptosis
lpr deficiency
gld deficiency
bcl-2 overexpression
Serum amyloid protein deficiency
DNAse I deficiency
C1q deficiency
Signaling molecules
CD19 overexpression
CD22 deficiency
Lyn deficiency
SHP-1 deficiency

Other apoptosis genes have also been implicated in the induction of autoimmunity. Transgenic mice overexpressing bcl-2 have long-lived lymphocytes and enhanced immune responses to immunization, and when the transgene is present on certain genetic backgrounds, spontaneously develop antinuclear antigens and immune complex glomerulonephritis (309). Enforced bcl-2 expression allows recovery of cross-reactive anti-dsDNA, antipneumococcal antibodies from the primary response of nonautoimmune hosts immunized with a pneumococcal cell wall antigen (310). Furthermore, normally anergized or deleted autoreactive anti-DNA B cells could be recovered from mice transgenic both for bcl-2 and an anti-dsDNA heavy chain (311). Hormones can also modify the expressed B cell repertoire in mice transgenic for an anti-DNA heavy chain, and facilitate the recovery of high-affinity B cells (312). Estrogen upregulates bcl-2, and may be interfering with tolerance induction by this mechanism as well as by decreasing the strength of BCR signaling. Prolactin also permits the survival and activation of DNA-reactive B cells. It appears to act by increasing costimulatory pathways that can rescue B cells destined for apoptosis.

Another possible link between apoptosis and autoimmunity can be found in studies showing that altered clearance of apoptotic particles and persistence of nuclear material in the circulation may induce anti-DNA antibodies. Immunizing non-autoimmune mice intravenously with syngeneic apoptotic cells induces antinuclear antibodies with specificity for cardiolipin and ssDNA (313). Furthermore, these mice also develop renal immunoglobulin deposition. Recent studies demonstrate a role for complement receptors in clearing of apoptotic cells from the circulation, thus perhaps explaining the apparent paradox that humans with a deficiency in early complement components are more susceptible to SLE. Serum markedly enhances the uptake of apoptotic cells by phagocytes; components of both classic and alternative pathways of complement are responsible for the enhanced uptake (314). Phosphatidylserine on the apoptotic cell surface may activate complement, coating apoptotic cells with C3bi, which facilitates apoptotic cell uptake by complement receptors on macrophages and leading to the degradation of apoptotic material (314). Clearance of apoptotic cells via complement receptors may be important in maintaining self tolerance to nuclear antigens. Deficiency in complement receptors CD21/CD35 or complement protein C4 in Fas-deficient mice (315) and C1q deficiency in normal mice (187) accelerates or induces lupus-like features. C1q binding to apoptotic cells or exposure of anionic proteins on the surface of cells undergoing apoptosis, like annexin V, can lead to a pro-inflammatory cytokine profile of phagocytic macrophages or induce a preferential uptake of these cells by dendritic cells (DC), which can facilitate an autoimmune response (316 ,317).

Serum amyloid P may also play a role in handling of chromatin from apoptotic cells. Serum amyloid P deficient mice spontaneously develop antinuclear antibodies and severe glomerulonephritis, and display increased anti-DNA antibody levels in response to chromatin immunization (318). A similar lupus phenotype occurs in mice with a targeted deletion in DNAse 1, an enzyme that may be important in degrading DNA generated by apoptosis (188). Interestingly, one study has suggested that patients with SLE have significantly lower serum levels of DNAse 1 when compared to controls with nephritis from other causes (319).

In mice, the complete phenotypic expression of autoimmunity caused by the lpr defect (320) or the bcl-2 transgene (321) is highly dependent on the genetic background. It seems reasonable to speculate that Fas, Fas ligand, bcl-2, and other genes and regulators of apoptosis, in combination with additional as yet unidentified genes, may be sufficient to induce many of the phenotypic features of systemic lupus in humans, although it is evident that defects in Fas expression lead to a different human disease.

Abnormalities in signaling pathways can alter thresholds for induction of B cell tolerance. The BCR is associated with several molecules that comprise the B cell coreceptor complex. CD19 is part of the coreceptor complex, and plays a role in regulating signaling thresholds that modulate B cell activation and autoimmunity (322). CD19 overexpression leads to an increased strength of the BCR signal resulting in B cell hyperresponsiveness and breakdown of peripheral tolerance, as manifested by increased levels of anti-DNA antibodies and rheumatoid factor in mice (323). C22 is a B cell surface glycoprotein that becomes rapidly phosphorylated following BCR crosslinking. CD22 is a negative regulator of BCR signaling as shown by hyperresponsiveness to receptor signaling in mice deficient for the molecule (324). CD22 deficient

mice display a heightened immune response, increased numbers of B-1 B cells, and serum autoantibodies (325). Associated with CD22 are Lyn and SHP-1. Targeted deletion of the genes encoding either of these molecules also leads to autoimmune manifestations (326 ,327 ,328). The effects of alterations of these signaling molecules on regulation of tolerance and autoimmunity are evident in mice; however, a definite role for altered signaling in the autoimmune diathesis in patients with lupus remains speculative at this time.

Recent studies have also shown that an overabundance of molecules that rescue B cells from negative selection will lead to the development of a lupus-like serology in mice. BAFF is a B cell survival factor and is critical in the ability of transitional B cells to acquire a mature B cell phenotype and achieve immunocompetence. BAFF overexpression however, leads to the survival of autoreactive B cells that would normally be deleted at an immature stage of development. Presumably, BAFF receptor activation impedes the apoptotic pathway triggered by BCR engagement.

Activation of TLR9 in B cells by DNA-anti-DNA complexes can also rescue B cells from negative selection. Thus, the immune complexes that are characteristic of lupus probably contribute to sustaining the maturation and activation of DNA-reactive B cells that might otherwise undergo tolerance induction.

As mentioned above, increased prolactin can potentiate autoimmunity by upregulating CD40 on B cells and CD40 ligand on T cells. Engagement of CD40 is another mechanism for blocking the completion of an apoptotic program induced by BCR engagement of immature B cells. Not surprisingly, therefore, overexpression of CD40 in mice can also lead to autoantibody production. Several studies have suggested an increased expression of CD40 ligand on both T and B cells in SLE patients that may function to prevent B cell tolerance induction and to enhance activation (329).

Therapeutic Interventions

Classic therapeutic interventions in SLE are characterized by their lack of specificity for B cells making particular pathogenic antibodies. Besides the desired decrease in autoantibody production by B cells, these therapies also cause a more generalized immune suppression, with potentially devastating consequences. In recent years, there have been several new and intriguing developments in the treatment of SLE (Table 21-6). Important advances in the molecular biology of B lymphocytes and their regulation have increased our understanding of the immunologic mechanisms that mediate B cell tolerance and offer new opportunities and novel targets for therapeutic manipulation. While many of these approaches are not selective for autoreactive B cells, they may have the advantage of causing fewer deleterious side effects than conventional cytotoxic therapy. Furthermore, antigen-specific therapies may increase the selectivity of the intervention, offering efficacy while potentially decreasing unwanted side effects.

Table 21-6: Therapeutic Interventions in SLE

Nonantigen-specific therapies
Classic immunosuppressive therapies (corticosteroids, cytotoxics)
Rapamycin
Mycophenolic acid
Inhibition of costimulation (anti-CD40 ligand, CTLA-4-Ig)
BAFF/APRIL blockade
Anti-CD20 antibody (Rituximab)
Stem cell transplantation
Hormonal manipulation
Antigen-specific therapies
LJP 394 (tetrameric oligonucleotides)
Peptide-based
Immunoglobulin V region-derived peptides
Histone peptides
Peptide mimotopes of dsDNA

CTLA, cytotoxic T-lymphocyte antigen; BAFF, B cell-activating factor; APRIL, proliferation-inducing ligand

Nonantigen-Specific Therapies: Interfering with Cognate Interactions

Antiself antibodies in SLE clearly arise from T cell–dependent responses. Among the important accessory molecules in the B cell–T cell interaction, CD40 ligand (gp39) is expressed on activated T cells and binds to antigen-specific B cells to transduce a second signal for B cell proliferation and differentiation (Fig. 21-3) and, as mentioned above, to rescue autoreactive B cells from deletion at an immature stage. A short treatment of young SNF1 lupus-prone mice with a monoclonal antibody to CD40 ligand markedly delays and reduces the incidence of lupus nephritis for long after the antibody had been cleared (330). Furthermore, treatment of older SNF1 mice with established nephritis reduces the severity of nephritis and prolongs survival (331). Similarly, treating NZB × NZW F1 mice with anti-CD40 ligand leads to decreased anti-dsDNA antibody titers, less renal disease, and most importantly improved survival compared to the control group (332). Inhibition of other costimulatory molecules was also found to be beneficial in lupus. Selective inhibition of the interaction of costimulatory molecules B7-CD28 by cytotoxic T-lymphocyte antigen-4 (CTLA-4)-Ig (a recombinant fusion molecule between CTLA-4 and the Fc portion of an immunoglobulin molecule) blocks autoantibody production and prolongs life in NZB × NZW Fl mice, even when given late in the course of disease (333 ,334). This intervention prevents T cell activation, thereby preventing T cell–dependent B cell activation. Simultaneous blockade of B7/CD28 and CD40/CD40 ligand with a short course of CTLA-4-Ig and anti-CD40 ligand was significantly more effective than either intervention

alone (335). Clinical trials with anti-CD40L antibody in lupus were terminated because of a higher incidence of thrombo-embolic events (336). Investigation into the possible mechanisms leading to increased thrombosis revealed that human platelets express CD40L, which interacts with integrins (337 ,338). This interaction apparently is important to maintain stability of a platelet thrombus; in the presence of anti-CD40L antibody preformed platelet clots become unstable and release smaller thrombi. Mechanistic studies performed in a limited number of patients that received this antibody, demonstrated however, a decrease in the number of peripheral anti-dsDNA B cells as well as a decrease in the titers of anti-dsDNA antibodies (339). Clinical studies using CTLA-4-Ig in the treatment of human lupus are underway.

B cell–activating factor (BAFF) of the tumor necrosis factor family plays an important role in B cell survival and excess BAFF is often upregulated in SLE patients. There have also been encouraging results with BAFF blockade in murine SLE and clinical studies in human lupus are in their early stages (340). A vast clinical experience in the treatment of non-Hodgkin lymphoma has accumulated on the use of a humanized chimeric antibody (rituximab) specific for human CD20. This pan-B cell surface marker is expressed on pre- and mature B cells but almost undetectable on plasma cells (341). Recent small clinical trials of rituximab along with cytoxan and steroids in SLE suggest there may be significant benefit for diverse clinical manifestations but a randomized, placebo controlled trial has not yet been performed (342 ,343 ,344).

Nonantigen-Specific Therapies: Interfering with T Cell Proliferation

Mycophenolate mofetil inhibits inosine monophosphate dehydrogenase, an enzyme important in the de novo synthesis of guanine nucleotides. Inhibition of this metabolic pathway inhibits B- and T cell proliferation, and results in immunosuppression (345). While mycophenolate mofetil acts as a cytotoxic agent by inhibiting cell division, this effect is relatively selective, and limited to lymphocytes. In MRL/lpr/lpr (346) and NZB × NZW F1 (347) murine lupus models, mycophenolate mofetil improves renal disease, decreases serum anti-dsDNA antibody levels, and significantly prolongs survival. In a recent study in human lupus, mycophenolate mofetil showed beneficial effects in the treatment of lupus nephritis (348).

Rapamycin is a novel immunosuppressive macrolide drug, which inhibits lymphocyte proliferation. Rapamycin binds to a protein kinase important in regulating cell cycle progression (349). In MRL/lpr/lpr mice, treatment with rapamycin significantly reduces serologic manifestations of lupus as well as tissue damage (350). Treatment of NZB × NZW F1 mice with early nephritis (5 months of age) with a brief course of anti-B7 antibodies in combination with an 8-week course of rapamycin reduced mortality at 10 months from 60% to 0% (abstract FASEB).

Antigen-Based Therapies

There are two theoretical ways by which antigen conjugates might improve the course of disease in lupus. First, antigen conjugates may specifically block pathogenic autoantibodies from binding to their target antigen and initiating a tissue-destructive inflammatory cascade. Second, antigen conjugates may downregulate antigen-specific B cells and induce specific B cell tolerance, which is ordinarily induced by BCR ligation in the absence of costimulation. One such conjugate, polyethylene-glycol with tetrameric oligonucleotides, was administered to BXSB male lupus-prone mice (351). Treatment decreased the number of anti-dsDNA–producing B cells, decreased proteinuria, and significantly increased survival. Several studies in humans have been inconclusive although there does appear to be some decrease in the serum anti-dsDNA titers (352 ,353 ,354). A putative role for peptides in induction of anti-DNA antibodies was discussed above; these small antigens may also be suitable for therapeutic use. Intravenous treatment of preautoimmune SNF1 mice with nucleosomal peptides postpones the onset of nephritis, while chronic treatment of older mice with established disease improves survival (283). Immunization of mice with peptides derived from anti-dsDNA antibodies have been shown to activate autoreactive T cells that provide help for the production of autoantibodies (see above). Treating NZB × NZW Fl mice with several T cell peptide epitopes derived from an anti-dsDNA antibody induces T cell tolerance to these peptides, and results in significantly improved renal disease and prolonged mean survival (284). Similarly, mice treated neonatally with CDR-based peptides acquire resistance to subsequent induction of autoimmunity (282). Clinical trials with tolerogenic peptides are about to commence.

The recent technology of displaying random peptides in phage permits the identification of peptides that function as surrogate antigens to autoantibodies. The selected peptide does not necessarily have to be the actual sequence that is recognized by pathogenic antibody (although it can be). Whether peptide dsDNA mimotopes will be useful in inhibiting polyclonal antibody deposition and/or directly tolerizing pathogenic B cells in lupus mouse models is currently under investigation.

Summary

Sequences of many anti-dsDNA antibodies have been analyzed to see how they differ from the human and murine antibody response to foreign antigens. As expected from idiotypic studies in SLE, certain V region genes or families are used preferentially in the anti-DNA response. However, observations of restricted gene usage do not differ in principle from those made in the response of nonautoimmune animals to foreign antigen, in which a small number of V regions dominate the response to any particular antigen. No particular gene family is absolutely necessary for the production of autoantibodies; nonetheless, investigation is continuing into genetic polymorphisms in the Ig locus that are

associated with human lupus. It appears, however, that all individuals are capable of generating pathogenic autoantibodies; in autoimmune individuals, autoantibodies that have developed high affinity for autoantigen through somatic mutation are present in the expressed B cell repertoire. This appears to primarily reflect a defect in the mechanisms of self tolerance rather than an abnormality in V-gene repertoire, the process of gene rearrangement, or the process of somatic mutation. While a defect in central tolerance permitting exodus of autoreactive B cells from the bone marrow (perhaps through lack of proper receptor editing or through aberrant signaling) seems to occur in lupus, it is equally possible that the defect is in peripheral tolerance (in the regulation of B cells maturing in the germinal centers), where responsible mechanisms are not yet delineated.

The autoantibody response in SLE has the characteristics of an antigen-selected response. Cognate B-T–cell interactions are crucial to the maturation of pathogenic anti-dsDNA antibodies, which are primarily IgG, mono- or oligo-specific, and have high affinity for the antigen (dsDNA). Together with the higher-than-random R-to-S ratio in the CDRs of many anti-dsDNA antibodies, this suggests that the anti-DNA response is both driven and selected by an antigen. Pathogenic, IgG anti-dsDNA antibodies in SLE seem to arise from the conventional B cell lineage, possibly through somatic mutation of genes encoding protective antibodies. There is some speculation that natural autoantibodies, perhaps from the B-1 lineage, also could be precursors for anti-DNA antibodies.

While it is clear that more than one constellation of immunologic defects can result in the clinical syndrome collectively known as SLE, and almost certainly there is heterogeneity in the patient population, advances in understanding aspects of both B cell biology and disease pathogenesis have led to the development of new potential therapeutic modalities. Integration of inhibition of co-stimulation or antigen-specific therapies into the routine management of patients with systemic lupus does not seem to be far into the future.

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Chapter 22
Antinuclear Antibodies: An Overview

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Introduction

The antinuclear antibody test (ANA) is often used as a means to determine whether an individual presenting with joint problems has an autoimmune rheumatic disease (ARD) such as systemic lupus erythematosus (SLE) or a related disorder. A positive ANA is thus a serologic indicator of autoimmune disease although further immunologic investigations coupled with clinical findings are required to make a specific diagnosis. These further investigations may also provide relevant information concerning disease activity, end-organ involvement, and prognosis. Although initially identified on immunofluorescence microscopy (IFM) more than 100 nuclear antigen-antibody specificities have been identified using more sophisticated techniques, e.g., immunoblotting in patients with SLE and various ARD (1) reflecting the heterogeneity of the various nuclear targets. Given however, the vast number of targets available within the cell (around 2000) and the fact that very few specificities are found in more than 30% of patients; the diversity of autoantibodies produced is actually rather restricted. Distinct autoantibody profiles may provide a specific diagnosis.

The LE cell phenomenon, of phagocytosis of leukocytes exposed to lupus patients serum, described by Hargraves in 1948 (2) was the first serologic marker used in the diagnosis of SLE and has now been replaced by ANA testing. For the past 40 years ANA has been detected by IFM initially on rodent tissue sections (3 ,4 ,5) and more recently using human cell lines (6) that are capable of greater pattern recognition because of the larger size of individual cells and more discrete fluorescence of subcellular organelles while displaying species specificity.

Crucial to the interpretation of the ANA test is the recognition that the main patterns of staining on IFM are rarely specific or diagnostic of any individual autoantibody or disease. An exception to this is the centromere pattern seen with antikinetochore antibodies in limited cutaneous scleroderma. The presence and pattern of staining of ANA is indicative of the presence of an autoantibody which should be verified with more specific tests (see later) and considered in the clinical context to deduce if the diagnosis of SLE, another autoimmune or separate disease should be considered.

This chapter summarizes the history of ANA testing, choice of substrate, interpretation of IFM with links to target antigens and their clinical relevance, more specific diagnostic tests and a practical guide to interpretation. The aim is to provide an understanding of the strengths and limitations of ANA determination, its sensitivity and specificity, and above all the importance of a positive ANA result in deciding if the patient has SLE or an autoimmune disease at all.

History

The initial description of the LE cell test (2) constituted one of the first laboratory abnormalities found to be associated with SLE. Haserick in 1950 then demonstrated that the LE factor is a component of the gamma globulin fraction of serum proteins (7), while Miescher in 1954 reported that incubation of sera from lupus patients with a suspension of cell nuclei eliminated the capacity to induce the LE phenomenon (8). Thus the LE test was the first antinuclear antibody reactivity ever described, and has recently been shown to be directed against histone H1 as the major monomeric proteinaceous antigen (9).

Friou et al. in 1957/8 first described and partially characterized the phenomenon by which serum from patients with SLE would bind to nuclear antigens in fresh frozen tissue from humans and a range of vertebrate animals. Using fluorochrome labeled antisera it was shown that the component binding the nucleus was in fact antibody from the patient (3 ,10). These findings were subsequently confirmed by others (4 ,5) and clearly demonstrate that an autoimmune pathology was underlying the disease and heralded a new era of research into SLE and since 1971 has been one of the 11 ACR classification criteria (6 ,11).

Choice of Substrate

In the last 45 years a variety of substrates have been used to identify the presence of a positive ANA. Initially rodent tissues of varying types, e.g., mouse kidney, rat liver were popularized. More recently human cell lines, especially epithelial cells (HEp-2) derived from a human laryngeal carcinoma (12 ,13) have been widely adopted. HEp-2 cells have the advantage that, in addition to the easy visualization of large nuclei and nucleoli, as rapidly dividing cells they present antigens only expressed during certain stages of the cell cycle which enables a wide range of staining patterns to be

recognized on IFM. The cell lines can be cultured in the research laboratory or are available from commercial companies on slides. Analysis of the pattern, intensity, and even presence of immunofluorescence is very time-consuming requiring careful interpretation by a trained technician or pathologist that may still give rise to a wide variability between different laboratories experienced in performing ANA tests (14).

More recently attempts at improved standardization of ANA testing have been introduced using commercially developed enzyme-linked immunoabsorbent assays (ELISA). There are several different assays available some of which coat the ELISA plate with extracts of entire nuclei while others use only specific antigens. However, the results of these different assays vary significantly from each other and in some cases from ANA measured by IFM when tested upon the same serum. Those ELISAs with the highest sensitivity for the detection of SLE (in patients known to have the disease) have the highest false-positive rate, while those tests with a low false positive rate have a low sensitivity for the detection of lupus in the same patients (15).

A further disadvantage of the different ELISA kits that use specific antigens is that they cannot detect as yet unknown cellular antigens. In ELISA kits that utilize whole nuclear extracts to coat the plate it is difficult to monitor the binding of different antigens to the polystyrene plates. Thus IFM is likely to remain the test of choice using HEp2 cells for some time.

Patterns of Immunofluorescence and Links to Target Antigen

Demonstration of a positive ANA is merely the start of the journey. Given that the cell nucleus contains DNA, RNA, proteins, and enzymes together with some special structures such as the nucleolus and nucleosomes—the fundamental unit of chromatin, major attempts have been made in the past 40 years to define more precisely what is/are the antigenic targets of a serum that gives a positive immunofluorescence pattern.

Table 22-1: Patterns of IFM on Tissue Culture Staining

Pattern on IFM	Linked Antigen Specificities	Related Disease
Nuclear		
Homogenous	Chromatin, histone, dsDNA Ku	SLE, DIL PM-Scl-SLE
Rim enhanced	Lamins, nuclear pore complex	SLE
Speckled		
coarse	Sm, U1-RNP	SLE
Fine	Ro, La	SS, SCLE, CHB, NL
Distinct	Anti-p80 coilin, anti-p95	PBC
Nucleolar		
speckles	Scl 70, RNA polymerase 1	Scl
Homogenous	PM-Scl Ku	Scl PM-Scl-SLE
Clumpy	U3RNP	Scl
Centromere	kinetochore	Scl
Different patterns of staining	PCNA	SLE
Cytoplasmic	Ribosomal P protein	SLE

SCLE, subacute cutaneous lupus; CHB, congenital heart block; PBC, primary biliary cirrhosis; DIL, drug-induced lupus; PM-Scl-SLE, polymyositis-scleroderma-SLE overlap.

Six main patterns of IFM on HEp2 cells are recognized, one of which is directed against constituents of the cytoplasm (Table 22-1), not all of these patterns occur commonly in SLE patients. The pattern of staining often reflects the predominant antibody present in the serum. A homogenous pattern corresponds with antibodies binding to dsDNA (16) and/or histones (17 ,18), both of which are described in detail later. The peripheral (rim) pattern has been reported with antibodies to integral glycoproteins of the (1) inner nuclear membrane-lamin B (homogenous rim pattern) in SLE (19) and (2) nuclear pore complex, glycoprotein (gp)210 (punctate rim pattern) in patients with myositis (20), primary biliary cirrhosis (21 ,22), and chronic active hepatitis (23).The antibodies to lamin B found in the sera of patients with lupus may explain the peripheral rim pattern described in previous reports and which was thought to be due to antibodies to DNA (24). A speckled pattern is commonly found in patients with SLE indicating binding to a variety of nonhistone, small ribonucleoprotein (RNP) particles including: Sm (25), Ro, La, and U1RNP (26). The precise function of these particles is unknown but they may play a role in the processing of messenger RNA. In fact, the anti-Sm and anti-Ro reaction consists of multiple antibodies binding to multiple antigens. Anti-Sm antibodies bind to a series of proteins: B, B', D, E, F, and G complexed with small nuclear RNAs: U1, U2, U4–6, and U5 (27). The major antigenic peptides of the Ro RNA protein particle are described by their molecular weight, 52-kD and 60-kD Ro. Antibodies to Sm are virtually diagnostic of SLE and are found in 30% of black lupus patients and 5% of Caucasians, frequently in conjunction with anti-U1RNP antibodies. Anti-La antibodies are particularly

associated with SLE and Sjogren syndrome (SS), frequently in conjunction with anti-Ro antibodies, (16) and lupus patients with anti-La antibodies are less likely to have renal disease (28). Anti-Ro antibodies without anti-La are also detected in subacute cutaneous lupus erythematosus (SCLE), lupus of homozygous C2 and C4 deficiencies and neonatal lupus syndrome (29). Figure 22-1 show some examples of these common patterns of nuclear IFM in lupus patients.

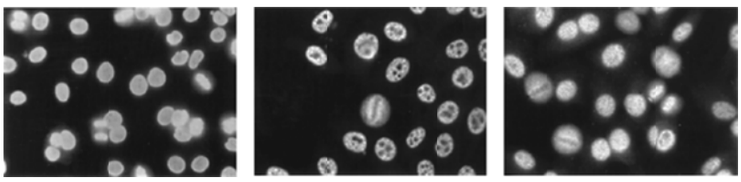

Figure 22-1. Patterns of IFM upon HEp-2 cells commonly seen in patients with SLE. **A,** Homogenous staining characteristic of antibodies to dsDNA. **B,** Coarse speckled staining typical of anti- U1RNP/Sm antibodies. **C,** Fine speckled staining characteristic of antibodies to Ro/La antigens.

Different patterns of cytoplasmic staining exist and are indicative of the distinct target antigens involved. Antibodies directed against components of the cytoskeleton are identified more frequently in ARD but are not specific to lupus (30 ,31). Approximately 15% of SLE sera display a fine speckled pattern on IFM, which has been identified as antibodies to ribosomal P protein. (32 ,33). Interestingly, the presence of an epitope that is antigenically related to the carboxyl terminus of ribosomal P proteins has been found on the surface of a variety of cells including human neuroblastoma cells, endothelial cells, and activated monocytes (34). Recently, antiribosomal antibodies affinity purified from patients with SLE were shown to increase the production of tumour necrosis factor α (TNF-α) and interleukin-6 by activated monocytes in vitro (35).The clinical significance of these findings however are unclear; since although anti-ribosomal P antibodies have been reported to correlate with neuropsychiatric manifestations (36) and severe depression (37) in SLE these associations were not evident in many published series and this proposed link remains controversial (38).

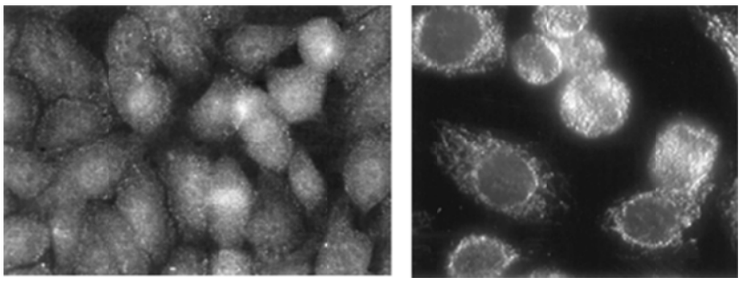

Figure 22-2. A, The fine speckled pattern of cytoplasmic IFM, characteristic of antibodies against ribosomal P and the aminoacyl t-RNA synthetases. **B,** In contrast the reticular, lacy cytoplasmic staining characteristic of antibodies to mitochondria typically seen in patients with primary biliary cirrhosis. The substrate in both cases was HEp-2 cells.

The finely granular cytoplasmic fluorescence seen with antiribosomal P antibodies is also seen with autoantibodies to antigens involved in translation and protein synthesis, the aminoacyl-tRNA synthetases: anti-Jo-1 (39), anti-PL-7 (40), anti-PL-12 (41), anti-EJ (42), anti-OJ (42), anti-KS (43), antibodies to signal recognition particle (44), and anti-KJ (45). Figure 22-2 demonstrate the pattern of staining typical of anti-Jo-1 antibodies. These autoantibodies have not yet been described in lupus being virtually confined to patients with autoimmune myositis especially those with accompanying interstitial lung disease (46).

Centromere and nucleolar patterns of IFM are rarely seen in SLE sera. If detected their presence should alert the physician

to an alternative diagnosis. Nucleolar patterns are mostly displayed in sera from scleroderma (SSc) patients or overlap syndromes. Anti-topoisomerase I antibodies, originally described as anti-Scl-70 (47) are found in approximately 25% of SSc patients and identify the diffuse cutaneous subgroup (DcSSc) with systemic involvement. Anticentromere autoantibodies (ACAs) targeted against the kinetochore are more prevalent, 52% to 82% in SSc patients with limited cutaneous disease (48 ,49). To date, more than six centromere proteins have been identified as bound by sera from patients with SSc, designated CENP-A (centromeric nucleoprotein A) through CENP-F (50). Figure 22-3 illustrates both centromere and nucleolar IFM patterns.

Rarely, a homogenous nuclear and nucleolar IFM pattern is caused by antibodies directed against anti-Ku, which were first described in Japanese PM-SSc overlap patients (51). Initially, there was some confusion with another precipitating antibody system, the anti-Ki, because of the serum used as prototype in the laboratories (16 ,52). The Ku antigen consists of two proteins of 66 and 86 kD, which bind tightly to DNA (53 ,54). Anti-Ku antibodies are found in 55% of Japanese PM-SSc patients (51), and 1% to 19% of SLE-PM-SSc patients and 1% to 14% of SSc patients in North America (46 ,55).

Anti-Ki antibodies described in 1981 (56) are probably identical to the sicca-lupus system (SL) identified in the same year (57) and found in 7% to 10% of unselected SLE sera by immunodiffusion studies (58). The antibodies recognize a 32 kD nonhistone nuclear antigen and display speckled nuclear patterns on IFM. Subsequent detection of anti-Ki antibodies by ELISA using rabbit thymus extract as substrate revealed frequencies of 19% and a fluctuation of the antibodies with disease activity in the sera from lupus patients (59). Further studies with recombinant Ki antigen on ELISA testing found a frequency of 21% of anti-Ki antibodies and an association with central nervous system (CNS) involvement in SLE patients (60). Despite these reported findings anti-Ki antibodies are not specific for SLE being found in sera of many other patients with ARD.

A rare but specific polymorphonuclear pattern corresponding to different phases of the cell cycle is detected in 3% of SLE sera (16) and is caused by antibodies to proliferating cell nuclear antigen (PCNA, previously called cyclin), an antigen involved in cell cycle regulation. Peak expression of PCNA has been shown to occur immediately before full DNA synthesis hence its name (61 ,62). These antibodies are rarely found in other diseases.

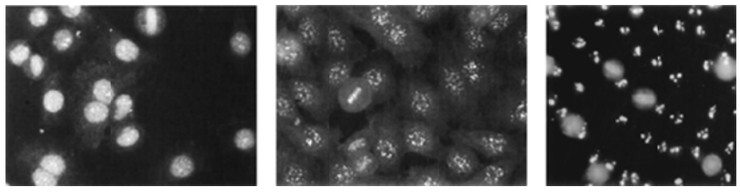

Figure 22-3. Immunofluorescence patterns on HEp-2 cells rarely seen in SLE. **A,** Fine granular nucleolar staining characteristic of anti-Scl-70 antibodies. **B,** The diffuse speckled nuclear pattern typical antibodies against the centromere in metaphase. **C,** Clumpy nucleolar fluorescence characteristic of anti-fibrillarin U3RNP antibodies.

Antibodies to RA-33 an antigen contained in nuclear extracts were initially described in sera from patients with rheumatoid arthritis (RA) but there was no correlation with the presence, absence or pattern of ANA staining on IFM (63). Subsequently RA-33 antibodies have been shown to be identical to the A2 protein of the heterogeneous RNP complex (64). It is now recognized that RA-33 antibodies identify a subset of SLE patients who have an erosive arthropathy (65).

Clinical Interpretation

In high titers, say dilutions exceeding 1:320, in an appropriate clinical setting, e.g., a young woman presenting with a malar rash, polyarthralgia, and leucopenia, the ANA test and interpretation of its significance are straightforward. For every positive ANA result in a case of SLE however, many more will arise because of drug therapy, old age, chronic infection, chronic liver disease, and other ARD. Very rare cases of ANA negative lupus are reported (see later) and a positive ANA may be found

in healthy individuals and asymptomatic first-degree relatives of lupus patients (60 ,61). Furthermore, ANA results vary widely depending on the substrate and immunohistochemical methods used for detection. Currently, the most widely described technique in published studies is IFM on HEp-2 cells. Evidence based guidelines for the use of immunologic laboratory tests in the rheumatic diseases have been published (27 ,50 ,66 ,67 ,68). These guidelines were developed by critical review of all relevant papers using published standards for studies of diagnostic tests, the use of which was then categorized as primarily diagnostic or prognostic. Information extracted from each paper was used to calculate a weighted average for sensitivity and specificity from which likelihood ratios were then derived and used to recommend the usefulness—or otherwise—of a test to practicing physicians (66).

In healthy individuals, age 20 to 60 years, ANA determined by IFM on HEp-2 cells has been shown to be positive at an increasing frequency with decreasing ANA titer. Thus 3.3% of putatively normal individuals are ANA positive at 1:320 dilution and up to 31.7% of the same population at 1:40 dilution (14). There is a steady rise in the prevalence of numerous autoantibodies with increasing age such that 10% to 37% of healthy elderly individuals (older than 70 years of age) have a positive ANA albeit of generally low positive titer (69 ,70). Therefore, ANA results should always include a description of the highest titre for which IFM is detected and ideally a description of the percentage of patients without any ANA-associated disease (controls) who have similar titers (67).

A positive ANA has been found in most patients with a systemic ARD to varying degrees; however it occurs with the highest frequency and titre in SLE (Table 22-2). The absolute level of the titre itself does not carry any prognostic significance, however higher titers are more likely to be significant in making a diagnosis of disease.

In some cases however, the ANA may be positive even before the diagnosis of SLE has been made. An evaluation of a prospectively assembled collection of frozen samples taken from more than 5 million U.S. armed forces personnel found a positive ANA (at a dilution of ≥1:120) in 101 of 130 patients with SLE up to 9.2 years before the diagnosis (71). Furthermore, this study found a progressive accumulation of specific autoantibodies before the onset of SLE; with ANA, anti-DNA, antiphospholipid, anti-Ro, and anti-La antibodies present earlier than anti-Sm and anti-nuclear RNP antibodies (71).

Thus the presence and staining pattern of a positive ANA alone is not sufficient to determine the presence of lupus or any ARD. Detection of specific autoantibodies is required by more specific tests and even then not all autoantibodies can recognise a specific disorder (72). Consideration of autoantibody profiles however increases diagnostic predictive value without loss of specificity or sensitivity. For example, the presence of antibodies to dsDNA and/or Sm are very useful for confirming the diagnosis of SLE, although a negative result does not exclude SLE (27 ,68). Since these autoantibodies are rarely found in other ARD they are very useful in distinguishing patients with SLE from those with other ARD, such as SS (associated with anti-Ro and/or anti-La) or SSc (ACA and/or Scl 70 antibodies) (73). The same autoantibody profiles have been tested in SLE patients and found to identify disease subsets. For example lupus patients with antibodies to dsDNA and/or Sm had a significant increase in malar rash, hypocomplentemia, renal and hematologic involvement while patients with anti-Ro and/or anti-La antibodies had a worse lupus rash and photosensitivity (74). In fact, the strongest autoantibody association with the presence and activity of SLE renal disease occurs with anti-dsDNA antibodies, which also correlate with overall disease activity in many but not all patients with SLE (68). Evidence-based guidelines, described earlier, found that anti-Sm and anti-RNP antibodies add little to the prediction of renal disease in SLE (27). A recent study of a large and consecutive cohort of 285 patients with SLE identified five clusters of autoantibodies with particular clinical associations. The most striking of these associations were of anti-RNP antibodies with Raynaud phenomenon, of anti-Ro and anti-La antibodies with sicca symptoms and of antiribosomal P antibodies with hemolytic anemia (75). Therefore more diagnostic and prognostic power is conferred when autoantibody profiles are considered as a whole in each patient.

Table 22-2: Positive ANA Tests in Various Conditions Using HEp-2 Cells as Substrate

SLE	Other ARD	Other Disease	Normal Population
98%	SSc 98%	Chronic active hepatitis 100%	13.3% at 1:160
	PM 90%	Drug-induced lupus 100%	
	SS 80%	Myasthenia gravis 50%	FDRs of SLE
	pJIA 70%	Waldenstrom macroglobulinemia 20%	patients 20%–30%
	RA 60%	Infectious mononucleosis 15%	
	PAN 18%	Diabetes 25%	

ARD, autoimmune rheumatic diseasel SSc, scleroderma; PM, polymyositis; SS, Sjogren syndrome; pJIA, pauciarticular juvenile idiopathic arthritis; RA, rheumatoid arthritis; PAN, polyarteritis nodosa; FDR, first-degree relative.

A wide variety of drugs can induce a positive ANA and/or symptoms of drug-induced lupus (DIL), most commonly hydralazine, procainamide, isoniazid, minocycline, and chlorpromazine. Of particular interest is the reported induction of ANA and/or anti-DNA antibodies in some patients treated with TNF-α inhibitors (76 ,77), despite which this class of drug has been successfully used to treat a small number of lupus patients in open label trials (78). The homogenous staining pattern seen on IFM with such drugs is caused by antibodies to histones targeted primarily against the H2A-H2B-DNA complex (79 ,80 ,81) or H2A-H2B alone (82). Anti-histone antibodies are also found in 30% to 80% of idiopathic SLE sera directed mainly against H1 and H2B (83), H3 (58), and the H2A-H2B complex (18). The presence of these antibodies is not associated with any particular clinical manifestations in SLE but have been correlated with disease activity (84).

Thus homogenous staining and the presence of antihistone antibodies alone cannot distinguish between idiopathic

lupus and DIL when a patient has appropriate symptoms having been exposed to a potentially lupus inducing drug. If other autoantibodies are present and the symptoms do not abate within a few months of stopping the drug accompanied by a decline in the ANA then DIL is unlikely.

More Specific Diagnostic Tests

Once the presence of autoimmunity is suspected clinically and then reinforced with a positive ANA more specific tests of antibody detection are required. Antibodies to the target antigen of interest are labeled with markers such as enzymes, fluorochromes, or radioisotopes in the tests commonly used notably radioimmunoassay (RIA), ELISA, and Western blotting, which are more sensitive than immunodiffusion techniques.

RIA is similar to IFM in that a labeled antibody, conjugated with a radioisotope in this case, is used to detect a specific antigen. The technique is very sensitive detecting antigen in the picogram range. An inherent drawback of RIA is the use of radioisotopes that require careful handling and expensive equipment to monitor radio-active emissions. Hence the ELISA was devised whereby antibodies against the antigen of interest are attached to an enzyme such as horseradish peroxidase or alkaline phosphatase instead of a radioisotope. Detection of the antibody (and thus antigen) is facilitated by the addition of a substrate and developer that will generate a colour reaction if the enzyme labeled antibody is present. The end point color can be read in a spectrophotometer or ELISA reader to give a quantitative estimation of the bound antibody and hence antigen. Advantages over RIA include the lack of radioisotopes and speed as a 96 well plate can be read in less than 1 minute.

Western (immuno) blotting takes proteins from an extract of cultured cells and separates them by polyacrylamide gel electrophoresis according to their charge. The separated proteins are then transferred electrophoretically to a nitrocellulose (NC) sheet that is a replica of the original gel and a solid support for antigen. The NC sheet can then be probed with either enzyme or radiolabeled antibody (85).

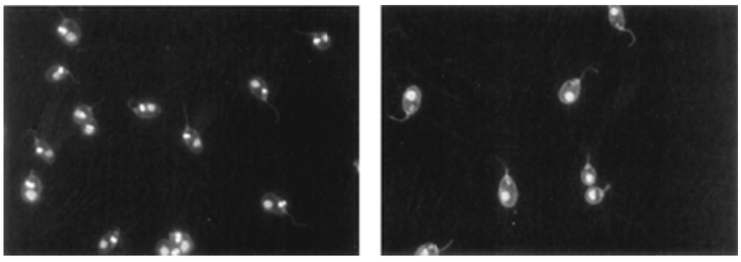

Figure 22-4. A, The Crithidia luciliae assay for antibodies to dsDNA is positive with immunofluorescence of the kinetoplast only. **B,** Fluorescence of the nuclei, in this example, or any structure other than the kinetoplast is not indicative of the presence of anti-dsDNA antibodies.

Identification of Lupus-Related Antibodies

Of the disease specific antibodies found in patients with SLE it is those targeting dsDNA that carry the most diagnostic power and clinical application. The presence of antibodies to dsDNA—especially of the IgG isotype—has been used as a serological marker for the presence of SLE for over 45 years. Between 60% to 83% of lupus patients are found to have anti-dsDNA antibodies when tested by one of the currently available assays and in some patients the titer of these antibodies is an excellent measure of disease activity. Despite some problems with regard to the type of assay used to detect these immunoglobulins and one or two potential challenges to their serological predominance—such as antinucleosome antibodies—on balance the continued inclusion of anti-dsDNA antibodies as a criterion for the diagnosis of lupus remains a reasonable proposition (86). Furthermore, their use as a potential biomarker has been proposed in future clinical trials to study the effects of new therapies for SLE (87).

Amongst the currently available methods of detecting antibodies to dsDNA the Farr (RIA) may be the most specific test for SLE and is the assay most likely to predict occurrence of disease flare especially glomerulonephritis. The Crithidia assay detects anti-dsDNA antibodies by their ability to bind to the kinetoplast of *Crithidia luciliae,* a protozoan organism with a circular dsDNA structure at one pole, and subsequent immunofluorescence. This assay is technically demanding, requiring careful training of technicians to correctly identify the kinetoplast rather than the nucleus or polar body upon IFM, as shown in Figure 22-4 . A major advantage is that it detects antibodies to dsDNA almost exclusively as opposed to the Farr and ELISA that may give positive results with ssDNA. However, the ELISA is widely available and relatively easy to

perform detecting both high- and low-affinity IgG antibodies to dsDNA (88).

Amongst the different ELISA kits available there are marked differences in sensitivity, specificity and predictive values in diagnosing SLE and determining disease activity (89). Hence laboratories may offer more than one test for detection of anti-dsDNA antibodies in certain instances. For example the Crithidia assay may be reserved for diagnosis and ELISA used to monitor antibody titers and thus reflect disease activity.

Recent studies have strengthened the notion that nucleosomes may be in vivo targets of anti-dsDNA antibodies in patients with SLE. Analysis of serum antibody activity against nucleosomes and dsDNA has shown both in human and murine lupus that serum anti-dsDNA reactivity is almost always associated with an antinucleosome activity (90 ,91). In 80% to 90% of patients with SLE the presence of antinucleosome antibodies appear to be a more sensitive marker of SLE than autoantibodies to any other antigen (91 ,92). It has been suggested that antinucleosome antibody titers fluctuate with disease activity (93), although this finding has not been consistently reproduced (94). These discrepancies probably arise because of different methods of nucleosome preparation, insufficient data looking at variations in individual patients over time and variations in the indices of disease activity used. Hence in the future the antinucleosome ELISA is very likely to be an additional immunologic test for diagnosing SLE but its prognostic value is yet to be fully established.

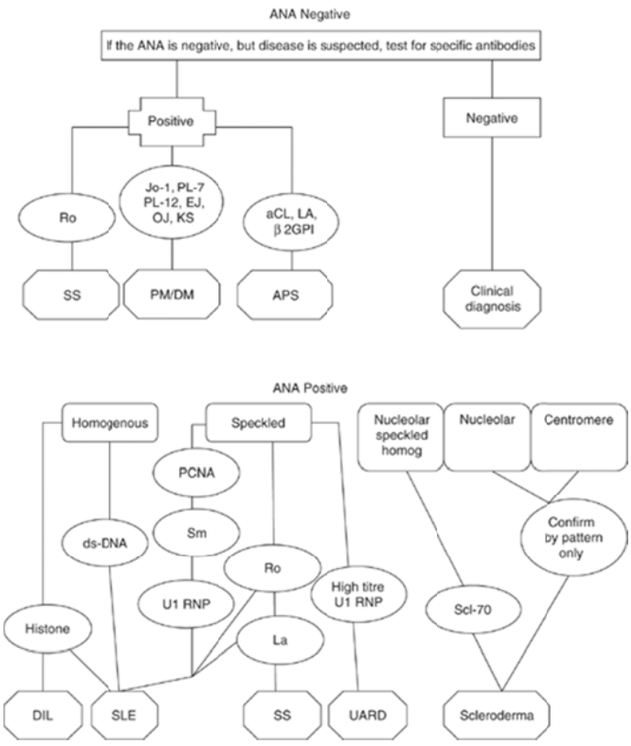

Figure 22-5. A practical guide to interpretation of the ANA test.

ANA Negative Lupus

True negative ANA tests in patients with lupus are now very rare, perhaps 2% of patients at most (95), with the advent of human tissue culture cells as the substrate. Previously more patients were falsely labeled as being ANA negative in up to 5%, using rodent (mainly mouse and rat liver) tissue substrate. Autoantibodies however are detectable by ELISA against Ro and La antigens, originally found in 90% of ANA negative patients sera (96), and now by using a more sensitive

ELISA virtually 100% of the same sera (97). The reason for this apparent dichotomy is the variable distribution of Ro and La antigens in cells of different species with significant quantities of the antigen detected by IFM in cells of humans, monkeys, and guinea pigs but absent to low amounts found in cells of rat, mouse, and chicken (98).

A previously positive ANA test in patients with lupus may become negative with disease remission that occurs either spontaneously or following immunosuppressive treatment. Such ANA negativity can occur in 10% to 20% of cases, especially those lupus patients who experience renal failure.

The disappearance of previously positive ANA does not remove the need for careful vigilance of clinical and laboratory markers of disease as the future course of the disease cannot be assumed to have burnt out. Apart from anti-dsDNA antibodies and possibly antinucleosome antibodies the titre of other autoantibodies and ANA is not a guide to disease activity.

Conclusion

Identification of the presence and pattern of staining of ANA on IFM of HEp2 cells is an essential first step in the immunologic diagnosis of lupus. The subsequent detection of specific autoantibodies and their profile helps to cement the clinical diagnosis of SLE, in the case of anti-dsDNA and/or anti-Sm antibodies, or suggest another ARD such as SSc if ACA or anti-Scl 70 are found. Figure 22-5 offers a practical guide to interpretation of the ANA test, which highlights the diagnostic decision pathway that must be taken each time an ANA test is ordered.

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Chapter 23
Antibodies to DNA

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Antibodies to DNA (anti-DNA) are the classic autoantibodies that characterize systemic lupus erythematosus (SLE). High-avidity immunoglobulin G (IgG) antibodies to double-stranded DNA (dsDNA) play a major role in inducing some of the disease manifestations of SLE (especially nephritis), are relatively specific for the disease, and are good markers of disease activity in some patients.

The History of Antibodies to DNA

The lupus erythematosus (LE) cell phenomenon identified the first autoantibody recognized in patients with SLE (1). LE cells result from the action on nuclei of antibodies to DNA protein complexes; the major antigen recognized is the nucleosome, a histone/DNA complex (2). Altered nuclei are then ingested by phagocytic cells. LE cells can be formed by phagocytosis of apoptotic bodies (which contain nucleosomes) induced by antinuclear antibodies (ANAs) (3).

The first reports of anti-DNA in the sera of patients with lupus appeared in 1957, discovered in several different laboratories almost simultaneously (2). The clinical importance of circulating anti-DNA was soon recognized. Certain subsets were found to be specific for SLE and correlated with disease activity and nephritis (4 ,5), and evidence mounted that anti-DNA causes some of the tissue lesions that are characteristic of SLE, especially lupus nephritis. Anti-DNA was eluted from tissue lesions (glomeruli, skin, choroid plexus) of patients and mice with SLE (6 ,7 ,8 ,9). In many murine models of SLE, disease was accelerated by increasing anti-DNA responses and prevented by blocking them (6 ,10 ,11 ,12 ,13 ,14).

The development of monoclonal antibody (mAb) technology permitted expanded studies of DNA antibodies (15), which have providedconsiderable information regarding the characteristics of different antibody subsets, the presence of anti-DNA in unstimulated immune repertoires of healthy individuals, and the features of individual antibodies that contribute to their pathogenicity. The central role of these antibodies in the disease process in some patients seems clear.

Antibodies to DNA as Part of the Normal Immune Repertoire: Natural Autoantibodies

There are many different individual antibodies to DNA. They differ in isotype, complement-fixing capabilities, avidity for DNA, antigenic specificities, charge, idiotypes (Ids), V region sequences, and propensity to deposit in different regions of the kidney (16 ,17 ,18 ,19 ,20) (Table 23-1). Healthy humans and mice make antibodies to DNA as part of their normal resting or natural immune repertoires (10 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28). These natural autoantibodies are largely IgM class, react primarily with single-stranded DNA (ssDNA), have low avidity for DNA and are weakly polyreactive. Analysis of the structure of natural anti-DNA shows that most are IgM encoded by germline DNA, with few or no somatic mutations. However, activation of the resting B cell repertoire in mice and in humans yields not only IgM low-avidity anti-ssDNA but also some IgM and IgG subsets that bind dsDNA (10 ,22 ,23 ,25 ,26 ,27). Therefore, the ability of human and murine B cells to make antibodies to ssDNA and dsDNA is not forbidden but rather is normal. In fact, IgM and IgG antibodies to ssDNA can be found in many healthy individuals and in many disease states other than SLE that are associated with B cell activation, such as infections, chronic inflammatory states, and aging. Administration of certain drugs and biologics (such as tumor necrosis factor alpha (TNF-α) inhibitors can induce IgM anti-dsDNA and in some patients IgG anti-dsDNA as well (28 ,29). In contrast, IgG antibodies to dsDNA are consistently more abundant in the repertoire of individuals with SLE than in healthy persons, and their presence in serum at high titers is indicative of SLE.

Polyreactivity is characteristic of natural autoantibodies. However, both IgM and IgG mAbs directed against ssDNA, dsDNA, or both can be quite specific for these antigens (30) or have multiple reactivities, including cross-reactivity with polynucleotides, Sm, La, the A and D polypeptides of ribonucleoprotein (snRNP), cytoskeletal proteins, histones, nucleosomes, laminin, α-actinin, phospholipids, the Fc of IgG, cell surface structures (on platelets, lymphocytes, and Raji cells), proteoglycans such as heparan sulfate, myelin, neuronal glutamate receptors, gangliosides, and bacterial polysaccharides and proteins (21 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49). Some of these cross-reactivities probably contribute to the pathogenicity of

individual antibodies, while others permit anti-DNA to bind undesirable foreign antigens.

Table 23-1: Different Subsets of Anti-DNA

Probable nonpathogens (the normal repertoire)
IgM
IgG noncomplement fixing
Low avidity for ssDNA
Wide cross-reactivity (low avidity) to
Polynucleotides
Sm/RNP
Cytoskeleton
Histones
Fc of IgG
Laminin
Proteoglycans
Phospholipids
Cell surfaces
Myelin
Gangliosides
Bacteria
Polysaccharides
Phospholipids
Proteins
Probable pathogens (the SLE repertoire)
Complement-fixing Ig isotype (IgG1 and IgG3 in humans, IgG2a and IgG2b in mice, some IgM)
High avidity for dsDNA and ssDNA
Ability to bind directly to glomeruli: α-actinin, planted nucleosomes, heparan sulfate, laminin, phospholipids
Cationic charge
High-avidity binding to
DNA
DNA/histone
Nucleosome
Ability to bind directly to neurons: glutamate/NMDA receptor
Ability to form immune complexes of correct size and charge to avoid clearance and fix to GBM
Enrichment in certain idiotypes
IdGN2
16/6
32/15
3I
O-81
Ability to penetrate cells and fix to cytoplasmic structures or to nucleus

dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; GBM, glomerular basement membrane; Ig, immunoglobulin; RNP, ribonucleoprotein.

Natural autoantibodies to DNA likely serve as a repertoire that, when a foreign antigen arrives, requires minimal somatic mutation to generate high-avidity antibodies against the bacterial or viral invader. Some IgG anti-DNA with pathogenic potential probably derive from a small number of somatic mutations in germline-encoded natural autoantibodies (50 ,51 ,52 ,53 ,54). Conversely, antibodies to bacteria can be precursors of antibodies to DNA, and these two types of antibody can share similar sequences. A single-point mutation changes the ability of an antibody to bind either pneumococcal phosphocholine or DNA (51), and some anti-DNA can bind bacteria directly, possibly via phospholipids (30 ,44). Immunization of a lupus patient with pneumococcal vaccine produced antibodies with the capacity to bind different antigens; some were specific for the bacterial phosphocholine, some for DNA, and some bound both antigens (54). Similarly, immunization of mice with bacteria, bacterial DNA, DNA from activated lymphocytes, or lipopolysaccharide can induce antibodies to DNA (10 ,33 ,34 ,55 ,56 ,57). Thus, the resting natural autoantibody repertoire serves as a sentinel that is designed to provide quick antibody protection against undesirable invaders of the host. Unfortunately, the natural repertoire also has the capacity to generate pathogenic autoantibodies; the difference between recognizing danger and recognizing self can be a matter of a single amino acid change. This was demonstrated in recent work in which single point mutations in two nephritogenic human mAb anti-dsDNA were mutated back to germline configuration; changes in one or two amino acids in either heavy (H) or light (L) chains of the mAbs destroyed ability to bind nucleosomes/DNA (58).

Evidence That Some Subsets of Antibodies to DNA Are Pathogenic

The preceding discussion reviewed the early evidence that anti-DNA play a role in the pathogenesis of SLE. That evidence includes the following: (a) anti-DNA is located in sites of tissue damage in mice and patients with SLE; (b) large quantities of high-avidity IgG antibodies to dsDNA in the serum are associated with active lupus nephritis and active clinical disease in humans and mice; (c) DNA administered as immunogen accelerates murine lupus nephritis; and (d) DNA administered as tolerogen delays the appearance of anti-DNA and nephritis in mice with lupus.

More direct evidence has come from experiments in which anti-DNA are administered to normal mice and cause renal dysfunction or frank clinical nephritis. For example, Dang and Harbeck (59) injected polyclonal antibodies from the serum of nephritic New Zealand black/white (NZB/NZW) F1 mice into young mice of the same strain; cationic populations of serum anti-DNA lodged in glomeruli and induced nephritis earlier than in controls. Raz et al. (60) perfused rat kidneys with various mAb anti-DNA and showed that some bound directly to glomeruli and caused proteinuria. Tsao et al. (61) implanted B cell hybridomas secreting mAb IgG anti-DNA into pristane-primed peritoneal cavities of healthy, young BALB/c mice; several mAbs deposited in glomeruli and induced proteinuria and azotemia. Madaio et al. (62) have identified mAb murine anti-DNA that, when transferred to healthy mice, bind to capillary loops of glomeruli and cause proteinuria. Ehrenstein et al. (63) inoculated severe combined immunodeficiency disease (SCID) mice with human mAb anti-DNA from patients with lupus; one mAb bound to glomeruli and induced proteinuria even though histologic damage was not evident. Later, Ravirajan and colleagues (64) showed that one human IgG anti-dsDNA

mAb grown as a hybridoma in SCID mice caused proteinuria and granular Ig deposition in renal glomerular capillaries. Finally, Tsao et al. (65) made normal mice transgenic for genes encoding both heavy and light chains of a murine IgG anti-dsDNA mAb. B cells in transgenic mice secreted the mAb, IgG anti-dsDNA appeared in the serum, IgG was deposited in glomeruli, and proteinuria appeared. None of these events occurred in nontransgenic littermates. Other laboratories have made mice transgenic for genes encoding heavy or light chains which, when paired with multiple different endogenous light chains, bind DNA; those mice develop glomerular Ig deposits and proteinuria (66).

Several groups have shown that some mAb anti-DNA bind directly to glomeruli, and they have defined some of the components of glomerular basement membrane with which those mAbs cross-react (laminin, heparan sulfate, α-actinin, nucleosomes planted in tissue) (35 ,36 ,37 ,38 ,46 ,47 ,48). Although other antibodies can play a role in inducing the nephritis of SLE, certain subsets of anti-DNA clearly induce nephritis and can be classified as pathogens.

Pathogenic Anti-DNA: Role in Tissue Deposition and Damage

It is generally accepted that antibodies to DNA that can fix to tissue (glomeruli, skin, blood vessels, receptors for glutamate on neurons, platelet membranes, endothelial cells, etc.) are likely to cause damage, although there are examples where deposition occurs but damage does not follow (49). Mechanisms for the deposition in tissue are as follows: (a) antigens and antibodies form circulating immune complexes of the correct size and charge to be trapped in basement membranes of glomeruli, skin, and blood vessels where they activate complement and bind FcγR receptors; (b) antibodies to DNA bind directly to DNA or DNA/histone/nucleosome complexes planted in target tissues (46), following which complement and FcγR are activated; (c) cationic subsets of anti-dsDNA bind to polyanions (e.g., heparan sulfate) in the proteoglycans of basement membranes on the basis of charge, again creating immune complexes in situ (67 ,68 ,69); (d) antibodies to DNA, or antibodies complexed to DNA/histone, can bind to non-DNA antigens that are present in basement membranes, such as alpha-actinin and laminin (36 ,37 ,38 ,47 ,49). A subset of antibodies to dsDNA may cross-react with the NMDA glutamate receptor and can mediate excitotoxic death of neurons, suggesting these antibodies may contribute to memory loss in some SLE patients (48 ,70).

There are several mechanisms by which DNA/anti-DNA complexes and tissue deposition of complexes or anti-DNA cause injury; injury has at least two components, inflammation and sclerosis, which may differ between individuals with lupus (71). Activation of complement causes chemotactic signals for inflammatory cells that release destructive enzymes. Additionally, tissue damage can be mediated through activation of activating FcR (72) that bind Ig (FcγRI and FcγRIII), or prevented by activation of an inhibitory FcR (FcγRII) (73 ,74). Some anti-DNA bind to living cells, mediate antibody-dependent cell-mediated cytoxicity (ADCC), or enter cells by binding to the myosin receptor from which they are transported via caveolin to cytoplasm or nuclei, altering cell functions such as protein synthesis and activation (20 ,75 ,76 ,77 ,78 ,79 ,80 ,81). Anti-DNA can cause release of cytokines by human mononuclear cells, including IL-1ß, IL-6, IL-8, IL-10, and TNF-α (82). Some antibodies to DNA have enzyme activities (83 ,84). Some anti-DNA induce apoptosis of target tissue with creation of apoptotic bodies containing nucleosomal and spliceosomal antigens, further driving autoantibody production and immune complex formation (85 ,86). DNA-reactive B lymphocytes participate directly in tissue damage via antigen presentation to T cells and cytokine release (87). DNA/anti-DNA activate plasmacytoid dendritic cells which release IFN-α and drive the pro-inflammatory pathways associated with innate immunity. DNA/anti-DNA activate B lymphocytes directly via Toll-like receptor 9 (TLR9) (88 ,89). These various abilities are probably related to the structure of each mAb, and to the genetic characteristics of each individual which influence autoantibody persistence, tissue deposition, and the potential inflammation and sclerosis that result.

Importance of Isotype, Complement-Fixing Ability, Avidity for DNA, and Ability to Form Immune Complexes

Several investigators have shown that subsets of anti-DNA that are associated with active disease and with nephritis (in both humans and mice) are predominantly of the IgG isotypes which fix complement well (21 ,59). These are IgG2a and IgG2b in mice, and IgG1 and IgG3 in humans. Such antibodies usually bind both dsDNA and ssDNA; only a small proportion binds dsDNA alone.

Studies of polyclonal anti-DNA eluted from glomeruli of humans or mice with nephritis showed that glomerular populations of Ig are enriched in Ig with high avidity for DNA compared to anti-DNA in sera and urine (8 ,9 ,90). However, studies of mAb anti-DNA have shown less relationship between avidity for DNA and the ability of a transferred mAb to cause glomerulonephritis in normal mice (91 ,92). In fact, mutations introduced in VH regions of mAb that changed avidity for DNA did not correlate with changes in pathogenicity or ability to bind glomeruli in vitro. It is possible that avidity for cross-reacting antigens is more important than avidity for DNA in determining pathogenicity, or that high avidity offers a mAb some pathogenic advantage, but this property alone may not be sufficient to induce disease.

The ability of anti-DNA to form immune complexes of the correct intermediate size favors the ability of that antibody to be pathogenic. Large antigen/antibody complexes are engulfed by the phagocytic system and removed; small complexes are excreted in the urine, so both populations are unlikely to cause disease. In addition to size, conformation of the complex, overall charge, and charge of individual components of the complex, and ability to activate complement

and FcR after being trapped in tissues are all important in pathogenicity, as discussed above.

The Importance of Charge

Many regions of the glomerular basement membrane are polyanionic. Therefore, antigens, antibodies, or immune complexes with cationic charges can bind to glomerular basement membrane via charge (21 ,67 ,68 ,69). If a cationic anti-DNA is trapped by this mechanism and DNA is available, then an immune complex forms in the glomerular basement membrane, complement is activated, and damage occurs. One group has reported that one antibody with a cationic charge in an immune complex is sufficient to permit trapping in the glomerular basement membrane (69). Several investigators have reported enrichment of cationic anti-DNA populations in IgG of glomerular eluates from mice with lupus nephritis (21 ,59 ,67 ,68), although one group disagrees (93). A study of Japanese patients with lupus nephritis showed that cationic clonotypes of anti-DNA (pI 7.0 to 8.5) were found in glomeruli but not in circulating immune complexes (94). Studies of the ability of mAb anti-DNA to induce nephritis in mice have shown that some mAb with neutral pI (7.0 to 7.5) as well as mAb with cationic pI (7.5 to 8.5) can induce nephritis in normal mice (38 ,61). As with high avidity, the presence of cationic charge on a particular anti-DNA probably confers a pathogenic advantage, but some antibodies without cationic charge are perfectly capable of causing disease. Thus, predicting whether a particular mAb anti-DNA is a pathogen or not is difficult and depends on multiple features of that antibody.

The Importance of Idiotypes

The idiotypic characteristics of anti-DNA also may contribute to pathogenicity (21 ,68 ,94 ,95 ,96 ,97 ,98 ,99). Idiotypes are antigenic sequences in the V regions of antibodies; they induce B and T cell antiidiotypic responses that are important in regulating antibody production.

Idiotypes and idiotypic networks are reviewed elsewhere in this volume. Public idiotypes that characterize many antibodies to DNA have been identified in both murine and human lupus. Certain idiotypes have been found in tissue lesions (IdGN2, 16/6, 32/15, 3I, 0-81) of patients with lupus (21 ,68 ,94 ,95 ,96 ,97 ,98 ,99). One human public idiotype or antiidiotype (the 16/6 system), when injected into certain strains of normal mice, can activate an idiotype-antiidiotype network. This results in production of multiple idiotype-positive autoantibodies (including anti-DNA), and the mice develop nephritis (100). Administration of certain peptides from the VH regions of the 16/6 idiotype mAb anti-DNA induce regulatory $CD4^+CD25^+$ T cells and reduce the ability of pro-inflammatory T cells to circulate to target tissue (101). One of these peptides is in clinical trials in human SLE. Experiments in murine models of SLE have shown that anti-DNA and nephritis can be suppressed by administration of antiidiotypes (102 ,103). Therefore, pathogenic anti-DNA subsets are enriched in certain idiotypic markers, and those idiotypes can be targets of regulation that permit the abnormal upregulation of pathogenic subsets of anti-DNA. One study showed that in patients with lupus nephritis, anti-DNA and antiidiotypes leak into the urine (90). The highest avidity anti-DNA and anti-Id were found in glomerular eluates, moderate avidity anti-DNA and anti-Id in serum, and low-avidity anti-DNA and anti-Id in urine.

The Importance of Cross-Reactivity with Tissue

The ability of anti-DNA to cause disease may result from cross-reactivity with structural components of glomeruli or other target tissue rather than (or in addition to) the binding of DNA planted in tissue or passive entrapment of DNA–anti-DNA complexes. For example, several investigators have shown that various mAb anti-DNAs that persist in glomeruli after transfer and cause renal dysfunction can bind laminin, α-actinin and proteoglycans such as heparan sulfate (either alone or complexed with antigen) (36 ,37 ,38 ,43 ,44 ,47 ,48 ,49 ,104).

Subsets of anti-DNA that bind DNA/histone complexes (including nucleosomes [NUCs]) may be particularly enriched in pathogens (35 ,43 ,44 ,104 ,105 ,106 ,107 ,108 ,109). Histones or NUCs are trapped in glomerular basement membrane, particularly in collagen type IV (108), and anti-DNA can fix to those structures via planted histones. Some mAb anti-DNAs can bind to heparan sulfate in glomerular basement membrane only after they bind histone; thus, a complex of anti-DNA/DNA histone is required for pathogenesis (38). Approximately 70% of patients with SLE have antibodies to nucleosomes; the IgG3 subclass of those antibodies correlates with disease activity and nephritis (109). Several studies (110 ,111 ,112) have suggested that IgG antibodies to nucleosomes correlate better with nephritis and active disease than do IgG antibodies to dsDNA, although others disagree (113). Levels of nucleosomes are elevated in sera of patients with SLE but do not correlate with disease activity (113 ,114 ,115). At the time of this writing (2006), testing for anti-dsDNA is still the gold standard for measuring the population of DNA/protein-reactive antibodies found in each SLE patient.

The source of DNA/histone that is planted in glomeruli might be NUC released by apoptotic cells during active SLE. NUC-size DNA is found in the circulation of such patients (113 ,114 ,115). Several investigators have shown that antibodies to NUC appear early in murine lupus; they suggest that NUCs are antigens that induce antibodies that later mature/mutate to anti-dsDNA (106 ,107). This induction would be in addition to that provided by bacterial DNA, DNA from activated lymphocytes (56), mutation of antibodies to bacteria or viruses, and so on.

To study the totality of antibodies that bind to glomeruli, an in vitro method was developed to estimate the pathogenicity of antibodies by incubating polyclonal or mAb Ig with permeabilized rat glomeruli isolated on

filters, or with an extract of those glomerular basement membranes in an enzyme-linked immunosorbent assay (ELISA) (108 ,116 ,117 ,118). This work suggests that some anti-DNAs bind to glomeruli via DNA and/or DNA/histone or NUC combinations fixed to collagen type IV in glomerular basement membrane. Additionally, several non–DNA/NUC-binding antibody populations can play a major role in the nephritis of SLE. Many investigators have reported that non-DNA–binding antibodies to the following antigens can be associated with nephritis in human and murine lupus: ssDNA, laminin, heparan sulfate, Ro (SSA), Sm/RNP, RNA, RNA polymerase I, gp70 (mice only), C1q, and ribosomes (36 ,48 ,49 ,104 ,118 ,119 ,120 ,121 ,122 ,123).

The Potential Importance of Antibodies Penetrating into Living Cells

Several investigators have reported that some anti-DNA (including polyclonal populations isolated from the serum of patients or mAb from mice with SLE) can bind to the surface of living cells, enter those cells, and bind nuclear or cytoplasmic structures (20 ,75 ,76 ,77 ,78 ,79 ,80 ,81). There are probably at least three types of such anti-DNA subsets (79). One binds to cell surfaces and can mediate complement-induced cytotoxicity; the second penetrates cells and binds to cytoplasmic antigens; the third penetrates cells and binds to nuclei. In our library of murine mAb IgG anti-DNA, some pathogenic mAb have these properties, and others do not. F(ab)2 fragments of these anti-DNA also bind and enter cells, so they are not entering via Fc receptors. DNase treatment of cells and antibodies does not impair their ability to access the interior of cells, so DNA as a receptor is not a likely entry point. One receptor that binds anti-dsDNA and permits cell entry is brush-border myosin I (80). Anti-DNA entering via myosin receptors enters the cytoplasm via caveolin (75), interacts with DNase-1, and inhibits endonuclease activity, before moving to the nucleus and attenuating apoptosis. The antibodies can then recycle back to the cell surface. After cell entry, several other abnormalities can occur, including cell activation in an abnormal pattern (81), and decrease of protein synthesis (75 ,79).

All of these interactions between antibodies to dsDNA and living cells are likely to profoundly alter cell functions and to present altered antigens from damaged cells to the immune system to promote autoantibody expansion.

Antibodies to DNA and Nucleosomes

Increases in numbers of apoptotic cells and impairment in their clearance promotes autoantibody production, including anti-DNA and anticardiolipin, and some of these antibodies may deposit in glomeruli (85 ,124 ,125). Antibodies to DNA and to nucleosomes bind NUC released from cells undergoing apoptosis. Since the generation of apoptotic cells in both lymphocytes and neutrophils is increased in patients with SLE, more antibodies to DNA and nucleosomes are induced (86). Additionally, clearance of apoptotic cells is abnormally slow in patients with SLE, permitting persistence of these surface autoantigens (126). Therefore, we have the scenario in which damage to cells caused by lupus autoantibodies drives those cells into apoptosis, and the apoptotic state itself promotes production of more harmful antibodies to DNA, to cardiolipin and to snRNP, thus creating a vicious circle. Furthermore, the combination of nucleosome and IgG antinucleosome acts on normal peripheral blood mononuclear cells (PBMCs) in vitro to induce the release of interferon-α (IFN-α) (127). Since IFN-α is immunostimulatory, it may play an additional role in keeping disease activated. Elevations of IFN-α–inducible genes in peripheral blood cells are a “signature” for SLE in humans (128 ,129). Normal human PBMCs cultured with polyclonal or monoclonal antibodies to DNA from patients with active SLE showed diminished proliferation and enhanced secretion of interleukin-1ß (IL-1ß), IL-8, TNF-α, and IL-10 (82). Polyclonal IgG anti-DNA from SLE patients incubated in vitro with human umbilical vein endothelial cells caused increased expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on those cells (130), which could be a mechanism for vascular damage. This is more evidence that additional activities of anti-DNA include influencing tissue damage by altering the release of pro-inflammatory cytokines or of molecules that mediate adhesion of potentially harmful phagocytic cells.

Direct Roles of T and B Lymphocytes in Cell Damage

Although we traditionally think of the role of B cells in lupus as a source of pathogenic autoantibodies, and those antibodies as being necessary for disease, B cells play an additional role. Medical research laboratory (MRL)/Fas lymphoproliferative (lpr) mice expressing a mutant surface Ig but unable to secrete Ig still developed nephritis. The disease was characterized by cellular infiltration within the kidney, indicating that functional B cells produce adverse local effects, either by contributing directly to damage or by presenting peptide antigens that activate cytotoxic T cells (131). The role of B cells as inciters of inflammation via antigen presentation that activates T cells was also suggested by the observation that patients with SLE who improved after treatment with anti-CD20 mAb had not only B cell depletion but also decreased surface markers of activation on circulating $CD4^+$ (helper) T cells, compared to nonresponders (132).

T lymphocytes in the setting of lupus lesions also play a role beyond providing help for local autoantibody production. Multiple T cells and macrophages infiltrate the kidneys of patients and mice with lupus nephritis (133). Chemotactic factors (MCP-1) and adhesion molecules (ICAM-1) facilitate recruitment of these cells into areas of inflammation. Once there, the cells induce increased expression of major histocompatibility complex (MHC,

HLA-D in humans) class II and CD40L on renal tubular epithelial cells, coupled with upregulation of CD40L and IL-2R on infiltrating T cells. Doubtless these properties of infiltrating T cells contribute to the renal damage of SLE.

The Importance of Toll-Like Receptors in Pro-Inflammatory Responses to Anti-DNA.

TLR were first recognized as activating receptors on dendritic cells (DC). Of the several types of TLR (134), TLR9 bind oligodeoxynucleotides (ODN), of which there are also several types. It is now known that B lymphocytes express TLR9. Recently a substantial body of evidence has developed showing that certain DNA/nucleosome/anti-DNA complexes can bind to TLR9, with subsequent activation of plasmacytoid DC and B cells, thus upregulating IFN type 1 and 2 responsive genes, and increasing the pro-inflammatory environment that favors development of clinical lupus (80 ,88 ,89). Interfering with this pathway [by knocking out IFN type 1 receptors (135) or occupying TLR with CpG ODN that are suppressive rather than activating] prevents or suppresses lupus-like disease in genetically susceptible mice (136).

The Structure of Anti-DNA

The structures of many human and murine mAbs to DNA have been determined (38 ,50 ,51 ,52 ,53 ,58 ,61 ,91 ,92 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148) and reviewed (146) (Table 23-2). As reviewed elsewhere in this volume, the heavy chains of Igs are encoded by rearrangements of variable (VH) region gene segments with diversity (D) and joining (JH) segments that combine with constant (CH) regions for the Ig isotype. Light chains are formed by rearrangements of VL and JL with C-κ or C-λ. Joining of the heavy and light chains forms an intact Ig molecule. Diversity in antibody binding is generated by (a) variations of the information contained in germline DNA within different individuals; (b) different rearrangements of V, D, and J segments for heavy chains, and of V and J segments for light chains; (c) addition of nucleic acids by terminal deoxyribonucleotidyl transferase (TdT) at the junctions between variable and constant regions in heavy or light chains; (d) junctional insertion of inverted mono- or dinucleotides that are independent of TdT; and (e) somatic mutations of single nucleic acids in rearranged heavy and light chains. Generally, antigen-driven antibody responses undergo increasing numbers of somatic mutations with each cell division, and such mutations increase the affinity of the antibody for the stimulating antigen. An additional modification, B cell editing, can occur after B cells are mature and “committed” to production of a given antibody. In this process, the B cell shuffles heavy and light chains until a combination that does not bind self with high avidity is found; that combination is then expressed on the cell surface (149 ,150). In a model of lupus nephritis induced by rendering mice transgenic for the H and L chains of a nephritogenic anti-dsDNA, the disease eventually disappeared, as did circulating anti-dsDNA, primarily because of B cell receptor editing (65).

Table 23-2: Structure of Anti-DNA

Genes encoding immunoglobulin
Germline DNA—no unique information
Rearrangement of germline
No unique rearrangements
Many different VH, D, JH, VL, and JL can be used
Enriched in VH J558 in mice, VH3 in humans
Somatic mutations—can be none, a few, or many
Amino acid sequences in heavy and light chains
CDR of heavy and light chains are enriched in Arg, Asp, and Tyr
Ser-Tyr found frequently in CDR1 of VH
Tyr-Tyr-Gly-Gly-Ser-Tyr found frequently in CDR3 of VH
Region gene segments: VH, variable heavy; D, diversity; JH, joining heavy; VL, variable light; JL, joining light.

In the mAb anti-DNA studied to date, no unique germline DNA encoding Ig is used to make anti-DNA. Most DNA-binding mAbs use rearrangements of germline DNA that are similar to those used in antibodies to foreign antigens, especially bacteria (16 ,17 ,51 ,64 ,137 ,138 ,140 ,141 ,142 ,151 ,152). Some mAb anti-DNAs are constructed from germline rearrangements with no or few mutations (153 ,154), but most IgG anti-dsDNA contain somatic mutations (50 ,51 ,61 ,104 ,137 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,150 ,151 ,152 ,153 ,154 ,155 ,156 ,157 ,158). There is one example of a murine MAb directed against phosphocholine (i.e., a dominant response in BALB/c mice to immunization with pneumococcal polysaccharide) that underwent a productive single-point mutation that eradicated the ability to bind phosphocholine but introduced the ability to bind DNA (137 ,142). Conversely, back-mutation experiments of two human nephritogenic anti-dsDNA showed that in one case a single mutation in an Asp residue in a CDR region of the H chain, and in the other case a small number of point mutations in the L chain caused the molecule to lose all capacity to bind dsDNA or nucleosomes (58). It seems likely that the resting IgM anti-DNA repertoire can be used to introduce somatic mutations that drive the immune response toward high-avidity IgG antibacterial or anti-DNA antibodies, and that high-avidity anti-DNA can arise from the mutation of antibacterial antibodies. These observations suggest that pathogenic IgG high-avidity anti-dsDNA can arise from stimulation with foreign antigens, such as bacteria, rather than from antigenic stimulation by mammalian self-DNA. This possibility is supported by the observation that immunization with phosphocholine coupled to protein carrier elicited anti-dsDNA antibodies in a normal mouse (152). Humans without SLE were immunized with pneumococcal vaccine (Pneumovax) and developed an antipneumococcal response that was characterized by a public idiotype found in patients with SLE; however, those

idiotype-positive antibodies did not bind DNA. In contrast, a lupus patient immunized with pneumococcal vaccine developed B cells that secreted antibodies specific for phosphocholine, for DNA, or cross-reactive with both (54).

Certain structural characteristics are common to many mAb anti-DNAs. Although many different V, D, J heavy-chain regions and many different light chains can be used to construct anti-DNA, there is repeated use of certain VH and CDR3 regions (e.g., VH J558 in NZB/NZW and MRL/lpr mice, VH3 in human MAb anti-DNA) (143 ,144 ,155 ,157 ,159 ,160 ,161). In fact, studies in MRL/lpr and NZB/NZW F1 lupus-prone mice suggest that each mouse makes autoantibodies from a small number of clonal B cells, which are then expanded and mutated to form anti-DNA (16 ,143 ,144 ,162).

To summarize, there is limited clonality of anti-DNA, but it is not stringent. Fifteen to 20 heavy-chain regions encode most anti-DNA. The CDR regions of heavy and light chains in anti-DNA often are enriched in arginine, asparagine, and tyrosine (16 ,143 ,162 ,163). These amino acids can form hydrogen bonds with the phosphate backbone of DNA. Studies of computer models and crystals of DNA/anti-DNA complexes have suggested that Arg, Asp, or Tyr project from CDR regions of the Ig molecule into the antigen-binding groove, where they can contribute to high-avidity binding of DNA (64 ,164). However, there are some anti-DNA in which these amino acids play little, if any, role in DNA binding. Although not an absolute requirement, several laboratories have noted that the D regions of anti-DNA antibodies are read in an unusual frame, and CDR3-D regions are long (165). Certain amino acid motifs occur commonly in mAb anti-DNAs, such as Ser-Tyr in CDR1 of VH and other sequences in the D region of the heavy chain. Our observation (38) that the CDR regions of VH in pathogenic anti-DNA compared with nonpathogens is enriched in arginine, lysine, aspartate, and glutamate residues (i.e., positively and negatively charged amino acids) suggests that charge interactions play a role in pathogenicity, possibly by determining tissue structures with which the mAb can cross-react. Arginine residues in CDR and some framework regions of heavy and light chain are important to DNA binding in some anti-DNA, as shown by analysis of mutants in which R residues have been substituted, resulting in loss of binding or changed avidity for DNA (91 ,92 ,166). On the other hand, there are some mAb anti-DNAs that do not have Arg residues in the positions thought to influence DNA binding. For many mAb anti-DNAs, the heavy chain determines the ability to bind DNA, although only certain light chains in combination with that heavy chain permit the binding to occur (166 ,167). Most murine and human IgG high-avidity anti-dsDNAs have undergone numerous somatic mutations, suggesting that specific antigens are driving B cell maturation and secretion (16 ,17 ,19 ,54 ,64 ,168 ,169).

In summary, there are no unique germline DNA sequences, heavy- or light-chain rearrangements, or amino acid motifs that are specific for anti-DNA. Many different heavy- and light-chain rearrangements can encode such antibodies. However, certain V region genes are used more frequently than would be expected, and certain amino acids or motifs are commonly found. The structural differences between pathogens and nonpathogens are not yet understood. Anti-DNA can arise spontaneously from germline DNA without antigenic stimulation; most IgG anti-dsDNAs are mutated and probably arise in response to many different antigenic stimuli as discussed above. Finally, there are experiments in which large quantities of anti-DNA are made and bind to glomeruli, but renal damage with clinical nephritis does not occur (49 ,71). They illustrate that at least some inflammatory pathways must be intact for immune deposition to produce tissue damage and disease.

The Origins of Anti-DNA: Genetic Predisposition, Antigenic, and Environmental Triggers

The ability of an animal or person to make pathogenic, high-titer IgG antibodies to DNA (or DNA/histone) is influenced by genes. Other chapters in this volume detail the genetic predispositions to SLE in humans and in mice; only the summaries will be given here, as they pertain to anti-DNA. In patients with SLE, Tsao et al. (170) showed positive correlation between IgG antibodies to nucleosomes and the 1q41 region on human chromosome 1, a region that also contains a gene or genes that predispose to SLE in mice. Several investigators have shown associations between human leukocyte antigen (HLA) molecules and anti-DNA in patients with SLE, specifically with HLA-DR3 (171), DR2 (172 ,173), and DR7 (174). Their respective linked HLA-DQ alleles may also responsible for the observed associations. In humans, a genome scan stratified for presence or absence of anti-dsDNA in SLE multiplex pedigrees showed linkages at chromosome regions 19p13.2 (in European Americans) and 18q1 (in African Americans) (175). The contributing genes in these regions are not known; candidate genes in or near 19p13.2 include DNase2, DNA methyltransferase 1, and calreticulin.

Studies in murine lupus have identified several candidate genes or loci associated with anti-DNA or antichromatin that occur on several different genes (176 ,177 ,178 ,179).

In mice with a New Zealand background, some of the ability to make anti-DNA depends on genes in the MHC class II region, with combinations such as H-2d/z being particularly susceptible (180). On chromosome 1 in mice with an NZW and NZB background (NZM2410), a gene region designated SLE1ab contains a gene or genes that allow a mouse to break tolerance to nucleosome (150 ,168 ,177) and produce anti-NUC. The gene region dysregulates STAT3 and ras-ERK signaling, resulting in increased IL-6 production by B cells and B cell hyperactivation associated with increased production of ANA (150). SLE1ab seems to be a critical gene; alone it cannot result in clinical immune nephritis, but when combined in a normal mouse with another predisposing gene, such as a gene related to T cell hyperactivity (SLE3) or an autoimmunity accelerating gene on the Y chromosome of another lupus mouse model (BXSB), Yaa, clinical nephritis

occurs in the majority of mice (178 ,179). In contrast, SLE3 plus Yaa do not produce clinical nephritis. The lupus-enhancing capacity of SLE1 is opposed by a gene region from an unaffected mouse (NZW), designated SLEls, located in or near the MHC region (179). The lbw2 gene on chromosome 4 (from NZB mice) in NZB × NZWF1 mice promotes B cell hyperactivity which in a mouse that can make anti-DNA results in more B cells making anti-DNA and more severe disease (181). In conclusion, in mice and humans, antibodies to nucleosome and DNA probably require more than one gene for full expression, as well as additional genes that promote development of clinical disease.

Potential Environmental Inducers of Antibodies to DNA

In addition to the genetic component, it is likely that certain environmental stimuli permit full expression of the gene, probably by initiating an immune reaction that then leads to formation of highly mutated and potentially pathogenic antinucleosome or anti-DNA. Table 23-3 lists the possible antigens that might elicit IgG anti-dsDNA pathogenic antibodies. Possibilities include the following: (a) anti-DNA is induced by DNA of bacteria or viruses and cross-reacts with self-DNA; (b) self-DNA becomes altered (e.g., by viral infection or exposure to ultraviolet light or the process of apoptosis) and therefore becomes immunogenic; (c) an antigen containing DNA plus protein is the initial immunogen, and some of the resultant reactivity happens to be directed toward naked DNA; (d) the initial immunogen is a peptide from another self-antigen, and the immune response spreads to produce multiple autoantibodies, including anti-DNA; and (e) the initial immunogen is not self but has a sequence or conformation similar to that found in self-antigens (such as EBNA1 from EBV virus), including DNA, and the resultant anti-DNA recognizes a similar conformation.

One thinks first of infectious agents, given the examples of single mutations in anti-DNA or in antiphosphochloline antibodies resulting in an antibody of the opposite specificity (137). It is likely that several bacteria and viruses can participate. Although initial antibody after immunization or mice with bacterial DNA is anti-ssDNA, only a few mutations, or recombinations between H and L chains, are required to develop anti-dsDNA (57). Some anti-DNA cross-react with *Klebsiella polysaccharides* (182), with *Escherichia coli* galactosidase (41), and with phospholipids of streptococci and staphylococci (40). Further, the structure of many mAb anti-DNA molecules is similar to that of antibacterial antibodies (137 ,151). Bacteria can supply DNA or lipopolysaccharides that induce anti-DNA (33 ,57 ,183). Pyun et al. (33) showed that immunization of normal mice with *E. coli* DNA induces IgM and IgG anti-DNA. We have mentioned previously that immunization with pneumococcal polysaccharide can induce antibodies to DNA in people who have SLE, and a single point mutation in antibody to pneumococcal polysaccharide can produce DNA-binding (34 ,137). Therefore, it is possible that some bacteria provide antigens that induce anti-DNA responses in humans. Bacterial DNA contains more hypomethylated regions (e.g., CpG) than mammalian DNA; hypomethylation of DNA can trigger autoreactive T cell activation and has been implicated in the pathogenesis of SLE (183 ,184). However, MRL/lpr lupus-prone mice raised in a germ-free environment were not protected from disease, although if they were fed an ultrafiltered antigen-free diet their disease was less (185). It is probably useful to think of certain bacteria as capable of flaring if not inducing SLE, and as inducing anti-DNA in genetically susceptible individuals.

Table 23-3: Possible Antigens

Inducing Anti-DNA
Nucleosomes
May be made available in surface blebs of lymphocytes, neutrophils, or glomerular cells undergoing apoptosis; also released from apoptotic, but not necrotic, cells; there is some evidence that nucleosomal DNA is the initial DNA-containing antigen recognized by the immune system
DNA/histone complexes, or DNA complexed with other proteins
Altered self-DNA (e.g., enriched in CpG motifs); this may occur when lymphocytes are activated
Bacterial DNA (either linked to protein, or forming complexes with Ig which activate plasmacytoid dendritic cells, or complexes which activate B cells directly via TLR9)
Viral infection (especially EBV, producing spreading of initial anti-viral antibody to include DNA-reactive antibody)
Bacterial polysaccharides
Phospholipids
May be made available on surfaces of cells undergoing apoptosis, or in bacteria
Other autoantigens (e.g., Sm, Ro, La or certain peptides associated with these particles)
B- and T-lymphocyte responses initially specific for immunodominant proteins in these Ags undergo degeneration and spreading so that the cell receptors recognize additional self-antigens, such as DNA
Protective immune responses to external antigens (e.g., antibodies to phosphocholine of pneumococcus also bind DNA)
Antibodies to external antigens with anti-Ids that bind DNA
Antibody to virus or bacteria may raise an antiidiotypic antibody that recognizes DNA; thus, protective response to an external antigen also generates autoreactive cells

There has been recent renewed interest in viruses as etiologic agents of SLE and/or its autoantibodies, including anti-DNA. An interesting observation in children with SLE showed that the chance of the patients being infected with Epstein-Barr virus (EBV) is about 50-fold higher than the chance in matched controls of children without lupus attending the same clinics (186). Although this virus is nearly

ubiquitous among adults in the United States, positive serology for EBV and antibodies to EBNA were significantly more frequent in adults with SLE compared to controls (187), and several other investigators confirmed the finding that EBV-infected cells and/or antibodies are more common in SLE patients than in controls (188 ,189). Furthermore, expression of EBNA-1 in normal mice elicits anti-DNA production (190). Another virus that might be involved is polyomavirus, which has been carefully studied in SLE by one group (191 ,192 ,193). Polyoma virus has a transcription factor T antigen that as immunogen induced antibodies to DNA in mice. Activation of that virus, with release of T antigen, is frequent in patients with SLE. T antigen circulates in sera of some patients, complexes with nucleosomes, and stimulates proliferation of $CD4^+$ T lymphocytes from both normals and lupus patients. These $CD4^+$ T cells could help syngeneic B cells produce anti-DNA and anti–T antigen. This interesting work awaits confirmation from other laboratories. In another example of a potential lupus-inducing virus (194), BK virus administered to rabbits induced antibodies against self eukaryotic DNA if the infection became productive, but not if it was contained, again suggesting that activation of a virus is key to providing the antigens required to induce anti-DNA.

Pristane is another external substance that can induce anti-DNA and lupus when injected into genetically susceptible strains (195). BALB/c mice so treated develop IgG anti-DNA and antichromatin, as well as IgG antibodies to nRNP, Sm and Su. Immune complex disease develops in glomeruli. Human exposure to pristane is exclusively by the oral route, and there is as yet little evidence that pristane induces lupus in people.

Antigens from Self Molecules May Induce or Sustain Antibodies to DNA

The interesting observation of Arbuckle et al. (196) that autoantibodies precede the onset of clinical SLE in most patients by as much as 9 years, and that anti-DNA are among the earliest to appear, suggests that people destined to develop clinical SLE either modulate anti-DNA over time to introduce more pathogenic subsets, or fail to regulate autoantibodies normally, or both. Given the recent evidence that EBV infection may be an environmental trigger for SLE (187 ,188 ,189 ,190 ,197), it is possible that EBNA antibodies may easily mutate to anti-DNA, although to our knowledge that has not been demonstrated directly. However, the human Sm B/B' protein contains a highly stimulatory, proline-rich sequence that is nearly identical to an amino acid sequence in EBNA. When used as an immunogen in mice, rabbits, or nonhuman primates the Sm peptide induces T cell activation, then B cell responses to that peptide, then to related peptides, then to unrelated peptides on the same molecule, then to unrelated peptides on different molecules, culminating in IgG anti-dsDNA and proteinuria (198). Under conditions of oxidative damage, e.g. during inflammation, this epitope spreading is even more extensive (199). Similarly, immunization with a peptide from the protein SmD1 complex induced both anti-Sm and anti-DNA in the NZB/NZW F1 lupus model, activated helper T cells and accelerated disease (200 ,201). Similar epitope spreading resulting in production of anti-DNA has been demonstrated after immunization with other external and autoantigens, including pneumococcal polysaccharide and Ro. This topic has been reviewed recently (202).

Another autologous source of antigens that promote development of anti-DNA in individuals predisposed to SLE is the autoantibodies themselves. Several groups (203 ,204 ,205 ,206 ,207) have shown that certain peptides derived from autoantibodies, presented in MHC class II molecules by B cells or other APC, are recognized by $CD4^+$ helper T cells. Those T cells are activated by the peptides, and in turn activate B cells making Ig anti-dsDNA. Thus, an antibody to dsDNA provides its own peptides that activate T cell help for that B cell, resulting in sustained, rather than regulated production of a pathogenic autoantibody. T cell stimulatory peptides have been demonstrated from the VH and VL areas of antibodies to DNA. These T cell determinants occur primarily in autoantibodies, with similar regions at similar locations in the molecule in both murine and human anti-DNA (208). It seems likely that autoantibodies persist and resist downregulation when they contain effective T cell determinants, and B cells with those IgG receptors are thus selected for survival.

Finally, histones associated with DNA also contain peptides that are T cell determinants—determinants that activate T cell help for IgG anti-dsDNA production in both humans and lupus-prone mouse strains. Since many histones have amino acid sequences that are conserved across many species, the T cell determinants that stimulate murine and human cells are similar (209).

In summary, although nucleosomes are effective antigens in initiating antibodies that bind dsDNA, responses to multiple external and self antigens can also lead to T cell help and B cell production of IgG anti-dsDNA–containing populations that are pathogenic, particularly in a permissive genetic environment.

Apoptotic Cells as a Source of Immunizing Autoantigens

It is likely that many of the autoantigens that induce lupus autoantibodies, including pathogenic subsets, arise from apoptosis of autologous cells, particularly T and B lymphocytes. The surfaces of such cells contain blebs, released into tissues as apoptotic bodies, within which lie nucleosomal DNA/histone complexes of the size found in sera of patients with SLE (but not controls) (113 ,114 ,115). In a preceding section we discussed the role of anti-DNA/DNA complexes in activating DC and B cells, which expand inflammation and autoimmunity. Apoptotic blebs also contain the snRNP, Ro and La antigens that stimulate autoantibodies in some patients. In addition, the membranes of cells undergoing apoptosis

change conformation in a way that permits the antigenic “heads” of phospholipid molecules to flip toward the outside of the membrane, where they can be recognized by the immune system. Apoptotic cells administered as immunogens to mice induce antibodies to DNA, RNP, Ro, and cardiolipin (85). Furthermore, patients with SLE have larger numbers of lymphocytes and neutrophils undergoing apoptosis than do matched healthy or diseased controls, and they have significantly less ability to phagocytose apoptotic bodies (86 ,87 ,126). The normal removal of apoptotic bodies requires interactions between the bodies, complement components, serum amyloid P, and phagocytic cells. Genetically engineered normal mice with genes encoding C1q or serum amyloid protein (SAP) “knocked out” develop SLE-like disease with increased numbers of apoptotic bodies in tissues (210 ,211 ,212). It is reasonable to conclude that any condition that permits increases in apoptotic cells or allows them to persist longer than normal predisposes to SLE and to the production of antibodies to nucleosomes and DNA.

Immunoregulatory Abnormalities That Permit Sustained Production of Pathogenic Anti-DNA in SLE

Helper T cells are critical to SLE. They drive the IgM to IgG switch that enhances pathogenesis of autoantibodies, and they sustain autoantibody production and disease. Individuals with SLE have the ability to make pathogenic subsets of anti-DNA and either the ability to continually upregulate antibody production, the inability to downregulate it, or both. Chapter 9 reviews these mechanisms in detail. In the NZB × NZW F1 mouse model of SLE, nephritis does not develop until young mice switch their IgM anti-DNA production to IgG anti-DNA (213). A major role of helper T cells in stimulating the Ig class switch has been shown (214), and, in fact, elimination of helper T cells virtually prevents disease in both the NZB × NZW and BXSB models of lupus (215,216,217). $CD4^+$ and T cells have been cloned from NZB × SWR F1 mice that can make B cells from that animal secrete cationic IgG anti-dsDNA (218). The T cell receptors of those clones are enriched in positively and negatively charged amino acids in both T cell receptor (TCR)-α and TCR-ß chains (219,220), and some of the T cells are activated by nucleosomes (221). Pathogenic anti-DNAs react with DNA/histone and are enriched in charged amino acids in regions that contact antigen; many antigens with which they react are highly charged. Thus, charged amino acids in peptides from anti-DNA molecules are likely to be presented to TCRs containing oppositely charged amino acids. In that way, T cell help is activated for antibodies to DNA. T cell help is provided by many different types of cells in murine and human SLE. Helper T cell phenotypes may be , , or $CD4^-CD8^-$, and TCR may be α/ß or γ/δ. If we understood why these cells are all skewed toward help, without normal regulation, we would be closer to understanding this disease. It is clear that a T cell or B cell initially specific for a single self-antigen soon stimulates other T and B cells that greatly broaden the repertoire of reactivity toward self. This happens by two mechanisms: T- and B cell degeneracy, in which the receptor on one cell can bind many antigens of different sequences; and T- and B cell epitope spreading, in which increasing numbers of cells with different but related specificities are recruited into an active state. As a result, the autoimmune repertoire can become quite diverse and a given patient can have autoantibodies to many apparently unrelated antigens (222,223,224).

Immune tolerance is a mechanism that controls autoreactivity in normal individuals. There are several mechanisms of tolerance. In central tolerance, T cells in the thymus and B cells in the bone marrow are eliminated if they have high avidity for self antigens, expressed within those organs. Some such cells “leak” into the periphery, where they also encounter tolerance mechanisms. Those include (a) deletion (often a consequence of cell activation with strong signals engaging TCR or BCR), (b) anergy, a state in which first signals are received by lymphocyte TCR or BCR but the cells neither activate nor die (this is common when second signals via CD28 and CD40 are not present, which can occur in the presence of tolerized dendritic cells which do not express the CD80, CD86 and CD40L ligands for second signaling), (c) induction of regulatory cells (either NK-T cells, $CD4^+$, or $CD8^+$ T cells), and B cell receptor editing of autoreactive BCR. In normal mice transgenic for various mAb anti-DNAs, B cells expressing these antibodies are subject to all known tolerance mechanisms (225 ,226 ,227). In contrast, B cells are resistant to tolerance in lupus-prone mice (228 ,229 ,230). Induction of regulatory T cells, which are ineffective in lupus-prone mice, can overcome the B cell abnormalities of lupus mice. For example, activation of NK-T cells can prevent murine lupus (231). Regulatory T cells of the $CD4^+CD25^+CTLA4^+$ subsets prevent autoimmunity, including lupus-like disease, in normal mice. Experiments have suggested that all mice are predisposed to autoimmunity, which is only held in check by natural, thymic-educated regulatory $CD4^+CD25^+$ T cells (232 ,233 ,234). $CD4^+CD25^+$ T cells are low in number in patients with SLE (235) and their function is defective in some models of murine lupus (236). These Treg can be stimulated by appropriate peptides, or by in vitro incubation with IL-2 and TGF-ß and thereafter they can suppress autoantibodies and disease (236 ,237 ,238 ,239). Similarly $CD8^+$ T cells are ineffective suppressors of autoantibody-producing B cells in murine and human lupus (231 ,240 ,241). However, several strategies, including immunization with peptides from anti-DNA, can be used to induce/activate $CD8^+$ T suppressor T cells in murine lupus, and these cells are capable of suppressing anti-DNA and nephritis (242 ,243). Work is in progress to adapt these regimens that induce immune regulation to human SLE.

Solving the puzzle of the absence of function in regulatory T cells is an important challenge to lupus investigators.

In summary, APC, B cells, and $CD4^+$ helper T cells are hyperactive in humans and mice with SLE. In contrast, regulatory networks designed to downregulate high antibody

responses (idiotypic networks, NK T cells, $CD4^+CD25^+$ Treg, $CD8^+$ suppressor T cells, and tolerogenic APC) are underactive. As a result pathogenic autoantibodies are made in high titer and persist long enough in time to cause organ damage (205). Each of these abnormalities is discussed in detail in other chapters, and a recent review is available (243).

Structure of DNA

DNA occurs primarily as a B helix in solutions with physiologic tonicity and pH. Figure 23-1 shows the structure. Two polydeoxyribose-phosphate backbones spiral around central base pairs of purines and pyrimidines. The backbone is readily available to react with antibodies; the bases are contained in major and minor grooves. The binding sites of an anti-dsDNA can straddle the backbone and interact with bases in those grooves.

Chapter 22 shows the structure of nucleosomes.

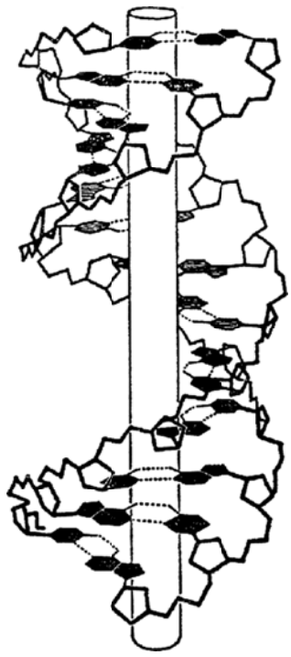

Figure 23-1. The DNA molecule in B helix conformation. Purine and pyrimidine bases in the center of the helix are solid black. Pentose sugars in backbone are open pentagons. Phosphate groups are along the heavy black lines on the outside of the helix. In SLE, anti-DNA may react with nucleosides, oligonucleotides, nucleotide sequences, or the sugar-phosphate backbone. (From Adams ROP, ed. *Davidson's The Biochemistry of the Nucleic Acids.* New York, NY: Academic Press, 1976 , with permission.)

Specificities of Anti-DNA for the DNA Molecule

Anti-dsDNA can react with base pairs, including dG-dC and dA-dT. Anti-dsDNA can recognize the ribosephosphate backbone alone or particular conformations in dsDNA (167 ,182 ,244 ,245 ,246). Multiple different conformations occur: dsDNA may form twisted, supercoiled, closed circular molecules; relaxed circular forms; left-handed Z-DNA segments; and cruciform structures. These polymorphisms are associated with different base-pair sequences and with the physicochemical characteristics of the environment. It is not known which of these reactivities is associated with the most pathogenic antibodies. However, variation in methylation of DNA, particularly with increased numbers of deoxymethylcytosines, increases the immunogenicity of DNA. GC-rich regions can be nuclease resistant, and these combinations increased immunogenicity. Such a combination is found in nucleosomal DNA in apoptotic cells (183 ,247).

When dsDNA is denatured, compact single chains are formed, which can present bases, nucleotide sequences, backbone, short regions of base-paired secondary structures, and short helices. Anti-ssDNA therefore can react with bases, nucleosides, nucleotides, oligonucleotides, and ribosephosphate backbone. Anti-ssDNA react predominantly with individual bases and even better with polynucleotides containing multiples of that base. The largest proportions of anti-ssDNA in sera react with guanosine and thymidine, but others recognize polyA, polyC, polyI, Z-DNA, ssRNA, RNA-DNA hybrids, and poly (adenosine diphosphate (ADP) ribose) (244 ,245).

Anti-DNA induced by immunization with bases, nucleosides, or oligonucleotides usually is highly specific for the immunogen. In contrast, spontaneous anti-DNAs that arise in individuals with SLE usually are polyspecific and react with nucleosomes, multiple oligonucleotides and several forms of DNA. This wide reactivity could result from similar epitopes shared by multiple different molecules (probably conformational) or a single antibody-combining site having multiple contact regions for unrelated epitopes. An example of shared epitopes would be the cross-reactivity of many mAbs for DNA and cardiolipin (44 ,248). There are shared phosphodiester groups in the ribosephosphate backbone of DNA and in phospholipids. The role of bacteria, viruses, apoptotic materials and autoantigens in inducing anti-DNA is discussed above.

When the structure of DNA is altered by cell activation or inflammation, it may become immunogenic. Structural differences might account for bacterial DNA inducing anti-DNA in humans. Krieg et al. (249) showed that unmethylated cytosine phosphyrol guanine (CpG) motifs in bacterial DNA can directly trigger B cells to proliferate and secrete antibodies. Because these motifs are more abundant in bacterial or viral genomes than in mammalian genomes, this property may confer on vertebrates an advantage to fight off microbial infection. These findings also suggest that the increased levels of hypomethylated CpG in DNA of patients

with SLE may play a pathogenic role in inducing a polyclonal B cell activation. Additionally, hypomethylated CpG motifs in T cells promote their activation (183 ,184).

Desai et al. (250) showed that mammalian DNA (usually a weak immunogen) becomes strongly antigenic when combined with a highly charged fusion protein. This might be similar to the association of DNA with histones in nucleosomes, and nucleosomes may initiate a series of dendritic cell, B cell and T cell events that culminate in production of IgG anti-dsDNA, as discussed in an earlier section. DNA found in the circulation of patients with SLE is small in size (100 to 150 base pair [bp]), and units of 200 bp and their multiples (113 ,114 ,115). DNA of these sizes is usually found in nucleosomes. Cell apoptosis releases DNA of this size; cell necrosis does not. Therefore, in SLE, cell apoptosis may be induced by immunologic abnormalities, and the nucleosomes released from those cells provide the antigenic stimulus that results in somatically mutated, high-affinity IgG anti-DNA antibodies.

The last hypothesis—that anti-DNA recognizes a conformation shared with the original, unknown immunogen—has been used to explain the ability of an anti-DNA to recognize molecules with no known structural similarity to DNA (251). This hypothesis will require progress in studies of three-dimensional conformations for verification. To date, one anti-ssDNA has been crystallized (164), but there are no reports of crystals containing anti-dsDNA with antigen.

DNA may be different in individuals with SLE from that in normal individuals. The quantities of DNA released by cultured lymphocytes from humans and mice with SLE are significantly greater than the quantities released by normal cells (252 ,253). The circulating DNA in patients with SLE also is richer in guanosine and cytosine than DNA from healthy controls (254), and in hypomethylated regions (183 ,184). Such changes could increase the immunogenicity and ability to form Z-DNA.

Degradation of DNA

The degradation of DNA may be slower than normal in individuals with lupus, because their nucleases may be impaired. Based on studies in normal mice (253), DNA probably is cleared in two phases. First, large pieces of DNA (more than 15 bp) rapidly bind to various organs (primarily the liver for ssDNA and other soft tissues for dsDNA). Second, DNA is degraded in the circulation and in tissue by nucleases. ssDNA is cleared more rapidly than dsDNA (20 minutes vs. 40 minutes, respectively); the second, digestion phase of clearance is similar for both.

Several conditions may prolong the half-life of DNA. Hepatic uptake of DNA is saturable with excess DNA or excess immune complexes, which results in a prolonged half-life. If DNA is present in small size, organ binding is impaired. In patients with active disease, excess quantities of circulating DNA and of small immune complexes have been detected; therefore, clearance of antigenic DNA is probably impaired (253).

Another factor that allows DNA to persist in patients with SLE is its existence as protected fragments (255). Small DNA fragments (30 to 40 bp), are bound by the two arms of IgG anti-DNA and thus protected from both organ binding and the action of nucleases.

DNA may serve as a target antigen beyond its participation in immune complexes. There is a DNA receptor on a number of cells, including glomeruli (256 ,257), and DNA bound into that receptor might well be a target of pathogenic anti-DNA. Additionally, the ability of DNA or DNA/histone to be implanted in tissues, particularly collagen IV, laminin, and heparan sulfate in basement membranes, may target antibodies to organs that are damaged in patients with SLE (35 ,38 ,42 ,43 ,104 ,105 ,108 ,118 ,257).

In summary, DNA, DNA/histone complex, or small immune complexes containing protected fragments of DNA may persist for abnormally long periods in individuals with SLE for a variety of reasons. Such a situation could result in prolonged antigenic stimulation and in the availability of DNA to bind to target tissues or to circulating anti-DNA, thus perpetuating the disease process.

DNAse-1 is the major nuclease present in serum, urine, and secretions that degrades DNA, particularly at sites of high cell turnover. DNAse-1–deficient mice were generated. They developed ANA and Ig deposition in glomeruli, with clinical glomerulonephritis (258). These observations, coupled with the information that levels of DNAse-1 in plasma of patients with SLE are usually low, led to clinical studies in which recombinant human DNAse-1 was administered to patients with SLE. The trial contained 17 individuals who received a single IV dose of 25 to 125 μg per kg of recombinant human DNAse-1 followed by ten subcutaneous doses or placebo. Serum DNAse-1 concentrations achieving hydrolytic activity of DNA sustained for a few hours after IV, but not subcutaneous administration. Serum antibodies to DNA were unchanged, as were cytokine levels of soluble interleukin-2R (sIL-2R), IL-6, IL-10, and TNF-α (259).

Polymorphisms in genes encoding DNAses that are associated with antibodies to DNA and lupus nephritis in patients have been described by one group (260 ,261).Mutations of DNAse-1 gene resulting in premature termination of mRNA have been reported in two Japanese SLE patients supporting the association between low activity of DNAse-1 and the development of SLE in patients (262).

The Characteristics of Immune Complexes Containing DNA and Anti-DNA and Their Clearance

Immune complexes containing DNA have been found in the circulation of as many as 50% of patients with active SLE, depending on the sensitivity of the method (114 ,263 ,264). The DNA in these immune complexes probably consists primarily of nucleosomes (113 ,115 ,263 ,264). For other immune complexes, small soluble complexes in slight antigen excess that can fix complement are the most pathogenic.

For DNA, complexes in slight antibody excess may be the most stable and therefore most pathogenic (264 ,265 ,266). The complexes should be bound by Fc and CR1 receptors and cleared by the mononuclear phagocyte system. However, during periods of active SLE, clearance of immune complexes is defective. Chapter 12 discusses the complex abnormalities. Abnormalities include a combination of high quantities of complexes, decreased numbers of receptors, saturation of available receptors, and defective phagocytosis of the complexes fixed to receptors (267 ,268). Genetic abnormalities in quantities of CR1 receptors have been reported (268), but the low numbers on cells of patients with SLE also may reflect stripping of occupied receptors. No mutations in CR1 or in Fc receptors have yet been found in patients with SLE. However, polymorphisms in FcγRIIA and RIIIA have been associated with SLE; those polymorphisms probably encode molecules less able to bind Ig strongly compared to other alleles (269 ,270 ,271 ,272). There is also defective FcγRIIB, a receptor which is a downregulator of B cells (273). Therefore, inheritance of inefficient-binding-alleles of these Fcγ receptors could impair the clearance of most immune complexes that cause disease in SLE. IgG1 is usually the most abundant isotype in glomerular Ig deposits. As discussed above, immune complexes containing cationic antigen or antibody, or protected DNA fragments that cannot be bound by cells that clear DNA, are probably important pathogens. These features stabilize the complex and permit its persistence.

Methods of Measuring Antibodies to DNA

Several techniques are available to measure anti-DNA in serum or plasma (274 ,275 ,276 ,277 ,278) (Table 23-4). There is no universal standardized assay used by all service laboratories, in contrast to antibodies to cardiolipin, for example. Therefore, the techniques used by each service or research laboratories are critical to accurate interpretation of test results. Some assays detect only high-avidity subsets of anti-DNA that are enriched in pathogens; these tests have low sensitivity but strong association with nephritis and clinical disease activity. Others detect both low- and high-avidity anti-DNA; these are more sensitive but less likely to be associated with clinical correlations. The following tests measure primarily high-avidity antibodies: precipitation, complement fixation, and the Farr assay. A second group of tests detects both high- and low-avidity antibodies: ELISA, *Crithidia luciliae,* hemagglutination, and radioimmunoassays with precipitation of DNA-containing immune complexes by polyethylene glycol (PEG) assay. In general, the first group is less sensitive than the second, being positive in only 50% to 60% of patients with SLE, but correlates better with disease flares and high risk for glomerulonephritis. The second group is more sensitive, being positive in 70% to 85% of patients with SLE; however, they correlate less well with disease activity and manifestations. These differences account for some of the discrepancy in the literature regarding the clinical utility of anti-DNA testing.

Table 23-4: Methods of Measuring Anti-DNA

Test	Sensitive	Specific	Correlates with Disease Activity and Nephritis	Availability
Precipitation	+	+++	+++	Poor
Hemagglutination	++	+	+	Poor
Complement fixation	++	+++	+++	Poor
Farr assay	++	+++	+++	Fair
PEG assay	++	++	+	Poor
Crithidia luciliae immunofluorescence	++	+++	++	Good
ELISA	+++	++	+	Good
EliA	+++	++	++	New

ELISA, enzyme-linked immunosorbent assay; PEG, polyethylene glycol.

The tests most commonly used in service laboratories are Farr, ELISA, and *C. luciliae*. In the Farr assay, radiolabeled DNA is added to diluted serum or plasma, and Ig is precipitated by ammonium sulfate. Radioactivity in the precipitate indicates binding of DNA by anti-DNA; unbound DNA is not precipitated. DNA can be purified to contain primarily dsDNA, although a few single-strand nicks develop during the assay. This assay has limited availability because of the need to use radiolabeled materials. Both IgG and IgM high-avidity precipitating antibodies are captured in the Farr assay, which probably is the best of the available tests to detect primarily high-avidity antibodies to DNA. In a recent placebo-controlled prospective clinical trial of a oligonucleotide/tetramer tolerogen (LJP394, Riquent, Abetimus) in patients with lupus nephritis, responders were found primarily in the group with anti-DNA that bound the tolerogen with high avidity, and most of the patients who improved were in the groups with falling titers of anti-DNA by Farr assay, whether they received active agent or placebo (279 ,280). This illustrates the important relationship of

high-avidity IgG antibodies to dsDNA in some people with lupus nephritis, and the utility of the Farr assay.

In the ELISA assay, DNA is bound to wells in plastic microtiter plates. In general, ssDNA sticks directly to the wells. The adherence of dsDNA is variable, and some laboratories coat the wells with negatively charged molecules such as protamine sulfate, poly-l-lysine, or methylated bovine serum albumin before dsDNA is added to ensure uniform entrapment of the antigen. However, there are commercial microtiter plates available to which dsDNA adheres well, without requirement for addition of charged lining molecules. Diluted patient plasma or sera are incubated with DNA in the wells. After several hours, the wells are washed and the bound Ig incubated with enzyme-labeled antihuman Ig, IgG, or IgM. The binding of the second antibody is detected by a color change following addition of a substrate on which the enzyme acts, the color being read in a spectrophotometer. This assay allows measurement of both low- and high-avidity antibodies and the detection of total IgG, IgG isotypes, IgM, and total populations of anti-DNA. The substrate fixed to the wells can be highly purified dsDNA (which can be from mammalian or bacterial sources); a few single-strand nicks develop during the incubations. Commercial substrates often contain significant quantities of ssDNA as well as dsDNA; therefore, low quantities of anti-DNA detected in this assay may be primarily directed against ssDNA. Clinical correlations are best if they are confined to the interpretation of high titers of IgG anti-dsDNA detected in this manner. There is considerable variability in the sensitivity and specificity of various manufacturers' ELISA kits for the measurement of anti-DNA and other autoantibodies (274 ,275 ,276). It is therefore most useful to both clinicians and researchers if assays are done repeatedly in the same laboratory using the same procedure or kits from the same sources.

Several groups have analyzed the association between antibodies to nucleosomes (measured in ELISA) and clinical correlations. They have noted strong association between IgG3 anti-DNA, disease activity, and nephritis in their lupus patients (109 ,110), and in general have reported that these antibodies are more sensitive and specific for change in disease activity. However, the test is not widely used. These examples illustrate the importance of every detail of ELISA assays, beginning with the antigen used, in influencing results. International standardization of these assays would be welcome.

The *C. luciliae* test takes advantage of the presence of a kinetoplast containing circular dsDNA in this flagellate. Test sera or plasma are incubated with the organisms on a glass slide. After washing, fluoresceinated antihuman Ig, IgG, or IgM are added. After appropriate incubation, Ig bound to the dsDNA structure is detected by examining the glass slide for fluorescence using an ultraviolet microscope. Although even this circular DNA structure can contain one or two single-strand nicks, this assay is the most specific for detecting antibodies against pure double-stranded DNA. Therefore, positive tests are highly specific for SLE. However, in some laboratories, the sensitivity is not as good as the ELISA assay. The Crithidia assay can be modified to detect complement-fixing antibody subsets, but in the standard test both low- and high-avidity anti-dsDNA are measured. In some comparative studies, antibodies to DNA by Crithidia assay are similar to those detected by ELISA or Farr in terms of specificity, sensitivity, and prediction of disease flares (278). The assay is technician-dependent, because there is an autofluorescent structure in the flagellate that can be confused with the dsDNA-containing kinetoplast.

In summary, several different assays for anti-dsDNA are available commercially; each has advantages and disadvantages. No standardized test is used uniformly in all service laboratories. The ideal anti-DNA assay would detect all IgG high-avidity, complement-fixing anti-dsDNA; however, none of the assays available in most service laboratories does so. Therefore, the physician should determine which assay is used in the laboratory to which the specimens are sent and understand the specificity, sensitivity, and clinical correlations of that method. With methods that detect most populations of anti-dsDNA (and some anti-ssDNA), weakly positive tests can be obtained in patients with chronic liver disease, rheumatoid arthritis, nonlupus connective tissue diseases, drug-induced lupus, infections, and aging.

Measurements of Antibodies to dsDNA: Utility for the Clinician

Detection of anti-dsDNA often is useful in the diagnosis and management of patients with SLE (Table 23-5). Their interpretation is limited by lack of a standardized assay to measure them, inability to equate results of assays with the most pathogenic subsets of anti-DNA, and the fact that antibodies other than anti-DNA participate in the tissue lesions of SLE. Antibodies to ssDNA should not be measured, because they have no specificity for the disease.

Table 23-5: Clinical Utility of Measuring Anti-DNA

High titers of anti-dsDNA
Have approximately 90% specificity for SLE
Often indicate clinically active disease and increased risk for nephritis
Low titers of anti-dsDNA
Can be detecting anti-ssDNA
Can be found in
Drug-induced lupus
Rheumatoid arthritis
Sjögren syndrome
Other CTD
Chronic infections
Chronic liver disease
Aging

CTD, connective tissue disease.

Tests for antibodies to dsDNA are useful in establishing the diagnosis of SLE; 60% to 83% of patients with SLE have

positive tests for these antibodies by the Farr, Crithidia, or ELISA assays at some time during their illness (274 ,275 ,276 ,277 ,278 ,281). The presence of anti-dsDNA fulfills a criterion for the American College of Rheumatology classification of patients as having SLE (282 ,283).

The ability of tests for anti-dsDNA to predict exacerbations of clinical disease, or various organ involvements, is controversial. Some studies suggest strong correlations between increasing levels of these antibodies and subsequent disease activation (281 ,284). Other studies suggest that such correlations are weak (285 ,286). A minority of patients have high titers of IgG anti-dsDNA for prolonged periods of time without developing exacerbations of disease or glomerulonephritis (287). In general, when tests for anti-dsDNA are performed at regular intervals using the same methods and laboratories, rising titers suggest increased risk for disease exacerbation, particularly of nephritis and/or vasculitis (207 ,281 ,284). Sometimes renal flares are preceded by drops in anti-DNA, probably because the antibodies are leaking into the urine. It is likely that combinations of laboratory changes, particularly rising anti-DNA and falling complement levels, are better than either one alone. It comes as no surprise that the clinician must combine results of careful laboratory testing with symptoms and signs to make correct therapeutic decisions. Currently there is an aggressive search for improved biomarkers of active, worsening or improving disease in patients with SLE (207).

In summary, measurement of anti-dsDNA in sera has two useful clinical applications (Table 23-5). First, high titers of these antibodies have a specificity of more than 90% for SLE; therefore, they are useful in making the diagnosis. Second, rising levels should alert the clinician to the possibility of an imminent disease flare, and high levels (especially associated with low levels of serum complement) suggest increased risk for lupus nephritis or vasculitis. We recommend that in each patient with SLE, the physician establish whether the pattern of serum anti-DNA titers correlates with clinical manifestations and disease activity. If such correlations are present, serial measurements of anti-DNA are useful.

Future Directions: Targeting Antibodies to DNA in Therapy of SLE

Many effective, widely immunosuppressive therapies for SLE such as glucocorticoids, mycophenolate and cytotoxic drugs are associated with falls in titers of anti-dsDNA that precede or accompany clinical improvement. There has been considerable interest in developing therapies that specifically target pathogenic autoantibodies and leave the normal immune repertoire intact.

There has been some success in experimental studies with LJP394 (Riquent, Abetimus), which is a DNA tolerogen (279 ,280). LJP394 is a molecule in which four oligonucleotides are held on a tetrameric scaffold. The idea is to cross-link two Ig anti-DNA receptors on B cells; this gives a weak first signal without a second signal, so that the B cells are anergized (not activated) and stop producing anti-dsDNA. Administration as a toleragen was effective in BXSB lupus-prone mice. Recently, in a clinical trial in patients, the number of flares of nephritis was significantly reduced in the patients tolerized with weekly intravenous injections of LJP394 compared to those who received placebo—in the subset of patients with anti-dsDNA that bound the toleragen with high avidity (279). The toleragen was given once a week intravenously. A follow-up study is in progress (2006)p.

Other attempts to reduce anti-DNA antibody as disease treatment have included immunoadsorption of anti-DNA using columns designed to specifically trap anti-DNA and immune complexes (288 ,289 ,290). Passing SLE serum over phenylalanine or human anti-DNA or dextran sulfate columns reduced anti-DNA titers and was associated with clinical improvement in at least half of patients. Administration of DNAse-1 was not effective in initial studies. The future of therapies that target certain autoantibodies is exciting, since many of the autoantibodies of SLE are interconnected, and suppressing one might have beneficial effects on quantities of the others.

Finally, there has been some success in reducing antibodies to DNA by administering peptides from the VH regions of murine or human anti-DNA (207 ,291). These peptides induce regulatory/suppressor T cells which delay the appearance of autoantibodies and nephritis. One of the peptides, Edratide, inhibits anti-DNA production by human SLE cells transferred to SCID mice (291). It is currently in clinical trials.

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Chapter 24

Antibodies to Histones and Nucleosome-Related Antigens

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Discovery of Histone-Reactive Antibodies

Histone-reactive antibodies played a major role in the demonstration almost six decades ago that systemic lupus erythematosus (SLE) is an autoimmune disease. The initial recognition that autoimmunity underlies diseases such as SLE is generally attributed to the observations of Hargraves et al. (1) on the lupus erythematosus (LE) cell phenomenon in which nuclear material derived from dead cells is phagocytosed by neutrophils in vivo. This phenomenon was reproduced in vitro by Hamburger (2) and Miescher and Fauconnet (3) by mixing normal neutrophils with SLE plasma, and Holman and Deicher (4) showed that the responsible factor in SLE plasma was an antinuclear antibody (ANA). Absorption of SLE sera with calf thymus nucleohistone and histone-DNA complexes but not with pure DNA greatly reduced the LE-cell phenomenon (5), suggesting that the specificity of this ANA was related to histone-reactive antibodies. Several groups showed that histones were necessary for maintaining the antigenicity of nuclei for the LE cell factor (4 ,6 ,7), and direct demonstration of the antigenicity of free histones was obtained by Holman et al. (8) and Kunkel et al. (9) using a complement-depletion assay. Thus, histone-reactive antibodies hold a preeminent position in the recognition of the autoimmune nature of SLE and lead to the discovery of many other ANA.

Much of the subsequent, older work on histone-reactive antibodies in SLE was limited to histone-containing nucleoproteins. Histones complexed with sonicated DNA were shown to form immunoprecipitins with SLE sera using classic Ouchterlony analysis (10). Many SLE patients had complement-fixing anti-DNA and anti-(histone-DNA), which tended to increase along with clinical disease (11). Various nuclear immunofluorescence patterns (rim, fibrillar, homogeneous) were attributed to antibodies to nucleoprotein (10 ,11). Over 50% of SLE sera had antibodies to soluble nucleoprotein that were not removed by absorption of sera with DNA (12), but the fine specificity of these autoantibodies was not determined until years later (13). It was generally inferred from these early studies that antibodies that bind nucleoprotein are antibodies to histone-DNA complexes, and this specificity is equivalent to the LE-cell factor. Studies by Hannestad et al. (14 ,15) suggested that a complex of histones in the absence of DNA possessed antigenic activity for the LE factor. More recently it has been shown that antibodies to histone H1 are primarily responsible for LE cells (16 ,17).

Histone Organization in the Cell

Histones are normally found in eukaryotic cells associated with DNA and organized in a huge macromolecular structure called chromatin, which is the native form of nucleohistone in the cell. In addition to DNA and histone which constitute 80% of its mass, chromatin contains non-histone proteins, RNA and other macromolecules. Many constituents of chromatin are targets for autoantibodies in SLE—histones, DNA, RNA, and proteins such as the centromere proteins, nucleolin and high-mobility group proteins (18). Histones and DNA constitute the repeat subunit of chromatin called the nucleosome (19 ,20), and the linear array of nucleosomes held together by a continuous strand of approximately 2×10^7 base pairs of DNA forms the polynucleosome, the primary chromatin fiber of approximately 100ö in diameter. This thin filament has a tendency to supercoil into higher-order solenoid-like structures of increasing diameter (21), compacting the DNA 5,000- to 10,000-fold as depicted in Figure 24-1 . In a typical chromosome approximately 7 millimeters of DNA is condensed to 1 micron by the organizing capacity of about 100,000 nucleosomes.

The nucleosome consists of two molecules of each of the “core” histones, H2A, H2B, H3, and H4, along with one H1 molecule and approximately 200 base pairs of DNA. Acid-solubilized histones are monomeric; the five major polypeptides can be distinguished by polyacrylamide gel electrophoresis and can be separated by gel permeation chromatography (Fig. 24-2). DNA of 146 base pairs wraps with 1.75 superhelical turns around an octamer of histones H2A, H2B, H3, and H4, and this core particle is connected to the adjacent core particle by a segment of the continuous stand of DNA designated as the linker. All the histones participate in limited contacts with DNA at 10 base pair

intervals wherever the minor grove of the DNA double helix faces the histone core (22 ,23 ,24), resulting in 14 regions on the twisting DNA anchored to arginine side chains of the histones (25). These multiple interactions account for the stability of the nucleosome and constrain the DNA to curve tightly around the octamer. Histones H1 or H5 appear to bind close to the exposed face of one of the two H2A molecules, creating asymmetry in the nucleosome that provides a ramp where DNA exits and is directed to the adjacent nucleosome (26). The amount of linker DNA shows wide interspecies and even inter-tissue variation so that nucleosomes may contain from 166 to 231 base pairs of DNA.

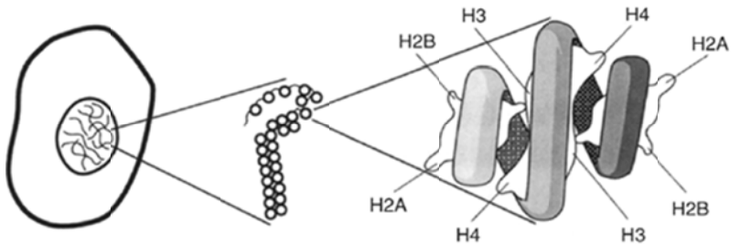

Figure 24-1. Higher ordered structure of histones in chromatin. On the right is a diagrammatic representation of the core particle of the nucleosome in which two turns of DNA wrap around an octamer of histones consisting of two H2A-H2B dimers flanking an H3-H4 tetramer. Separation of dimers from the tetramer is exaggerated for clarity—the native core particle is a compact structure stabilized by non-covalent bonds (25,27). Association with H1 completes the nucleosome (not shown), and an array of polynucleosomes supercoil into a solenoid-like structure depicted in the middle diagram. Additional supercoiling into even higher ordered structures is presumed to take place until chromatin fibers are eventually visible in the light microscope as mitotic chromosomes.

Digestion of chromatin with micrococcal nuclease preferentially cuts the less protected internucleosomal, linker DNA; H1 tends to dissociate and is removed by treatment with 0.5 M NaCl (Fig. 24-3). Unlike native chromatin, H1-stripped chromatin is soluble in physiological solutions. Partial digestion of chromatin with micrococcal nuclease produces a mixture of core particles, nucleosomes and oligonucleosomes, resulting in a “ladder” representing DNA of various sizes. The natural tendency of histones to interact with each other is of fundamental importance in the stability of the core particle, and dissociation of DNA by 2.0 M salt releases the histone octamer. Decreasing salt concentration causes dissociation of the component H3-H4 tetramer from the two H2A-H2B dimers (28). An artificial complex between the H2A-H2B dimer and DNA can be formed in vitro at physiological conditions; this complex is an important antigenic target in patients with SLE and drug-induced lupus.

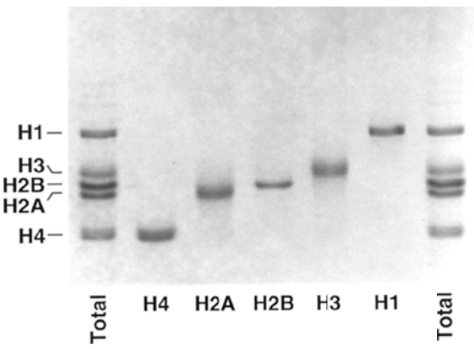

Figure 24-2. The five major histone classes. Total histones were derived by acid extraction of calf thymus chromatin and subjected to SDS polyacrylamide gel electrophoresis (29). The individual histone classes can be purified on a preparative scale by gel exclusion chromatography (30).

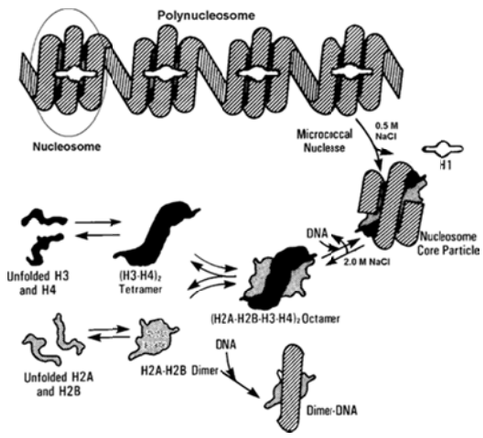

Figure 24-3. Dissociation of the polynucleosome. The nucleosome core particle is released by digestion of chromatin with micrococcal nuclease and treatment with 0.5 M NaCl. DNA can be reversibly released from the core particle in 2.0 M NaCl, producing the core histone (H2A-H2B-H3-H4)2 octamer. Decreasing the salt concentration causes dissociation of the component H3-H4 tetramer from the two H2A-H2B dimers; at very low salt concentration or low pH the individual, monomeric histones are produced. The (H2A-H2B)-DNA complex is stable in physiological conditions.

Structure of the Histones and Their Variants

The amino acid sequences of all five histones and numerous variants from many animals and plants have been determined (31 ,32). Histones H3 and H4 are two of the most conserved proteins (33)—bovine H3 and H3 from the pea plant differ by only 4 of 135 amino acids, and bovine and pea H4 are identical except for two conservative amino acid substitutions. Human and calf H4 are identical including the presence of three posttranslationally modified residues (34). The primary structure of human spleen H3 is identical to calf thymus H3 except for serine in place of cysteine at position 96 and for the presence of two minor variants in human H3 (35). The primary structure of H2B also displays remarkable similarity in the C-terminal half of the molecule among a wide variety of animals, but considerable variation in the amino acid sequence of the N-terminal half of H2B has been detected within the animal kingdom (32). However, human spleen H2B is identical with calf thymus H2B except for two minor variants in human H2B, each of which has a single amino acid substitution (36). The entire molecule of H2A appears to be more highly conserved than H2B. Calf thymus H2A is identical with human spleen H2A except for a minor variant having a histidine deletion at residue 123 or 124 (37). The vast majority of histone variants are identical among mammalian species, suggesting their importance in the biology of the cell. Because of this near identity between human core histones and their counterpart from calf thymus (the material of choice for chromatin extraction), bovine histones can generally be considered as valid autoantigens for analyzing human autoantibodies.

The H1 class of histones is considerably more complex and heterogeneous. An internal segment of approximately 70 amino acids displays extensive homology among the animal H1 histones sequenced, but the N-terminal 40 residues and C-terminal half of the protein show substantial primary structure divergence (32). H1 includes at least eight variants which differ in primary structure, and many of these, especially human, have only recently been sequenced or identified only by deduction from the nucleotide sequence of cloned genes. Mammalian H1° is present mainly in terminally differentiated, quiescent cells and shows extensive homology with histone H5, which is found in phylogenetically lower organisms and is generally isolated from avian nucleated erythrocytes. Human H1° and chicken H5 share eight essentially identical segments of 6–21 amino acids if only one amino acid deletion, addition or substitution is allowed per segment (38). However, these histones show less than 10% homology with the main H1 class (39). The nomenclature for the H1 family of histones has not been formally standardized, so structural comparisons between species are presently ambiguous. However, all the H1 histones including H1° and H5 share a nearly identical 16 amino acid long region in the globular domain (human H1.1 94–108, human H1.2 91–105, human H1° 79–94, bovine H1.1 91–106, chicken H5 80–95) as well as a highly conserved octapeptide in the C-terminal domain (human H1.1 151–158, H1.2 153–160, and H1° 152–159, chicken H5 148–155) (40). The significance of the H1 variants has been the subject of intensive study for years and appears to be related to the role of H1 in affecting the higher order structure of chromatin and, consequently, the overall transcriptional activity of the genome. Although extensive antigenic cross-reactivity can be expected among the H1 variants, the complexity of this histone class and the difficulty in physically separating and identifying these variants presently confounds the interpretation of studies involving autoantibodies to the H1 family of histones.

The different histone classes have very limited amino acid sequence similarities (41 ,42), but these similarities may account for some cross-reactions among antihistone antibodies. H2A shows homologies with H4, H2B, and H3, each in different parts of the H2A molecule, an octapeptide in H3 is similar to a decapeptide in H4, and H3 and H2B share an identical tetrapeptide (see (43) for sequence). Although all the histones are rich in basic amino acids, especially in their amino terminal regions, extensive, uninterrupted strings of such residues are generally not observed. There is a pentapeptide in H2B and H4 consisting of all basic amino acids, but tandem arrays of greater than three lysine, arginine or histidine residues do not occur in any histone, including H1. This may explain why naturally occurring antihistone antibodies display only weak or negligible cross-reactivity with basic homopolypeptides.

Within each class of histones, numerous subtypes or variants have been identified (44). Variants occur among different biological species as well as within a species, such as between stages of development, among different tissues and even within a homogeneous cell type. This heterogeneity in primary structure can be the result of transcription from multiple genes for a histone class with slightly different coding sequences or because of postsynthetic modifications. Posttranslational modifications occur on only a portion of the total histone pool of a cell or tissue, resulting in a variety of subtypes being present simultaneously.

Phosphorylation

Phosphorylation occurs in the N-terminal region on certain serine residues of all histones, threonine on H3 and H1, histidine on H4 and lysine on H1. H1 is extensively phosphorylated on both the N- and C-termini, especially during the S phase of cell division (45). Phosphorylation reduces the net positive charge and is thought to alter histone-DNA interaction (46), but whether this disruption of DNA-histone interactions contributes to the enhanced template activity of chromatin is unknown.

Acetylation

From two to four lysine residues may be acetylated in histones H3, H4, H2A, and H2B, as is the amino terminus of H2A, H4 and H1 (45). Unacetylated protruding amino terminal tails may make contact with DNA and/or histone of the adjacent nucleosome (25), thereby “locking” the polynucleosome into a higher order structure (Fig. 24-2) that suppresses transcriptional activity. Transcription factors associated with histone acetyltransferase activity resulting in added acetyl groups to the protruding histone tails neutralize the positive charge of the reacting residues, thereby disrupt this compact structure, allowing access to the transcriptional machinery (47).

Methylation

A portion of all the histones is methylated at certain lysine residues and probably at the guanidine nitrogen of arginine. Methylation is a relatively stable modification and occurs after DNA synthesis in the late S or G2 phase after chromatin biosynthesis, suggesting that this modification is not essential for the assembly of chromatin. On the other hand, methylation has been suggested to be important in chromatin condensation, other mitotic events, and gene transcription.

Poly(ADP-Ribosylation)

The covalent addition of an ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD) to proteins is catalyzed by poly(ADP-ribose) polymerase (48). The major acceptor proteins for poly-(ADP-ribose) are H1 and H2B although ribosylation of H2A, H3and H4 also have been reported. This modification has been postulated to play a role in DNA replication and repair, cell differentiation and apoptosis (49). An increase in the activity of poly-(ADP-ribose) polymerase has also been correlated with the appearance of DNA strand breaks produced by nucleases and other agents (50). This is interesting in light of observations that metabolites of drugs that are associated with drug-induced lupus induce extensive DNA strand breakage (51) and increase poly(ADP-ribose) polymerase activity in certain cells (52).

Table 24-1: Structural Domains within the Histones*

Histone	Number of Residues in Parent Molecule	Major Trypsin Resistant Domain	
		Residue Number	Designation
H3	135	27–129	P1
H2A	129	12–118	P2
H2B	125	21–125	P2
		24–125	P3
H4	102	18–102	P4
		20–102	P5
H1	212–222	36–121	P0 (G-H1)

*Compilation is based on (62) and refers to calf thymus histones. Histones are arranged in order of increasing electrophoretic mobility of the trypsin-resistant domain.

Ubiquitination

Approximately 10% of calf thymus H2A is covalently associated at lysine 119 with ubiquitin, a protein of 76 amino acids, resulting in a variant of H2A possessing an electrophoretic mobility equivalent to approximately twice the size of the parent molecule (53 ,54). Approximately 1% of H2B is ubiquitinated at lysine 120 (55). Poly-ubiquitinated H2A is a chain of at least four ubiquitin molecules bound to the amino group of H2A lysine 119 (56). Although ubiquitination in the cytoplasm tags proteins for degradation by the proteasome (57), ubiquitin in histones appears to be important in docking transcription factors or maintaining the structure of transcriptionally active chromatin (58).

Significance of Primary Structure Variation

Clearly, both very subtle variants as well as highly altered forms of histones have been identified among higher organisms. Even single amino-acid differences in histone variants could potentially be recognized immunologically (59), and side-chain modifications such as ribosylation and ubiquitination are profound. Autoantibodies to poly-(ADP-ribose) (60) and to ubiquitin (61) have been reported in SLE, but since these modifications are found on many other proteins, their relationship to antihistone antibodies is unclear. The significance of posttranslationally modified histones as well as of the histone variants with different amino acid sequences in the elicitation of antihistone antibodies or the antigenicity of the targeted histones is unknown.

Domain Structure of Histones

Histones in chromatin (but not the individual proteins) show a remarkable resistance to trypsin digestion considering their high content of lysine and arginine, the amino acids immediately N-terminal of the specific cleavage site of trypsin. Table 24-1 shows the trypsin-resistant “limit digest” products can be separated by sodium dodecyl sulfate (SDS) gel electrophoresis and their compositions. Since similar polypeptide products are observed after trypsin digestion of

nuclei, chromatin or core particles but not of histones in low ionic strength environments, resistance is attributed to protection of potential cleavage sites by the tertiary or quaternary conformation of the histones in the nucleosome. DNA does not afford principle protection of histones from trypsin because the DNA-free octamer in 2 M NaCl displays a similar resistance to trypsin digestion. In fact, the trypsin-sensitive sites are the highly positively charged regions previously (incorrectly) believed to be stably bound to the DNA phosphates: the 11–30 amino acid residues of the N-terminal ends of the four core histones and the C-terminal 11 residues of H2A and six residues of H3.

Biophysical studies on trypsinized core particles indicate that they are largely indistinguishable from their native counterparts (63). The trypsin-sensitive tails are highly mobile with no stable conformation or binding sites on DNA, although they have been suggested to provide relatively non-specific electrostatic shielding of DNA phosphates, stabilizing higher order chromatin structure (64). Apparently, these domains are accessible to the enzymes involved in histone posttranslational modification because almost all the sites for phosphorylation, acetylation, methylation and ubiquitination are at the trypsin sensitive regions. It has also been claimed that these domains comprise the predominant epitope for antihistone antibodies in SLE, but as discussed in later section, significance of antihistone fine specificity is an oversimplification.

All the H1 variants including H5 and H1° bear a similar three-domain structure as previously described. The highly conserved central globular domain is involved in the critical function of redirecting the DNA as it exits the core particle, and the flanking regions which are rich in the basic amino acids arginine and lysine presumably play a role in internucleosomal interactions involved in chromatin condensation into higher order structure (26). Histone H5 possesses an unusually large arginine-rich C-terminal domain believed to condense chromatin to the transcriptionally inactive form present in avian erythrocytes. The otherwise closely related H1° retains the lysines in the C-terminal domain characteristic of the main H1 family, but is associated with chromatin in terminally differentiated cells that have only selected genes that are transcriptionally active. The highly conserved 16-mer in the globular domain and octamer in the C-terminal region of all members of the H1 family are presumably involved in critical structural/functional roles. Although autoantibodies to H1 have been reported frequently (see the later section on H1), the domain structure and the complexity of the H1 family complicate interpretation of these data.

Assays for Antihistone Antibodies

To appreciate data on the clinical and pathologic significance of histone autoantibodies, an understanding of the methods that are used to demonstrate them is important. In most clinical laboratories, an indirect immunofluorescence (IIF) assay is used as a screening test to identify ANA (65). In SLE most histone autoantibodies can be detected by this test and are commonly correlated with a homogeneous or diffuse IIF staining pattern (Fig. 24-4). However, this technique commonly produces misleading information regarding antihistone antibodies. The homogeneous or diffuse pattern also is seen with other autoantibodies, notably those directed against dsDNA (66). Additionally, sera that have antibodies to certain histone classes (e.g., H1, H3, H4) or hidden determinants (cryptotopes) on native or on denatured histones may show only weak or negative ANAs (67 ,68 ,69 ,70). Therefore, the identification of histone antibodies must rely on more specific assays.

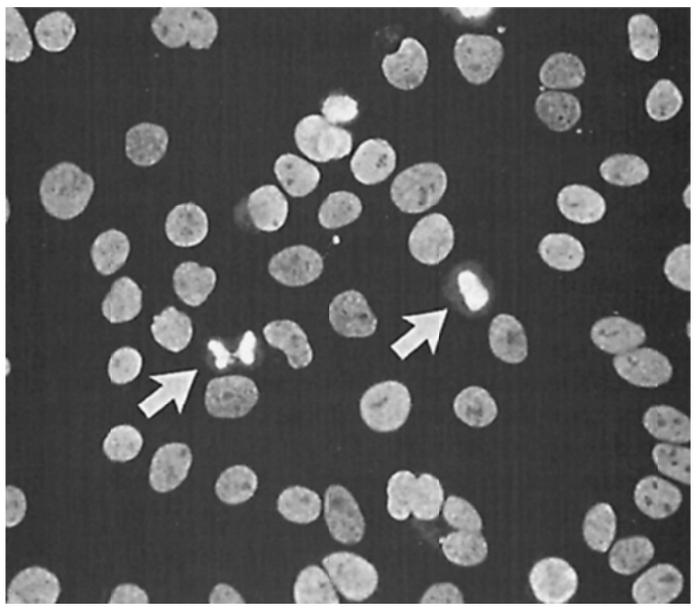

Figure 24-4. Indirect immunofluorescence of autoantibodies from a patient with SLE produces a homogeneous pattern of nuclear staining on human epithelial (HEp-2) cells. Note that staining is particularly intense over chromosomes of dividing cells (arrows) and that the cytoplasm displays no detectable staining.

Historical Perspective

The first assay for antihistone antibodies was an adaptation of the microcomplement fixation method using lysis of sensitized red blood cells as the indicator (9 ,71). Antihistone antibodies were reported to be infrequent in SLE using this assay (72) and reactive predominantly with histone H1, although a few sera displayed binding to all histones (71). It was also inferred from these and other studies (10 ,11 ,12 ,73) that a complex of histones with DNA was required to generate antigenicity. The stringent requirements of this assay (74), the procomplementary nature of histones (75) and its apparent insensitivity (76) have led to abandoning the complement-fixation assay for detecting antihistone antibodies and a general disinterest in antihistone antibodies.

Renewed interest in antihistone antibodies followed the modification of the IIF assay by Tan et al. (77) to render

it specific for histone-binding antibodies. Histones were extracted from mouse kidney tubule nuclei by 0.1 N HCl, preventing the binding of antihistone antibodies. The histone content of such nuclei can be partially "reconstituted" by incubation with a pure histone solution. Sera with antihistone activity display binding to histone-reconstituted nuclei and to untreated mouse kidney but not to acid-extracted sections. This assay was used to demonstrate that high titers of histone antibodies are found in patients with SLE (78 ,79) and procainamide-induced lupus (79). While this assay is highly specific for antihistone antibodies, it cannot be used with sera that contain anti-DNA antibodies, which display background binding to acid-extracted nuclei. It later became clear that the assay had limitations in that sera with antihistone activity predominantly to H3 and H4 displayed negligible reaction in the immunofluorescence assay (70 ,80), presumably because of the failure of these histones to bind to DNA with this technique. Thus, the immunofluorescence assay is largely selective for antibodies reactive with H1, H2A, H2B, and may preferentially detect epitopes requiring a histone-DNA complex.

Current Assays for Antihistone Antibodies

Solid-phase assays have largely replaced older assays for antihistone antibodies. Histones, histone complexes, and chromatin readily adsorb to polystyrene, and antibody binding can be quantified with a class-specific enzyme-, fluorescent-, or radio-labeled anti-immunoglobulin. The first solid phase assays detected antibodies to histones raised in rabbits (81 ,82), in human SLE (80 ,83 ,84) and in murine lupus (85). Most studies on antihistone antibody in the past two decades have relied on purified histones in solid phase enzyme-linked immunosorbent assays (ELISA). Subnucleosome structures have also been adapted to ELISA formats (86), which allow measurement of autoantibodies requiring these higher-ordered structures. Antihistone antibodies can also be measured by immunoblots (Western blots) of histones separated by SDS polyacrylamide gel electrophoresis. However, this technique may not detect autoantibodies reacting with native epitopes on histones and cannot detect antibodies to epitopes generated by histone-histone or histone-DNA complexes. More recently addressable laser bead immuno-assays have been used to detect chromatin antibodies.

Problems and Discrepancies in Measuring Antihistone Antibodies

Discrepancies in the literature on the prevalence and fine specificity of antihistone antibodies are common (see next section). The quality of histones used as antigens is highly variable, and commercial sources were often degraded or contaminated with nonhistone proteins. Inhibition of endogenous proteolysis during histone isolation is necessary to obtain intact histones, as shown in Figure 24-2 . It is difficult to remove all traces of DNA, a serious problem when assessing sera that may also contain anti-DNA antibodies. Additionally, the propensity for histones to bind DNA in biological fluids can result in artifacts such as false-positive reactions with anti-DNA antibodies (see below). Commercial sources of chromatin (nucleosome-related antigens) are not currently available, so assays for antibodies to the higher ordered structures in nucleohistone which are common in SLE are more difficult to develop in the laboratory. Other technical concerns common to many immunoassays such as differences in procedures to determine the cutoff between normal and pathologic sera, diagnostic criteria, ethnic or regional variation in patients, prescribed therapeutic agents (especially the use of corticosteroids which may suppress immune responses), and secondary antibodies as detecting reagents contribute to interobserver variation. A collection of normal human sera generally establishes the cut-off value used to assign an experimental sample to a positive category, but borderline values are always difficult to interpret in quantitative immunoassays, and these low level reactivities may sometimes be a result of increased nonspecific binding as a result of elevated gamma globulins commonly observed in sera from patients with rheumatic diseases. Finally, most studies use histones or chromatin prepared from calf thymus or chicken erythrocytes; ideally, human histones should be used for studies of true histone autoantibodies although, as discussed above, there is near identity between human core histones and their counterpart from calf thymus. As with many other areas in clinical laboratory medicine, attempts to standardize these variables through inter-institutional collaboration are a mandate of the Serology Subcommittee of the International Union of Immunological Societies.

Artifactual Antihistone Antibodies

Special problems arise when measuring antihistone antibodies in complex biologic fluids. Three features of this antigen-antibody system contribute to ambiguous results: (1) histones have a net positive charge and readily bind soluble polyanions such as DNA in physiologic medium or sulfated macromolecules on membranes, (2) DNA and/or histones are not uncommon contaminants of antibody-containing fluids or of other components of the assay, and (3) anti-DNA and antihistone activities commonly co-exist in SLE sera. Soluble DNA in human serum can be responsible for false-positive antihistone antibody signals by forming a macromolecular bridge between anti-DNA antibodies and histone bound to the solid phase. These interactions result in anti-DNA antibody binding to histones, indistinguishable from bona fide antihistone antibody activity (see Antilymphocyte Antibodies). This phenomenon can be clearly seen when harvesting tissue culture supernatants from a hybridoma secreting anti-DNA antibodies (87). DNase treatment of the antibody preparation removes the antihistone activity, consistent with the involvement of DNA in generation of artifactual antihistone activity.

Similar artifacts can occur in serum. Subiza et al. (88) demonstrated that DNase pretreatment of SLE sera resulted

in a significant decrease in antihistone antibody activity in 7/11 sera. Affected sera invariably had anti-DNA antibodies (whose activity increased after DNase digestion) and the histone binding activity could be regenerated by addition of DNA. These results would suggest that a significant portion of the antihistone activity in SLE may actually be a result of DNA-anti-DNA immune complexes. However, Suzuki et al. (89) observed a DNase effect on the antihistone activity in only 27% of SLE sera; this decrease was relatively small and uniform across all histone classes. Therefore, it appears that measurement of antihistone activity in carefully prepared samples of SLE sera gives largely valid results even in the presence of anti-DNA antibodies.

Nucleohistone in bovine milk (a common blocking medium) or serum can bind to histone bands transferred to nitrocellulose, introducing artifacts (90). Histones were isolated from bovine serum and milk by affinity chromatography on DNA-cellulose (91), and this material mediated the binding of antihistone antibodies to solid phase DNA (87). This type of artifact was recently confirmed with nucleosome-reactive autoantibodies obtained from a peptide phage display library due to DNA and histones in the milk blocking agent (92). Indirect evidence strongly suggests that DNA exists in serum in the form of mono- and oligonucleosomes (93 ,94), which can be immunoprecipitated with antihistone antibodies (94). Therefore, blocking media from these natural biological fluids should be avoided when measuring antihistone antibodies. This type of phenomenon may also have pathologic significance as suggested by the report of Schmiedke et al. (95) that circulating nucleohistone binding to the negatively charged residues on heparin sulfate in the glomerular basement membrane (GBM) may mediate the binding of DNA and anti-DNA antibodies to the glomerulus.

Recently, Teodorescu and associates have explored the basis for binding of immunoglobulin from SLE and rheumatoid arthritis (RA) sera to solid phase histone in ELISA formats. Surprisingly, much of the histone binding of IgG from SLE sera was eliminated by digestion with pepsin (96), which removes the Fc region of IgG, inconsistent with the manner by which bona fide antibodies bind to antigen. Immune complexes, common in SLE and the Felty syndrome variant of RA, also displayed a propensity for binding solid phase histone (97 ,98), and immune complexes are also affected by pepsin digestion. While the histone binding of immunoglobulins from RA sera correlated with immune complex and rheumatoid factor activity (98), the bulk of the histone-binding activity in SLE sera resided in the monomeric IgG fraction (99). This suggested that IgG with anomalous features was responsible for much of the antihistone activity in SLE sera. While these observations raise serious concerns about the nature and significance of antihistone antibodies, autoantibodies reacting with chromatin/nucleosomes bound strictly through the classical antigen combining site (99). Further studies will be required to gauge how widespread this anomalous antibody reactivity is and to evaluate its impact on the measurements and characteristics of antihistone antibody activity, including the reports described below.

Terminology

There is considerable confusion in the literature regarding terminology for histone-reactive antibodies. The term “antihistone antibody” should be reserved for assays that employ purified, monomeric histones such as Western blot and some solid phase assays; it can be assumed that the bulk of the “auto-epitopes” in purified histones are expressed in denatured regions of histones because DNA-free histone does not exist in vivo. As discussed below, antibodies to denatured purified histones detected by most assays are common and have little diagnostic value. Other assays which use histone-DNA complexes, including LE cells, fixed cells, deoxyribonucleoprotein, chromatin, soluble (H1-stripped) chromatin, (poly)nucleosomes, and (H2A-H2B)-DNA complexes would more likely be measuring auto-epitopes in native regions. It is possible that there is overlap of epitope content between denatured histones and native (DNA-bound) histones especially in some assays (77). However, for the most part antibodies to histones and antibodies to nucleosome-related antigens should be considered distinct. In the context of autoimmunity the terms chromatin, nucleosome, and polynucleosome can be used interchangeably (100).

Prevalence and Disease Association of Antihistone and Anti-Nucleosome Antibodies

Overall Disease Association

Table 24-2 lists reports of antihistone antibodies in various diseases. Although antihistone antibodies have been observed in a variety of (generally rheumatologic) diseases, most studies have focused on SLE or DIL. Reported prevalences ranged from 17% to 95% in SLE (average = 51%) and 67% to 100% in DIL (average = 92%). Antihistone antibodies have also been consistently observed in RA (average prevalence = 11%) and juvenile chronic arthritis (JCA) (average prevalence = 51%). RA patients with ANA were 2 to 7 times more likely to have antihistone antibodies than ANA-negative RA patients (137 ,138 ,169). Other than SLE in which other ANA commonly co-exist, the bulk of the ANA in sera with antihistone activity is probably due to these antibodies, although this relationship has been formally demonstrated only in DIL and DIA (170). There have been several recent reports on histone-reactive antibodies in a substantial proportion of patients with scleroderma-related disorders especially localized scleroderma with generalized morphea (124 ,153 ,154).

Limited studies on antihistone antibodies in various other syndromes have appeared. In some cases a remarkably high occurrence of antihistone antibodies was observed, especially in primary biliary cirrhosis (159 ,160), autoimmune hepatitis (162), ANA-positive neoplastic diseases (136) and Felty syndrome. Patients with undifferentiated connective tissue disease displayed predominately IgM anti-H3 producing a variable large speckled ANA pattern (69 ,171). There have been isolated case reports of unusual

antihistone antibodies such as anti-H1° in patients with sensory neuropathy (172 ,173) and antibody to chymotrypsin digested H2B in a patient with vasculitis (68). While antihistone antibodies have been observed in rheumatic diseases such as Sjögren syndrome, mixed connective tissue disease, and vasculitis, these tend to be of low titer and represented by few reports. Antihistone antibodies in polymyositis/dermatomyositis have been reported in 17% of 46 patients (158), predominantly reacting with H1. Anti-H2B antibodies were reported in over half of HIV+ patients with persistent lymphadenopathy (167) or patients with squamous cell carcinoma (166), but these results have not been reported elsewhere. In a study of 249 patients with monoclonal gammopathies, Shoenfeld et al. (165) found that 34 (14%) were positive for histone antibodies, and 11/12 purified monoclonal antibodies demonstrated antihistone activity.

Table 24-2: Reported Prevalence of Antihistone/Anti-Nucleosome Antibody in Human Disease

Disease/Syndrome	Prevalence*	References
Rheumatic diseases		
SLE	24%-95%	(13,13,84,89,89,89,89,101,101,102,103,104,104,104,105,106,107,108,109,110,111,112,113,114,115,116,117,118,118,119,120,121,122,123,123,124,125,126,127,128)
DIL	50%-100%	(70,79,106,118,129,130,131,132,133,134,135,134)
DIA	22%-95%	(106,130,131,132,134,135,136)
RA	0%-80%	(117,137,137,138,139,140,141)
Vasculitis	31%-75%	(68,140,142)
Felty syndrome	79%	(137,143)
JCA	42%-75%	(67,141,144,145,146,147,148,149,150)
MCTD/UCTD	45%-90%	(69,108,122,128,140)
Sjogren syndrome	8%-67%	(140,151)
PSS	23%-67%	(122,123,124,126,152,153,154,155,156,157)
PM/DM	17%	(158)
Other diseases		
PBC	50%-81%	(111,159,160)
HC/AH	35%-50%	(159,161,162)
IBD/UC	13%-15%	(163,164)
Neoplastic diseases	14%-79%	(136,165,166)
MG	N.D.	(165)
HIV/AIDS	N.D.	(167,168)

DIL, drug-induced lupus; DIA, drug-induced ANA; RA, rheumatoid arthritis; JCA, juvenile chronic arthritis; MCTD, mixed (undifferentiated) connective tissue disease; PSS, progressive systemic sclerosis disorders; PM/DM, polymyositis/dermatomyositis; IBD/UC, Inflammatory bowel disease/ulcerative colitis; MG, monoclonal gammopathy; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; HC/AH, hepatic cirrhosis/autoimmune hepatitis; PBC, primary biliary cirrhosis, N.D., not determined
*Prevalence of elevated IgG and/or IgM antibody to total histone or to at least one histone class.

In addition to the occurrence of antibodies reactive with isolated histones, patients with lupus-like disorders commonly have autoantibodies to chromatin (nucleosomes) (Table 24-3). The first report found that approximately three fourths of untreated SLE patients from Singapore had anti-nucleosomal antibodies (13); somewhat lower prevalence and titer were observed in (American) SLE patients under medication treatment (13). Anti-nucleosome (and anti-nDNA) appear to be particularly sensitive to corticosteroid therapy (186). The bulk of this reactivity was a result of binding to the (H2A-H2B)-DNA complex in the higher-ordered structure of chromatin. Other studies from various locations throughout the world reported 38% to 86% sensitivity of antinucleosome antibodies in SLE (Table 24-3). Most patients with lupus induced by procainamide, penicillamine, isoniazid, acebutolol, methyldopa, timolol, and sulfasalazine also have anti-nucleosome antibodies, predominately reactive with the (H2A-H2B)-DNA complex, although a substantial number of patients have only been examined with lupus induced by procainamide. Approximately half the patients with drug-induced lupus related to quinidine and hydralazine had anti-([H2A-H2B]-DNA). Several groups reported anti-nucleosome antibodies in almost half the patients with autoimmune hepatitis (187 ,188). Antinucleosome antibodies have also been reported in 25% (124) to almost 50% (122 ,189) of patients with PSS (scleroderma), but the specificity of these antibodies may be different from bona fide antichromatin

antibodies as discussed below. Antinucleosome antibodies have generally not been seen to any significant extent in other rheumatic disease patients and are remarkably low in the normal population, resulting in an overall sensitivity for SLE of 63% and specificity for SLE compared to other rheumatic diseases of 95% (100).

Table 24-3: Prevalence of IgG Anti-nucleosome antibodies in Idiopathic and Drug-Induced Lupus

Disease	Antinucleosome Antibody Prevalence	Reference
SLE, oriental, untreated	78%	(13)
SLE, American, treated	59%	(13)
SLE, Spanish	69%	(151)
SLE, Mexican	100%	(128)
SLE, French	72%	(122,174)
SLE, German	56%, 62%	(123,125)
SLE, Korean	76%	(175)
SLE, Italian	38%, 86%	(126,176)
SLE, Hungarian	39%	(177)
SLE, English	60%	(178)
SLE, Irish	64%	(127)
SLE, Czech	73%	(179)
Procainamide-induced lupus	96%	(132,180)
Lupus induced by penicillamine, isoniazid, acebutolol, methyldopa, timolol, sulfasalazine	N.D.	(180,181,182,183,184,185)
Quinidine-induced lupus	53%	(132,180)
Hydralazine-induced lupus	43%	(132)

N.D., not determined

Systemic Lupus Erythematosus

Studies on the association of antihistone antibodies with disease activity or severity, with the predominant organ system affected or with specific clinical features have been inconsistent. Some reports showed no association between presence or amount of antihistone antibodies and any measure of disease activity (78 ,103 ,106 ,139 ,140 ,186) with the exception of a history of photosensitivity in one report (140) and joint disease in another (78). On the other hand, in one of the earliest reports on histone-reactive antibodies, Rothfield and Stollar (11) observed that 14 out of 15 patients with active lupus contained antibodies to histone-DNA complexes, whereas only 4 of 26 patients in remission had these antibodies. Subsequently, associations of antihistone antibodies with active disease were observed with solid-phase (110) and histone-reconstituted immunofluorescence assays (136). Population correlations of the presence of antihistone antibodies with neuropsychiatric involvement (190), skin and joint symptoms (84 ,109) or overall disease severity have been reported (104). However, no quantitative association has been observed between the level of antihistone antibodies and any specific array of clinical symptoms or the overall disease activity. Perhaps variants of SLE characterized by increased disease severity commonly display antihistone antibody because of the general linkage between symptoms and signs in SLE. This view is supported by the association of anti-histone or nucleohistone antibodies with antibodies to native DNA (103 ,112 ,114 ,191), a well established correlate of disease activity. Part of the confusion about this issue is that some studies excluded patients with anti-DNA antibodies and others used assays that favored detection of antibodies to (denatured) histones which display low disease specificity and other assays primarily detected nucleosome-specific autoantibodies.

In recent years studies that deliberately used nucleosomal antigens have shown strong symptom correlation in SLE and a clinical specificity as high as 95% to 99% (126 ,127 ,128). Antichromatin and anti-([H2A-H2B]-DNA) antibodies was significantly correlated with glomerulonephritis (13 ,174 ,192) and were more specific for this feature than anti-DNA. Antinucleosome antibody of the IgG3 subclass were present at high levels in patients with active SLE, associated with flares and significantly correlated with lupus nephritis (122) or Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score (191). Other groups also observed a higher prevalence and/or amount of antichromatin antibodies in SLE patients with kidney disease (125 ,151 ,175) or overall disease activity score (128 ,151 ,176 ,177 ,178 ,179), although association with disease activity was not seen in all studies (126). Clearly, however, screening antigens using native nucleosomal materials are a substantially better tool for monitoring

SLE than assays using DNA-free histones, and most studies concluded that it is a better marker for SLE than anti-DNA.

Drug-Induced Lupus and Drug-Induced Autoimmunity

Histone-reactive antibodies have been reported in 50% to 100% of patients with DIL (Tables 24-2 and 24-3), depending on the drug and the assay employed. The drugs most commonly implicated in DIL include procainamide, hydralazine, quinidine, and isoniazid, although a variety of other drugs have been implicated as well (see Chapter 42). However, most patients who are treated with procainamide and other lupus-inducing drugs eventually develop antihistone antibodies even though symptomatic disease occurs in only 10 to 20 percent of patients (131 ,134 ,193). Thus, most antihistone antibodies are apparently benign, consistent with the occurrence of histone-reactive antibodies in many rheumatic and non–rheumatic diseases (Table 24-2). However, examination of their class and fine specificity has revealed that antihistone antibodies in asymptomatic patients are predominantly IgM and display broad reactivity with all the individual histones (130 ,194), or are inhibited in binding histones by DNA (132 ,135). In contrast, patients with symptomatic DIL induced by procainamide and many other drugs including penicillamine, isoniazid, acebutolol, methyldopa, timolol, sulfasalazine, and quinidine develop predominantly IgG anti-histone antibodies that display pronounced reactivity with a H2A-H2B complex (131), especially when bound to DNA (132) (Table 24-3). In fact, anti-(H2A-H2B) has been observed to precede overt clinical symptoms and therefore may have predictive as well as diagnostic value (133 ,195). Anti-(H2A-H2B) has a sensitivity of close to 100% and specificity of >90% for symptomatic procainamide-induced lupus compared to asymptomatic procainamide-treated patients (131) and an even higher specificity when an (H2A-H2B)-DNA complex is used as the screening antigen (132). As mentioned above, antibodies to the ([H2A-H2B]-DNA) complex are remarkable because they are also an important feature of the immune response in SLE as well as several murine models of SLE (196 ,197 ,198 ,199 ,200) but not in canine lupus (201).

Rheumatoid Arthritis

The frequency of histone antibodies in rheumatoid arthritis (RA) has been reported to be as high as 80% (Table 24-2). Aitcheson et al. (169) found that 14% of unselected patients with RA and 24% of patients with RA and a positive ANA had histone antibodies as demonstrated by the histone extraction-reconstitution assay. Other studies have reported similar frequencies in unselected patients with RA and higher frequencies in patients with RA and a positive ANA (138 ,202). Unlike patients with SLE and DIL, the titer of histone antibodies tends to be low in RA, and RA patients with antihistone antibodies did not display more active disease than antihistone antibody negative RA patients (138). Antinucleosome antibodies are generally not detected in RA (127).

Felty Syndrome

In Felty syndrome antihistone levels were considerably higher than in non-Felty's RA, especially of the IgG isotype (137). Campion et al. (143) reported histone antibodies in the sera of 20 of 32 patients (68%) with Felty syndrome compared to 12% prevalence in RA and the 54% in SLE. The antibodies in this syndrome appear to be directed against conformational histone determinants, because reactivity was lost when the histones were denatured in detergent. The high frequency of histone antibodies in Felty syndrome is of interest because these patients commonly have high levels of rheumatoid factor, an autoantibody believed to cross-react with histones (169 ,203), especially H3 (169 ,202)

Juvenile Chronic (Rheumatoid) Arthritis

Antihistone antibodies have been extensively studied in juvenile chronic arthritis, and most studies agree that histone antibodies are found in at least 40% of JCA sera (Table 24-2). In early studies when the acid extraction-reconstitution technique was used JCA sera were found to bind histones infrequently (144). Using immunoblot techniques the predominant reactivity of JCA sera was with a 33-kd doublet thought to be H1 (150). Antihistone antibodies in JCA are predominantly IgM, are commonly directed against H1 and H3, and tend to react with DNA-free histones.

The studies of histone antibodies in JCA were of interest because of their potential to aid in the subclassification of this disease. Antihistone antibodies were reported in 93% (145) and 67% (67) of JCA patients with uveitis compared to 33% of patients without uveitis (145). However, associations with uveitis were not observed in other studies (141 ,150 ,204), although patients with uveitis (active or inactive) tended to have higher levels or antihistone antibody compared to those JCA patients with no history of uveitis (149). When other clinical subsets were compared, antihistone antibodies tended to be more common in pauci- or polyarticular types of JCA compared to systemic onset JCA (67 ,150 ,205). These antibodies react preferentially with DNA-free histones (146 ,204), similar to those in asymptomatic drug-induced autoimmunity, and may not substantially contribute to the ANA commonly occurring in these children. Although the association of anti-histone antibodies with HLA-A2 (205) created optimism that histone antibodies might identify a subset of patients with JCA, the evidence of earlier studies as well as a thorough subsequent study (147) do not favor an association of histone antibodies with patterns of disease onset or the course of disease.

Vasculitides

Antibodies to histones have been reported in 75% of patients with RA and vasculitis (140), and antibodies to H2B have been reported in a man with vasculitis (68). These reports are of interest because both vasculitis (206) and SLE (207) have been associated with the presence of myeloperoxidase antibodies (perinuclear antineutrophil cytoplasmic antibody [pANCA]). Because both histones

and myeloperoxidase are highly basic proteins, the possibility of crossreactivity between them may occur. In fact antibodies to neutrophil myeloperoxidase are found in patients with hydralazine-induced lupus (207 ,208), and these patients also commonly have antihistone antibodies (Table 24-2). However, procainamide-induced lupus is not associated with anti-myeloperoxidase antibodies. More recently, it was reported that antihistone antibodies in pANCA positive ulcerative colitis patients react with two nonoverlapping segments in the COOH-terminal of histone H1 associated with a recurring PKKAK amino acid sequence motif (164). However, this specificity was not significantly correlated with pANCA titer or disease status, indicating that this epitope is not a predominant specificity of serum pANCA.

Scleroderma Spectrum of Diseases

Antibodies to histones have been reported in 1/3 to 2/3 of patients from the scleroderma spectrum of diseases (Table 24-2). It was claimed that this reactivity is relatively restricted to (H2A-H2B)-DNA complex (164 ,189). This is surprising because sera from these patients generally do not have the typical homogenous ANA pattern of antinucleosome antibody (Fig. 24-4). It has been suggested that these antibodies are of low titer and/or are primarily reactive with denatured regions on histones or contaminating autoantigens (100), and several groups have failed to detect antibodies to native epitopes on nucleosomes in scleroderma-related disorders (123 ,209) or they have been of low titer in a minority of patients (126). On the other hand, patients with localized scleroderma appear to have a particularly high frequency of antihistone reactivity (152 ,154), especially against H1 and H3 (154 ,156). Reports suggesting that patients with scleroderma that have concomitant histone antibodies have a higher frequency of pulmonary fibrosis (152), a more severe form of the disease and a poor clinical outcome (157), have not been substantiated in another study (154). As in studies of SLE and JCA, part of the reason for these differences is related to patient selection and technical aspects of the antihistone assays. In particular it was recently shown that contamination with the autoantigen Scl-70 in chromatin preparations could account for essentially all the binding of scleroderma sera to nucleosomes (123).

Summary

Because of the numerous reports of antihistone antibodies in many diseases, antihistone antibodies cannot be considered a specific marker for any disease. Additionally, the presence of histone antibodies does not necessarily correlate with disease activity. It is unclear how much assay differences and the artifactual reactivities discussed previously contribute to the discrepancies within the literature and the poor disease specificity. However, autoantibody reactivity to higher ordered structures involving histones such as chromatin or nucleosomes has been consistently reported to be a highly sensitive and remarkably specific marker for lupus-like diseases. The common occurrence of antinucleosome antibodies in drug-induced and idiopathic lupus suggests that loss of immune tolerance to chromatin and its conformationally native constituents such as the (H2A-H2B)-DNA complex and, less commonly, native DNA is a sensitive and perhaps early indication of the immune dysregulation that characterizes these conditions.

Characteristics of Histone-Reactive Antibodies

Isotype

Antihistone antibodies can be found in all the major immunoglobulin classes in patients with SLE, but, as discussed in detail (43), there is little agreement as to the predominant isotype. There appears to be a significant correlation between the amount or at least presence of IgG (but not IgM) antihistone antibody and overall clinical disease activity (101 ,104), although there was no correlation between isotype and predominant organ system involvement (104). The isotype profile tended to remain constant over time (210) and for IgG antibodies consisted mainly of IgG1 and IgG3 with notably negligible amounts of IgG2 antihistone antibody (210 ,211). IgG1 and IgG3 are strong complement fixing subclasses, implicating these antihistone antibodies in pathogenic processes. A broader distribution of the IgG subclasses was observed in drug-induced autoimmunity in which antihistone activity commonly occurred in all four IgG subclasses (211). These patients as well as patients with frank drug-induced lupus are more likely than SLE patients to have IgG, IgA and IgM antihistone and anti-([H2A-H2B]-DNA) antibodies. These autoantibody isotypes often appear to arise simultaneously during procainamide treatment, although patients who remain asymptomatic fail to develop IgG anti-([H2A-H2B]-DNA) (133). Interestingly, as treatment with the lupus-inducing drug continued, a gradual shift to predominantly IgG1 antihistone antibodies occurred (211), similar to the isotype profile in idiopathic SLE. The substantial IgA autoantibody levels in procainamide- (133) and isoniazid- (135) treated patients is remarkable, suggesting induction of autoantibody synthesis within the gastrointestinal mucosal immune system where drug concentration is presumably highest. The rather slow development of autoantibodies, the apparent concordance of IgG, IgA and IgM isotypes and the perpetuation of IgM autoantibodies for many years in asymptomatic, procainamide-treated patients suggest that the mechanism underlying drug induction of autoantibodies is unlike a classical immune phenomenon.

Fine Specificities of Autoantibodies to Individual Histones

Histone heterogeneity and chromatin structure (see the previous sections) raise the possibility that useful information may be derived from examining antibody activity to individual

histone classes and variants, domain regions within individual histones (Table 24-1) or to subnucleosome particles. Most studies have focused on the five major histone classes, although antibody activity to histone variants such as H1° (172), H5 (67) and ubiquitinated-H2A (212) have been reported. The methods employed have been either analytical separation of total histones by gel electrophoresis followed by Western blot or ELISA using biochemically purified histones or histone complexes.

In general SLE patients with antihistone antibodies tended to have antibody to all histones, with pronounced reactivity to H1 and/or H2B (107 ,108 ,109 ,114 ,118 ,120 ,140 ,194). However, there has been only modest agreement within these studies in that anti-H1 (107 ,194) or anti-H2B (139) was sometimes observed to be a minor antibody, or other histones, especially H3 (101 ,107 ,140), were major antigenic targets for antihistone antibodies in SLE sera. Biases in assays, differences in patient population and small sample size contribute to these discrepancies. Furthermore, patient-to-patient variability is obscured by this type of analysis.

Table 24-4 summarizes the reported average profiles in individual patients of antibody activities to a panel of histones. The aforementioned predominance of anti-H1 and anti-H2B in SLE can be observed in the patient profiles of only half the reports; the other studies showed no particular profile because of substantial patient-to-patient variability. Many of these studies used non-class specific immunoglobulin detecting reagents, adding additional ambiguity because the specificity of antihistone antibodies may depend upon the immunoglobulin class being examined. Considerable disagreement on the characteristic antihistone antibody profile in DIL is also apparent (43). With procainamide-induced lupus, a discrete profile of reactivity was only discernible when the dimer of histones H2A and H2B was included as a test antigen, although reactivity with the H2A-H2B complex may also be because of antibody binding to the individual, constituent histones (134 ,193).

Table 24-4: Antibodies to Histone Classes in Individual Patients

Disease	Method	Ig Class	Histone Class Reactivity	Reference
SLE	ELISA	G,M	Variable	(83,89,116,213)
	ELISA	G	H2B ≥ H2A > H3 = H4	(108)
	WB	Undefined	H1 = H2B > H4 > H3 > H2A	(120,121)
	FIA	Undefined	Variable; H1 = H2B = H3 > H2A = H4	(140)
	WB	G,M	Variable	(89,214)
	ELISA & WB	G	H1 = H2B > H2A > H3 > H4	(109)
DIL-PA	RIA/ELISA	G	H2A-H2B complex >>H2A = H2B	(70,130,131)
	WB	Undefined	H2B > H3 = H1 > H4 = H2A	(215)
	WB & ELISA	Undefined	H2A-H2B complex > H2A = H2B > H4	(214)
DIL-HY	FIA	Undefined	Variable	(194)
	WB	Undefined	H3 > H2B = H2A = H1 > H4	(215)
	WB & ELISA	Undefined	H3 > H4 >> H2B > H2A	(214)
DIA-PA	ELISA	M	Relatively uniform	(130,213)

ELISA, enzyme-linked immunosorbent assay; FIA, solid phase fluorescence immunoassay; G, IgG antibodies; M, IgM antibodies; RIA, solid phase radioimmunoassay; WB, Western (immuno-)blot; DIL-HY, hydralazine-induced lupus; DIL-PA, procainamide-induced lupus; DIA-PA, procainamide-induced ANA in asymptomatic patients.

H2A and H2B

There is good agreement that antibody to H2A and/or H2B in most SLE sera does not bind in Western blot to the large trypsin-resistant domain of these histones (Table 24-1). Thus, only 15% (214), 8% (118) and 0% (216) of SLE sera with anti-H2B retained activity on the C-terminal H2B polypeptide (21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125), i.e., when the N-terminal 20 amino acids were removed. Direct evidence that the N-terminal region of H2B contained a predominant epitope was obtained by Hardin and Thomas (120) who showed that 63% of sera with anti-H2B reacted with the N-terminal cyanogen bromide polypeptide (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59), while there was no reactivity with the C-terminal (63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125) polypeptide. Similarly, removal of the N-terminal 11 and C-terminal 11 amino acids from H2A dramatically reduced its antigenicity, so only 20% (118) 15% (214) and 11% (216) of anti-H2A in SLE retained activity on H2A polypeptide (12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118). These studies would suggest that the major epitope on H2B contains peptide (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20) and on H2A peptide (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11) and/or (119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129). In fact in ELISA H2B (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25) was antigenic for 38% of SLE sera (113). Gohill et al. (118) reported that the H2A 38-mer (91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129) retained good antigenicity for SLE sera having anti-H2A activity, but Muller et al. (113) found that the smaller C-terminal H2A peptide (116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129) and N-terminal H2A peptide (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15) displayed only weak antibody binding for 13% and 29%, respectively, of SLE sera. There are considerable discrepancies in DIL as to the predominant epitopes within the trypsin-resistant domains, as discussed (43). However, the trypsin-resistant domains of H2A and H2B are clearly important because these regions contain the amino acid residues responsible for H2A complex

formation with H2B (62 ,217) creating the highly antigenic H2A-H2B dimer (70 ,130 ,131 ,214).

H3 and H4

The effect of trypsin digestion on the antigenicity of both H3 and H4 is similar. In Western blot, the H3-derived P1 domain (“A” band) (118 ,215 ,216) and the H4-derived P4/P5 domain (“C” band) (118 ,215 ,216) (Table 24-1) retained antigenicity for fewer than 10% of SLE and procainamide-induced lupus sera having anti-H3 and/or H4 activity (118 ,214 ,215 ,216). This would suggest that the major epitope for SLE anti-H3 and anti-H4 antibodies resides in the N-terminal 26 amino acid and/or C-terminal six amino acids of H3 and the N-terminal 17 amino acids of H4, assuming no tertiary interaction between these and other regions of the molecules, as would be expected (62 ,217). In fact in ELISA, H3 peptide (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21) and (130 ,131 ,132 ,133 ,134 ,135) bound 76% and 39% of SLE sera, respectively, and 54% of SLE sera bound H4 (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29) (113). However, H3 peptide (40 ,41 ,42 ,43 ,44 ,45) was also antigenic (113), and absorption experiments to show that these activities were related to antibody to the parent molecule were not reported.

In contrast to SLE and procainamide-induced lupus, 80% (215) or 100% (214) of hydralazine-induced lupus sera reacted with the trypsin-resistant domains of H3 and H4 to an extent similar to that of the parent histones. The dichotomy between the reactivity of antibodies in hydralazine-induced lupus with those in procainamide-induced lupus and SLE is also manifested on subnucleosome particles (see Prevalence and Disease Association) and would suggest a difference in the immunogenic stimulus driving the autoimmune responses in these disease groups.

H1

Chymotrypsin cleaves accessible peptide bonds on the C-terminal side of aromatic amino acids, and brief digestion splits H1 approximately in the middle of the central, globular domain at phenylalanine 106. In Western blot the C-terminal polypeptide (107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129 ,130 ,131 ,132 ,133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,150 ,151 ,152 ,153 ,154 ,155 ,156 ,157 ,158 ,159 ,160 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,182 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199 ,200 ,201 ,202 ,203 ,204 ,205 ,206 ,207 ,208 ,209 ,210 ,211 ,212 ,213 ,214 ,215 ,216 ,217 ,218 ,219 ,220) retained full antigenicity for 100% (109 ,115 ,218) or 86% (120) of SLE and 100% of procainamide-induced lupus sera (218) having anti-H1 antibodies (both IgM (218) and IgG (115 ,215 ,218)). In contrast, the N-terminal half (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106) displayed lower antigenicity in most (but not all (109)) studies in showing reaction with only 28% (115), 16% (218) and 14% (120) of SLE sera with anti-H1 activity. When Gohill et al. (218) tested the antigenicity of the globular domain (36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121) (generated by trypsin digestion of H1) using ELISA, no SLE or procainamide-induced lupus serum reacted. These results suggested that the bulk of H1 antigenicity lies in the C-terminal hydrophilic domain. In a study using synthetic 15-mer peptides, Stemmer et al. (219) observed definite skewing of reactivity of SLE sera with C-terminal peptides of human H1b, although N-terminal and globular domain peptides were occasionally antigenic as well. Nevertheless, these data suggest that the apparent lower antigenicity of the N-terminal domain reported using bovine (109 ,115 ,218) or chicken (120) H1 fragments applies to human H1 as well, presumably the bona fide immunogen driving the autoimmune response. The highly conserved octapeptide in the C-terminal region (see preceding section) might be a candidate epitopic region which would cross species barriers and is included in the second most antigenic peptide in H1b (219). It is unlikely, however, that there is an immunodominant epitope in H1, consistent with the heterogeneity of anti-H1 antibodies in murine lupus (220). Additionally, SLE sera appear to have substantial antibody heterogeneity within the H1 variants; of 9 SLE sera examined, H1.5 was recognized by all 9 patients, but from none to all of the five other H1 variants showed antigenicity by Western blot (221).

Antibodies to H1 from JCA sera have also received considerable attention. Of particular interest was the report by Pauls et al. (67) that a small proportion (5 of 51) of JCA patients had antibody activity to chicken histone H5 without concomitant reactivity with the major H1 class. A similar preferential reactivity with H5 compared to H1 with SLE sera was observed by Stemmer et al. (219). Since H5 is not present in human tissues, it was suggested that the anti-H5 immune response was driven by an unknown nonhistone cross-reacting antigen. However, as previously discussed, H5 has extensive homology with H1°, the variant found in terminally differentiated cells. In fact, Monestier et al. (149) showed that JCA sera with anti-H5 had even higher reactivity with human brain H1° Antibody binding to H1° from human brain without binding to the major H1 fraction was also detected in a patient with sensory neuropathy (172). There are seven sequences in human H1° not present in H1 which are nearly identical to sequences in chicken H5, and these are distributed mainly in the globular and N-terminal region (38), possibly accounting for cross-reaction between H1° and H5. In fact epitope mapping demonstrated that peptides within the globular domain of H5 had the highest antigenicity in SLE and procainamide-induced lupus (222). Antibodies in JCA displayed poor binding to the central globular domain of H1 whether or not the amino terminal segment (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32) was also present, while highest reactivity was observed on the C-terminal segment containing the globular domain (33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129 ,130 ,131 ,132 ,133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,150 ,151 ,152 ,153 ,154 ,155 ,156 ,157 ,158 ,159 ,160 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,182 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199 ,200 ,201 ,202 ,203 ,204 ,205 ,206 ,207 ,208 ,209 ,210 ,211 ,212 ,213 ,214 ,215 ,216 ,217 ,218 ,219 ,220) (149). This reactivity appears to be similar to that of SLE sera, but coblocking studies between IgM antibodies in JCA and IgG antibodies in SLE have not been performed. The C-terminal region of H1 also contained the epitopes for antihistone antibodies in patients with ulcerative colitis (164).

Significance of Antihistone Antibody Fine Specificity

The specificity of histone-reactive antibodies in SLE and in lupus induced by most drugs is consistent with some form of chromatin being the predominant in vivo immunogen driving these B cell responses. Antibodies in SLE and DIL

which react with intact histones but not with histones from trypsinized chromatin can be most readily explained as having been elicited by chromatin rather than by free histones because the trypsin-resistant domain structure of histones is preserved in the form of chromatin and nucleosomes but not in individual histones. However, subnucleosome particles and histones in the form of the DNA-free H2A-H2B dimer and the H3-H4 tetramer are also resistant to trypsin digestion. Furthermore, the argument that chromatin drives the autoantibody response is weakened by the observations that antibodies reacting with the trypsin-resistant, and largely inaccessible cores of H2A and/or H2B also occur in some SLE sera and were the predominant activity in procainamide-induced lupus (132 ,214). This discrepancy is especially pronounced with antibodies in hydralazine-induced lupus, which retain reactivity with histones after trypsinization of chromatin (214 ,215). IgM antibodies from patients taking hydralazine or chlorpromazine (132) as well as IgG antihistone antibodies from patients with JCA (146) bound strongest to DNA-free histones, less to subnucleosome structures, and very little or not at all with chromatin. Thus, immune tolerance to more native forms of nucleohistone is largely preserved in JCA and with antibodies induced by chlorpromazine, isoniazid and IgM antihistone antibodies in hydralazine-induced lupus. This is the same pattern of antibody binding found when normal mice were immunized with histones (223) and is more consistent with some unknown form of DNA-free histone driving the immune response to histones in these patients.

A more convincing argument that chromatin drives the bulk of the histone-reactive antibody response in SLE and most DIL can be made from the data that compares the antigenicity of various forms of histones. As previously discussed, antibodies from patients with SLE and lupus induced by 10 drugs (Table 24-3) as well as antibodies from murine lupus bound prominently to a structural epitope in the (H2A-H2B)-DNA complex. Some patients with SLE also bound native DNA and the $(H3\text{-}H4)_2$-DNA subnucleosome, but reactivity with individual histones was much lower (13 ,174 ,186). In murine lupus, antibodies to (H2A-H2B)-DNA were found early in disease, before antibodies to native DNA and $(H3\text{-}H4)_2$-DNA arose (196). Absorption with chromatin removed most of the antibody reactivity to subnucleosome structures, indicating that regions buried in chromatin were not antigenic in SLE, DIL or murine lupus. Thus, histone-reactive antibodies in SLE can be most readily explained by auto-immunization with chromatin accompanied by sequential loss of tolerance first to the (H2A-H2B)-DNA region and then to $(H3\text{-}H4)_2$-DNA and native DNA. Loss of immune tolerance to epitopes on DNA-free histones, which can be considered “denatured histones,” and to “denatured DNA” (and to other nuclear antigens) may accompany the immune disregulation associated with lupus-related disorders, but, because of the complexity of these epitopes and the heterogeneity of this immune response, only with nucleosome-reactive antibodies can a strong case be made for the putative in vivo existence of a chromatin-like immunogen.

Histone Antibody Genes

Studies on the sequences of autoantibody variable (V) region genes provide insight into the origin of autoantibodies. One study showed that the nucleotide sequences of anti-histone monoclonal antibodies from MRL-*lpr/lpr* mice were not clonally related and that diverse V, D, and J genes were represented (224). However, other studies demonstrated that monoclonal antibodies to (H2A-H2B)-DNA obtained from $MRL^{+}/+$ mice were clonally related and possessed numerous charged residues in the heavy chain as a result of somatic mutations and various V_HDJ_H rearrangement processes (200 ,225), indicating antigen selection rather than polyclonal activation. However, the replacement/silent mutation ratios even in the complementarity-determining regions (CDR) were much lower than the ratio expected for random mutations (200), suggesting that once B cells with sufficient avidity for chromatin arise, something else, presumably T cell help, causes their expansion. This view is supported by the detailed sequence comparisons of anti-(H2A-H2B) antibodies derived from an (SWR × NZB)F1 (198) and an (NZB × NZW)F1 (199) mouse; these antibodies showed very few somatic mutations, suggesting that chromatin-reactive T cells selected a single precursor of these B cells for expansion at an early stage in their development and limited further V-region mutations to an extent corresponding to only one week of antigen-driven auto-immunization.

The physicochemical properties of some histone antibodies have at least a superficial resemblance to anti-DNA antibodies in immunochemistry, suggesting an analogous, antigen-mediated selection process. For example the antihistone antibody MRA12 has five negatively charged amino acid residues in its CDR2 and an isoelectric of 6.0–6.3 (224). This anionic region presumably is responsible for binding to H1, reminiscent of the observations that cationic anti-DNA antibodies react with the anionic phosphate groups on DNA (226 ,227). Similar physicochemical features in human monoclonal antihistone antibodies were noted (228). A simplistic view of the role of these negatively charged residues is that they are responsible for binding to the basic region of histones. However, because the monoclonal MRA12 bound specifically to H1 and not other histones, factors other than charge-charge interactions likely are important for antibody specificity and binding.

T Cell Epitopes and Histone-Reactive T Cells

Production of IgG antibodies including autoantibodies usually requires that B cells are stimulated not only by antigen but also by proximal T cells that produce the requisite co-stimulation and cytokines. Lymphocytes from 54% of SLE patients showed a proliferative response to nucleosomes in vitro (125). Chromatin-specific T cells were isolated from mouse (229) and human (230) SLE, and histone-reactive T cell lines were obtained from SLE and normal individuals

(231 ,232). T cell clones derived from lupus-prone (SWR × NZB)F1 (SNF1) mice displayed proliferative and cytokine responses to nucleosomes but not their component macromolecules. Of the clones that could be mapped, five reacted with peptides corresponding to H4 (13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39) and/or H4 (73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90) and one with H2B (10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33) (233), and their specificity seemed to be conferred primarily by the α-chain of the T cell receptor (234). Interestingly, these T cell epitopes were similar to those commonly recognized by T cells from SLE patients; human lupus T cells also recognized H3 (91–105 or 100–114), H2A (34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48), and H4 (49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63) (235). However, when peptides were extracted from the class II MHC of lymph node cells from lupus-prone MRL-*lpr/lpr* mice, the only detected histone-derived peptide was H2A (84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103) bound to I-E^k (236) which induced a significant T cell response in young MRL-*lpr/lpr* mice (237). However, this response was weak, and H2A (84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103) was not related to the dominant T cell epitopes reported in SNF1 mice (233) or human SLE (235). The basis for this discrepancy is not clear.

Nucleosome-reactive clones provided helper activity to primary B cells for secretion of IgG “anti-histone/DNA” in vitro (233). A controlling role for such chromatin-specific T cells in the humoral response of BXSB mice was the demonstration that injection of chromatin in vivo directly into thymic lobes delayed the appearance of anti-chromatin and anti-DNA antibodies, presumably due to the enhanced deletion or other form of central T cell tolerance of spontaneously arising autoreactive T cells (238). Histone-reactive T cell lines have been developed from normal individuals after stimulation with nucleosome-polyomavirus T antigen complexes (232 ,239), demonstrating that quiescent histone-reactive T cells may be part of the normal T cell repertoire.

The notion that the antichromatin response is limited by the availability of T helper cells is supported by immunization studies. Normal mouse strains generally fail to produce autoantibodies reactive with native self-antigens using various immunization protocols (240). Thus, host B cells from (C57BL/6 × DBA/2)F1 mice produced anti-[(H2A-H2B)-DNA] antibodies after in vivo transfer of DBA/2 T cells because of the graft-versus-host (GVH) reaction (241), but only antibodies to nonnative regions in chromatin can be detected after immunization of this strain with various chromatin-related antigens (223). These results are consistent with the findings that immunoglobulin receptors for (H2A-H2B)-DNA are derived by limited somatic mutations of the normal B cell repertoire (198 ,200 ,225), but these cells or their precursors remain quiescent until appropriate T cell help becomes available. Apparently, DBA/2 T cells provide this help in the GVH model, but direct immunization does not break T cell tolerance to chromatin. However, when a reactive metabolite of the lupus-inducing drug procainamide was injected into the thymus of (C57BL/6 × DBA/2)F1 mice, anti-([H2A-H2B]-DNA) antibodies arose (242), consistent with the view that chromatin-reactive T cells, created by disruption of central T cell tolerance, have sufficient helper capacity to drive precursors of ([H2A-H2B]-DNA)-specific B cells to somatically mutate, expand, and secrete autoantibodies. Recently Kang, et al (243) showed that injection of remarkably low amounts of a dominant T cell epitope, H4 (71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94), reduced development of autoantibodies and prolonged the life of SNF1 mice, apparently because of production of regulatory T cells. Taken together a strong case can be made for the view that histone-reactive T cells are a necessary and probably sufficient force in the development of lupus-like autoimmunity.

Cross-Reactions of Antihistone and Antinucleosome Antibodies

Rheumatoid Factors

Polyclonal IgG RF with ANA activity were first clearly demonstrated by Hannestad and Johanessen (244), and an IgM RF with capacity to bind nucleosomes containing the core histones was isolated from another RA patient (245). Several subsequent studies verified and extended these findings (169 ,246 ,247 ,248 ,249 ,250), suggesting that RF with histone binding activity was quite common. Trivial explanations such as contaminating immune complexes were ruled out by the discovery of monoclonal RF produced in tissue culture with similar properties (251). Although the structural basis for these cross-reactions is unclear, it is possible that the combining site of an antibody is considerably larger than that needed to accommodate a single epitope, permitting distinct regions within the Fab part of an antibody molecule to react with unrelated epitopes. It may also be relevant the IgM RF+ B cells can be co-activated (with anti-IgM) by nucleosome/anti-nucleosome complexes, apparently involving TLR9 (252), although it is not clear if these B cells produced cross-reacting RF.

Antilymphocyte Antibodies and Nucleosome/Membrane Interactions

Of 27 LE-factor-positive sera, 26 contained ANA that bound to and could be eluted from viable leukocytes (253). These “X-ANA” reacted with nucleosomes (254), and absorption experiments indicated that ANA, LE factor and antilymphocyte activities were properties of the same antibody population (15). In addition to human leukocytes, LE factor also bound to mouse and rabbit splenocytes, rat hepatocytes and human endothelial cells but not human or chicken erythrocytes (255 ,256). X-ANA behaved as a subset of the cold reactive antilymphocyte antibody repertoire (255). The related capacity of anti-DNA antibodies to bind a “lupus-associated membrane protein” (LAMP) has been shown to be mediated by DNA or nucleohistone debris contaminating the anti-DNA preparations (257 ,258), although apparently DNA-independent anti-DNA antibody binding to cell membranes has been described (259). The capacity of mononuclear cells or granulocytes to bind X-ANA was not mediated by the Fc region of IgG or by the Fc or C3 receptors on leukocytes, and, in contrast to anti-LAMP

antibodies, nuclei derived from dead cells appeared not to be responsible for the capacity of leukocytes to immuno-adsorb X-ANA (254 ,260 ,261). The cell membrane antigen is similar to an epitope in the core histone octamer (in the presence or absence of DNA) but not in nucleosomes depleted of H2A and H2B (253), and solid-phase H2B or H2B peptide (6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18) reacted with antibody affinity purified on leukocytes (262). A peptide derived from H2B also appears to be present on murine B cells (263). However, studies with a panel of histones as well as antihistone and anti-DNA antibodies failed to detect other chromatin-related antigens on cell membranes (264).

Activation of monocytes by endotoxin in the medium can apparently induce expression of a receptor (265) which binds chromatin debris released into the medium (266). Additionally, nucleosomes were observed to interact with bone marrow derived dendritic cells (DC) independent of endotoxin contamination, resulting in DC maturation and inflammatory cytokine secretion (267). Various receptors are candidates for these effects including a 94 kDa protein in a cell membrane extract which bound DNA and nucleohistone (257), a 30 kDa protein which is a component of a DNA receptor on mononuclear cells (268 ,269), or a 50 kDa protein that acts as a nucleosome receptor on a fibroblast cell line (270). However, there is no evidence that SLE patients, in which anti-H2B and/or anti-DNA antibodies are commonly observed, display membrane-associated antibody on circulating mononuclear cells in vivo. It is possible that receptor association is transient, and nucleosomal material is engulfed by macro- or micro-pinocytosis (267 ,270). Regardless of its pathogenic implications, it is possible that membrane-associated chromatin could be involved in driving the autoimmune response. This view is supported by findings in NZB/NZW mice that endogenous retroviruses carry chromatin-like material apparently derived from host cell membranes during virion budding, and these viruses can induce antichromatin antibodies by immunization with adjuvant (271).

Murine Strains with Antihistone and Antinucleosome Antibodies

Strains of mice in which serum antihistone antibodies spontaneously appear during their natural life history include (NZB × NZW)F1 (85 ,220 ,272 ,272 ,273 ,274 ,275), MRL-*lpr/lpr* (105 ,196 ,220 ,273 ,274 ,275 ,276 ,277), MRL$^{+/+}$ (225 ,274 ,275 ,276), BXSB (196), (SWR × NZB)F1 (198 ,229) Palmerston North (105), Swan (105), NZB (220 ,276 ,277), ddY (278), Yaa (275), NZM2410 congenic lines (279) and (C57Bl/6 × DBA/2)F1 mice undergoing chronic GVH disease after injection of DBA/2 T cells (223 ,241 ,273 ,280 ,281 ,282 ,283). As with most human SLE and some DIL, predominant reactivity with linear epitopes in the trypsin sensitive region of the core histones was also observed with spontaneously arising antibodies in murine lupus (272 ,281).

Murine lupus has provided the opportunity to examine the kinetics, pathogenic potential, and origin of nucleosome-reactive antibodies in considerable detail. In a genetic dissection of murine lupus it was shown that a region on chromosome 1 (Sle1) consisting of several loci is responsible for the development of anti-([H2A-H2B]-DNA) antibodies in NZM2410 mice (284). The presence of anti-chromatin was also strongly linked to early death in the progeny of (NZB × NZW)F2 intercross mice (285). When the kinetics of autoantibody appearance in the lupus prone MRL-*lpr/lpr* and BXSB mice were examined over short time intervals, the earliest autoantibodies detected reacted with native chromatin rather than its constituents such as histones and DNA (196 ,197). Similar early appearance of antinucleosome antibodies were seen in MRL$^{+/+}$ mice along with anti-U1A-RNP antibodies; anti-DNA antibodies appeared much later (275). Anti-([H2A-H2B]-DNA) accounted for the bulk of the early anti-chromatin activity in MRL-*lpr/lpr* and BXSB mice (196). This serology was similar to that observed in 14 out of 40 newly diagnosed SLE patients, and the mouse and human sera efficiently coblocked, indicating a similar target epitope (13). Another 14 SLE sera had elevated reactivity to multiple antigens in chromatin including (H2A-H2B)-DNA, native DNA, H3-H4 tetramer, and various individual histones (13). This serology, in turn, was very much like that in older lupus mice which had rapidly developed antibodies to multiple epitopes on chromatin (196). These data suggests that, as in DIL, the autoimmune response in human and murine SLE is initially directed to the (H2A-H2B)-DNA component of chromatin, but unlike DIL, spreads to other regions in chromatin (and to other nuclear antigens).

Pathogenic Potential of Antihistone Antibodies

The pathogenic mechanisms in SLE and DIL have not been clearly elucidated, but there is a general consensus that complexes between autoantibodies and their cognate antigens play at least a contributing role. A pathogenic role for chromatin-reactive antibodies in lupus nephritis, but not antibodies to histones (286), is suggested by studies in experimental animal models of nephritis and in vitro assays of glomerular-binding immunoglobulin. Antinucleosome-nucleosome immune complexes bound to glomeruli in vivo and to the glomerular basement membrane (GBM) in vitro (287). However, in SLE such preformed immune complexes appear to be of minor importance (288). Although serum from 81% of SLE patients with nephritis showed elevated IgG binding to a preparation of basement membranes isolated from human glomeruli in an ELISA format (288), GBM binding activity was substantially decreased by DNase treatment of the GBM (but not the sera), and nucleosome preparations were especially potent in restoring antibody binding to GBM. These and other data (95 ,287 ,289 ,290 ,291 ,292 ,293) demonstrated that chromatin or nucleosome core particles bind through ionic interactions to type IV collagen and/or heparin sulfate in the GBM and to skin basement membranes (294), providing an in situ surface for binding chromatin-reactive antibodies. Approximately 20% of SLE

patients were reported to have increased levels of circulating nucleosomal material, although plasma chromatin did not correlate with disease activity or SLEDAI score (191).

Other studies, however, suggest that binding of anti-nucleosome (including anti-DNA) antibodies to chromatin deposits in the kidney does not by itself initiate glomerulonephritis. Sera from a substantial proportion of SLE patients without nephritis also showed capacity to bind glomerular structures (295) or GBM (288) in vitro. Furthermore, sera from patients with DIL displayed a range of in vitro GBM binding capacity similar to that of SLE sera (288). Since SLE but not DIL patients develop glomerulonephritis, antichromatin antibodies alone are unlikely to be sufficient to cause this pathology. A compromised capacity to clear immune complexes either from the circulation or following their deposition in the kidney has been described as a feature of SLE patients (296) and murine lupus (297), but it is difficult to distinguish cause from effect in this phenomenon.

SLE patients with active nephritis typically have substantial GBM-associated immunoglobulin, more severe clinical disease and poorer prognosis than SLE patients with low GBM-binding antibody (295). These patients usually have hypocomplementemia, suggesting that activation of the classical complement pathway by autoantibodies deposited in the kidney contribute to disease. While antihistone antibodies from SLE sera did not fix complement in the histone-reconstituted IIF assay (78), SLE sera showed strong capacity to deposit complement proteins C3, (298 ,299) C4 and properdin on nuclei of HEp-2 cells (298). SLE sera with antiribonucleoprotein autoantibodies had particularly good capacity to fix complement (78 ,298 ,299). These data along with the finding that IgG1 and IgG3 are the predominant isotypes of autoantibodies antibodies in SLE (122 ,211) suggest that the frequent heterogeneity of autoantibodies in SLE sera make for a particularly strong cocktail of complement-fixing ANA. In contrast sera from patients with DIL, which contain predominately antichromatin antibodies, did not show strong capacity to fix complement in IIF assays (299 ,300). However, depressed complement levels have been detected in patients with lupus induced by procainamide (301) and hydralazine (302), and a prospective study of a patient with procainamide-induced lupus demonstrated elevated C4d/C4 ratios during symptomatic disease (195), indicating activation of the classical pathway in vivo. It is likely, therefore, that while nucleosome-reactive antibodies are not well tolerated and contribute to in vivo complement activation and its accompanying inflammatory sequelae in SLE and DIL, the more global immune disregulation and complicated abnormal serology in SLE is necessary for tissue destruction involving autoantibodies.

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Chapter 25
Antibodies to Ro/SSA and La/SSB

Morris Reichlin

John B. Harley

Anti-Ro and anti-La are important autoantibodies in systemic lupus erythematosus (SLE). Respectively, they are found in just less than one half and nearly one fifth of these patients in concentrations sufficient for precipitin formation. These autoantibodies also are closely allied with particular clinical findings. They are such an intrinsic component of disease expression that one is led to the conclusion that an understanding of the immune response to these (or to any one of the other major autoantigens) would reveal the mechanism of the autoimmune dysregulation of lupus, if not the etiology of the disease as well.

History and Development

A precipitin, likely to have been anti-Ro or anti-La, was first described over 40 years ago in a patient with Sjögren syndrome (1). This observation was expanded on by Anderson et al. (2), who defined both antigens, which they called SjT and SjD. In addition, they observed a "lupus" precipitin that in retrospect is likely to have been an antinuclear ribonucleoprotein (nRNP) or anti-Sm specificity. Unfortunately, these observations lay fallow until the anti-Ro and anti-La specificities were independently described by Reichlin et al. (3 ,4). Anti-Ro and anti-La autoantibodies, as well as the autoantigens they bind, have been under continuous investigation since then. They also have been described as Sjögren syndrome A (SS-A) and Sjögren syndrome B (SS-B) antigens (5 ,6). Figure 25-1 present an Ouchterlony immunodiffusion showing an example of the anti-Ro and anti-La responses. Beyond this discovery, the most fundamental contribution has been the marriage of autoimmune serology with molecular biologic approaches for the analysis of these antigens (7).

Clinical Relationships

The clinical relevance of anti-Ro has been particularly well established. The data are less compelling for anti-La, although clearly this autoantibody also is important. Table 25-1 presents the associations of clinical findings with anti-Ro and anti-La, which do not in themselves constitute direct evidence of an immunopathogenic role of the autoantibody for any aspect of the disease. Nevertheless, and at the very least, these associations are important as aids in diagnosis and prognosis.

Photosensitive skin rash in lupus as an association with anti-Ro was first appreciated by Maddison et al. (8) and has been confirmed by Mond et al. (9). Lee and David (10) as well as Lee and Weston (11) also have established that anti-Ro is specifically deposited in human skin, and therefore is likely to be directly involved in the cutaneous injury of these patients.

Chest radiographs showing the changes of interstitial pneumonitis have been repeatedly found to be associated with anti-Ro (12 ,13). There is no evidence directly implicating anti-Ro in the pulmonary disease of lupus, however.

Idiopathic thrombocytopenic purpura (ITP) is well known to present before lupus can be diagnosed in some patients. These patients tend to have anti-Ro precipitins (14 ,15 ,16). Both anti-Ro and a positive antinuclear antibody test may be found at presentation with immune thrombocytopenia and precede fulfilling the classification criteria for lupus by at least as much as 14 years (14). How long before presentation with immune thrombocytopenic purpura these patients develop anti-Ro is not known.

Indeed, the temporal relationship of the appearance of anti-Ro or anti-La to the clinical presentation with lupus or Sjögren syndrome is not known. Anti-Ro or anti-La appears after diagnosis only in the rare patient. In addition, most mothers with anti-Ro and anti-La who have infants with congenital heart block or neonatal lupus dermatitis have never had clinical manifestations of any of the disorders associated with these autoantibodies (17 ,18). Many mothers, however, develop Sjögren syndrome or lupus in the years following birth of the affected infant (19 ,20). This leads one to suspect that anti-Ro and anti-La autoantibodies may arise years before the clinical illness appears, which has been convincingly established (21).

In addition to thrombocytopenia, other hematologic cytopenias have been associated with anti-Ro. Patients with lupus who in any way satisfy the hematologic criterion for classification of SLE (22), but particularly those with lymphopenia, tend to have anti-Ro (23). In Sjögren syndrome, thrombocytopenia, anemia, and lymphopenia are associated with anti-Ro (24). In rheumatoid arthritis, although anti-Ro is uncommon (i.e., approximately 5% of patients) (25), this autoantibody is associated with leukopenia when it is present (26). One study shows that anti-Ro binds directly to the granulocyte surface and is closely associated with granulocytopenia (27).

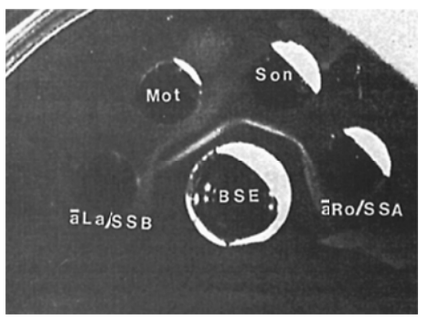

Figure 25-1. Anti-Ro and anti-La precipitins. The mother (Mot) has both anti-Ro and anti-La autoantibodies, while the affected son (Son) has only a faint anti-Ro precipitin. Precipitins from reference anti-La (aLa/SSB) and anti-Ro (aRo/SSA) sera also are presented against a bovine spleen extract (BSE). The anti-La precipitin is light and diffused in this example. This pedigree is described in Reichlin M, Friday K, Harley JB. Complete congenital heart block followed by anti-Ro/SSA in adult life: studies of an informative family. *Am J Med* 1988;84:339–344.

Table 25-1: Associations with Anti-Ro and Anti-La Autoantibodies in Systemic Lupus Erythematosus and Related Disorders*

Specificity	Clinical or Genetic Association
Anti-Ro	Photosensitive skin rash (lupus) Interstitial pneumonitis (lupus) Thrombocytopenia (lupus, Sjögren syndrome) Lymphopenia (lupus, Sjögren syndrome) Nephritis (lupus) C2 complement deficiency (lupus) HLA-DQ1/2 T cell receptor ß gene (lupus) Vasculitis (Sjögren syndrome) Thrombocytopenic purpura (Sjögren syndrome, subacute cutaneous lupus) Primary biliary cirrhosis
Anti-Ro and anti-La	Absence of nephritis (lupus) HLA-B8, DR3 (lupus) HLA-DQ1/2 (Sjögren syndrome) Rheumatoid factor (lupus, Sjögren syndrome) Sjögren syndrome Subacute cutaneous lupus erythematosus Neonatal lupus dermatitis Complete congenital heart block

*Associations are presented for systemic lupus erythematosus (lupus) unless the data are derived from patients with another diagnosis, such as Sjogren syndrome. While rheumatoid factor, Sjögren syndrome, subacute cutaneous lupus erythematosus, neonatal lupus dermatitis, and complete congenital heart block are frequently associated with both anti-Ro and anti-La precipitins, they also all occur in association with anti-Ro without anti-La. HLA, human leukocyte antigen.

There are data supporting a role for anti-Ro in nephritis as well. In a series of patients with anti-Ro precipitin, only those who had anti-Ro without an anti-La precipitin developed renal disease (28). Subsequent work has confirmed that patients with anti-La tend to be spared renal disease (23). Acid-eluted immunoglobulin from the renal tissue of two patients with anti-Ro precipitin contained anti-Ro that had been concentrated in the antibody deposited in the kidney (29). More recent data have shown that anti-Ro, anti-La, and anti-DNA all are concentrated in renal tissue (30). This is prima facie evidence to support a direct role for anti-Ro and other autoantibodies in the nephritis of some patients. Anti-Ro deposited in the tissue either as an immune complex or by virtue of binding to a specific antigen has the potential to be phlogistic and to mediate the inflammatory response that follows antibody binding.

Complete Congenital Heart Block

Over the past two decades, a compelling association has been demonstrated between complete congenital heart block and both anti-Ro and anti-La precipitating autoantibodies (17 ,31 ,32). The anti-Ro immunoglobulin G (IgG) clearly originates with the mother, who may be asymptomatic for an autoimmune rheumatic disorder. In a few cases, normal fetal cardiac conduction has been demonstrated before the third trimester (33 ,34). Heart block often appears late in the second trimester or early in the third trimester, coincident with the active transport of maternal IgG across the placenta.

Complete congenital heart block is found in approximately one of every 20,000 births (35). In the largest series of congenital heart block cases, anti-Ro and/or anti-La was found in 83% (17). Survey studies estimate that an anti-Ro precipitin may be present in as many as 1% of pregnant women (36 ,37). An expectant mother with an anti-Ro precipitin has an increased risk of bearing a child with complete congenital heart block, but even so, this risk remains small.

The mechanism of heart block is unclear. Cardiac conduction tissue is bound by anti-Ro more avidly than other cardiac cell types (38). Data from a set of fraternal twins who were discordant for heart block have shown depletion of anti-Ro in the serum of the affected twin, which is consistent with the possibility that anti-Ro is being specifically deposited in cardiac tissue (39). This observation strongly suggests that critical contributions are made toward the generation of heart block by the fetus in a way that varies among potentially affected pregnancies. Specific concentration of anti-Ro (both anti–60-kd Ro and anti–52-kd Ro) in cardiac tissue, but not in other tissues, has been demonstrated (40).

One theory of pathogenesis holds that the basic process is a nonspecific endomyocarditis that involves the atrioventricular node by extension at a time when the node is anatomically vulnerable (39). If true, then many fetuses of mothers with anti-Ro autoantibodies may have the endomyocarditis, while only a fraction of these involve the atrioventricular node and develop heart block. The association of anti-Ro and

anti-La antibody with endocardial fibroelastosis has been recently reported in 13 instances (41).

Much progress has been made in understanding the pathogenesis of complete heart block related to anti-Ro/SSA antibodies. Boutjdir et al. (40) showed that anti–52-kd Ro/SSA antibodies induce complete atrioventricular (AV) block in the human fetal heart perfused by the Langendorff technique and inhibit L-type $Ca2^{+}$ currents at the whole-cell and single-channel level. In addition, these workers immunized female BALB/c mice with recombinant 52-kd Ro/SSA protein and these antibodies crossed the placenta during pregnancy and were associated with varying degrees of AV conduction abnormalities, including complete AV block, in the pups (42). Similar results were reported by Viana et al. (43), in which affinity purified anti-52-kd Ro/SSA antibodies could induce complete AV block in isolated whole rabbit hearts. Finally, Miranda-Carus et al. (44) showed in mice that complete AV block was only seen in offspring from mothers immunized with 52-kd Ro/SSA, but not La/SSB or 60-kd Ro/SSA, although lesser AV conduction abnormalities were noted with the latter two antigens.

There are at least three ways that complete congenital heart block is relevant to lupus. First, pregnant female patients with lupus and anti-Ro, by virtue of having this autoantibody, have a one in 20 risk of having a child with complete congenital heart block (45). Second, the mothers of children with heart block are at an increased risk of developing a systemic autoimmune rheumatic disorder, even if the mother is asymptomatic when the child is born (19). Indeed, we have seen a patient whose lupus developed 26 years after the delivery of an infant with congenital heart block and that coincided with her retiring from Ohio to bask in the sunshine of Florida (46). Third, a study of adult patients with lupus has revealed an increased prevalence of conduction abnormalities among patients with anti-Ro precipitins (47).

Neonatal Lupus Dermatitis

Infants of mothers with anti-Ro also may develop neonatal dermatitis. This rash most often appears after birth. Areas that are exposed to sunlight predominate but are not exclusively involved. Skin lesions often are similar to those found in subacute cutaneous lupus with arcuate erythematous macules or papulosquamous lesions. Occasionally, they may leave hypopigmented skin, but the lesions generally resolve without sequelae. In infants, all lesions that appear usually develop and resolve together. As the maternal IgG is cleared from the infant's circulation, the rash also resolves. Additionally, in those infants who are not affected at or very soon after birth, the likelihood of developing the rash diminishes as maternal IgG is metabolized. Maternal IgG is almost undetectable in the infant by 6 months of age, and the onset of neonatal lupus dermatitis is unheard of at this stage. A few cases of neonatal dermatitis have been reportedly associated with an anti-nRNP precipitin in the absence of an anti-Ro precipitin (48).

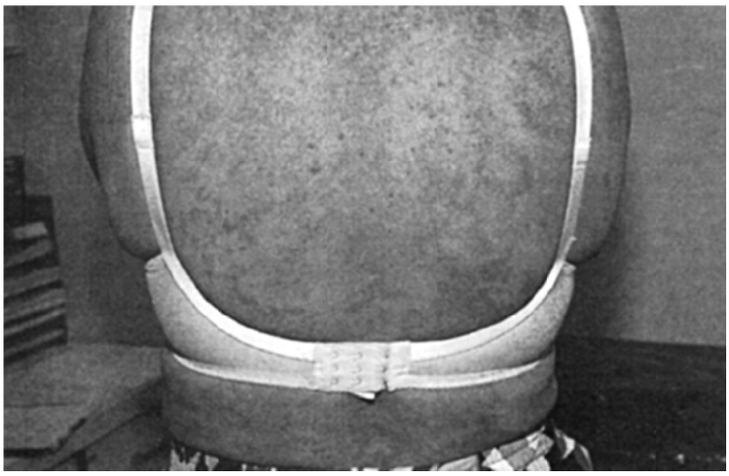

Figure 25-2. The rash of subacute cutaneous lupus erythematosus, showing erythematous macules and papulosquamous lesions. The typical arcuate lesions can be appreciated on the back of this middle-aged woman at presentation.

Subacute Cutaneous Lupus Erythematosus

The diagnosis of subacute cutaneous lupus erythematosus is established by the presence of one of the two typical and usually photosensitive rashes, annular or papulosquamous (Fig. 25-2), along with consistent histology. Approximately 75% of patients with subacute cutaneous lupus have anti-Ro precipitins (49). It has now been reported that anti-Ro precipitin negative patients with SCLE have elevated anti-Ro antibodies detectable with a sensitive and specific enzyme-linked immunoabsorbent assay (ELISA) (50). These patients may or may not satisfy the classification criteria for systemic lupus (22). The lesions are erythematous and may or may not be raised. In some patients, the lesions have central clearing. Lesions at all the different stages of maturity may be present simultaneously. Some of these patients develop petechiae or purpura, particularly of the lower extremities, biopsy specimens of which often reveal small vessel vasculitis. Those with vasculitis seem to be a subgroup with greater hypergammaglobulinemia who are more likely than the remaining patients to have Sjögren syndrome.

Sjögren Syndrome

Of the disorders that are associated with anti-Ro and anti-La, perhaps none is more intriguing than Sjögren syndrome. Depending on the assay performed and the population selected, from 40% to over 95% of these patients have anti-Ro, and from 15% to over 85% have anti-La (51). Dry eyes and dry mouth associated with a lymphocytic infiltrate of the salivary or lacrimal glands are not uncommon in a number of autoimmune rheumatic disorders, including SLE, rheumatoid arthritis, progressive systemic sclerosis, primary biliary cirrhosis, and autoimmune myositis. Hence, Sjögren syndrome is a feature shared by a minor proportion of the patients with each of these diseases, which suggests that they also must have fundamental aspects in common.

Primary Sjögren syndrome is considered to be present when the diagnostic criteria for Sjögren syndrome are satisfied (52) in the absence of another rheumatic disease with autoimmune features. Because the etiology and pathogenesis of all these diseases are unknown, and because there is great latitude in applying the diagnostic standards, the relative composition of patient groups is likely to vary greatly between investigators. This situation does not seem to have been improved by the current criteria for classification of rheumatoid arthritis (53), which are more broadly inclusive than the previous criteria (54).

There are a number of patients whose predominant disease process over time is Sjögren syndrome but who then develop features that are consistent with SLE (55 ,56 ,57). As a group, they are highly enriched for human leukocyte antigen (HLA)-DR3 and commonly have both anti-Ro and anti-La. The distinction between primary and secondary Sjögren syndrome is lost in this situation. Indeed, these patients are commonly referred to as having lupus-Sjögren overlap disease. The important point is that these and other patients with lupus or Sjögren syndrome form a continuous spectrum of disease expression, from classic primary Sjögren syndrome through the overlap with shared features to a more typical lupus process. The failure of existing nosology to distinctly separate patients demonstrates the inadequacy of present diagnostic practices, and it provides some of the impetus to understand etiology and pathogenesis.

Complement Deficiency Status

SLE also is associated with hereditary deficiencies of the early components of the classic complement cascade (58 ,59). Patients with complement component C2 deficiency tend to have an anti-Ro precipitin but not an anti-La precipitin. Anti-Ro may be more common in homozygous patients with C2 deficiency than in the remainder of patients with lupus (60). Patients with lupus and C2 complement component deficiency also tend to have a mild form of lupus with cutaneous manifestations, but with neither anti-double-stranded DNA (anti-dsDNA) autoantibody nor nephritis. Patients with lupus and the other early complement component deficiencies also have anti-Ro without an anti-La precipitin, but this has not been evaluated in a sample large enough to be conclusive (59).

Tissue Concentration of Anti-Ro and Anti-La

Anti-Ro and anti-La clearly are related to autoimmune rheumatic disease expression. In only a few situations has strong evidence been obtained for their phlogistic potential. In individual tissues, anti-Ro has been shown to be concentrated in the kidney, heart, and parotid gland (29 ,30 ,40 ,61). Affinity-enriched anti-Ro has been shown to deposit specifically in human skin transplanted onto nude and severe combined immunodeficiency disease (SCID) mice (10 ,11) as presented above.

Immunogenetics of Anti-Ro and Anti-La

Immunogenetic associations with individual autoantibodies have led to model building in an effort to understand the possible molecular events in the context of what has been learned about the immune response. The first relationship to be appreciated has been the association of anti-Ro with HLA-DR3 (62 ,63). Subsequently, it has been appreciated that anti-Ro also is related to HLA-DR2 in both lupus and Sjögren syndrome (64 ,65).

These multiple associations have been reconciled in two ways. First, a gene interaction effect has been defined between HLA-DQw1 and HLA-DQw2 such that patients with lupus or Sjögren syndrome who have both of these alleles tend to have anti-Ro. This has been extended in lupus to show that particular subsets of the DQA1 and DQB2 genes mediate this effect, which therefore is consistent with a gene complementation mechanism (66). One of the attractive possibilities that could explain these results is a DQ molecule composed of the predicted DQA1 and DQB2 genes and encoded by different chromosomes. There is no direct evidence for or against such a molecule in lupus or Sjögren patients. Others have performed experiments in cell lines and have obtained data suggesting that the predicted molecule is not favored (67).

Reveille et al. (68) have taken a more inclusive approach by attempting to define the primary sequence of HLA-DQ that is common to all patients with anti-Ro relative to that of a control population. They also have mapped the most powerful associations to the DQ locus. Nearly all patients with an anti-Ro response had a glutamine at amino acid position 34 of at least one of their DQ α-chains and a leucine at amino acid position 26 of at least one of their DQ ß-chains.

The HLA associations with the anti-La response are a little different. In primary Sjögren's syndrome, anti-La is related to HLA-B8, DR3, and DR2 (51 ,69 ,70), much as for anti-Ro. Indeed, the association of anti-La with the HLA-DQ1/w2 heterozygous state was as powerful as it was for anti-Ro (71). In lupus, however, the strongest association is with the B8, DR3 haplotype (23). The basis for this discrepancy is not known.

Models of Pathogenesis

Humoral autoimmunity is revealed by the presence of autoantibodies. It is much more difficult to be confident that human diseases with lymphocytic infiltrates but without autoantibodies also are autoimmune, but work in animal models has provided convincing and overwhelming evidence that this mechanism of disease pathogenesis is a practical possibility. Here, the prevailing suspicion is that T lymphocytes are mediating autoimmunity without stimulating B lymphocytes to differentiate and produce autoantibodies.

T lymphocytes appear to determine not only whether a cellular, as opposed to an antibody, response results from immunogen exposure, but also in many circumstances whether tolerance is maintained or broken. For these and other reasons, most investigators suspect that T lymphocytes have an obligate role in the immunoregulatory decision to synthesize autoantibody against protein autoantigens. Defining this role in human lupus has been difficult, as it has been in other inflammatory, and possibly autoimmune, disorders of unknown etiology. For example, in lupus, there is no evidence to suspect the linkage of lupus with α, ß, or γ T-lymphocyte receptor genes in multiplex families (72).

On the other hand, just as the histocompatibility associations are different for risk of disease than they are for production of individual autoantibodies, there may be analogous differences at the level of the T cell receptor. Recent work has shown that alleles of the T cell receptor ß-chain gene are related to the presence of anti-Ro in lupus (73). Interestingly, the association is most significant for those patients who have an anti-Ro precipitin without an anti-La precipitin, and it does not exist for those who have both anti-Ro and anti-La precipitins. Preliminary analysis is consistent with synergy between the T cell receptor association and the HLA-DQ alleles that are associated with anti-Ro (74). Other work with the 70-kd U1 ribonucleoprotein has shown that lymphocytes proliferate after exposure to this peptide when it is presented as a fusion protein, and that a region from the carboxyl terminus is more stimulatory than other regions of the molecule (75).

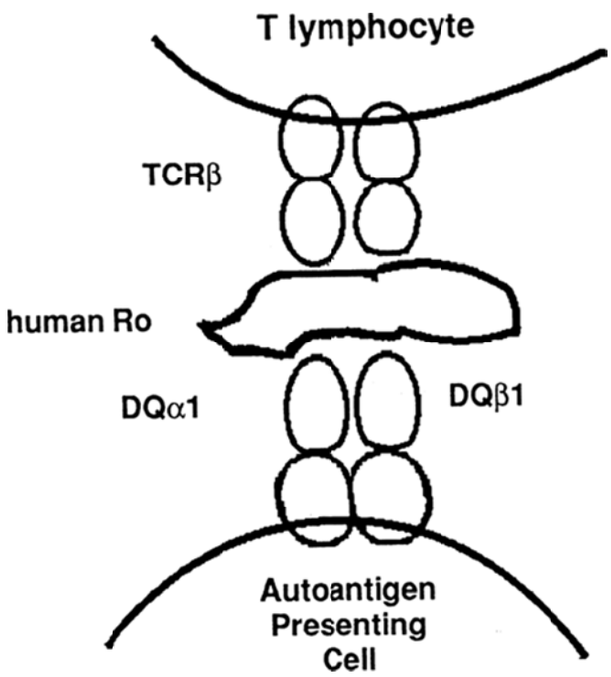

Figure 25-3. The trimolecular complex model for autoantigen presentation of the human Ro autoantigen based on associations of anti-Ro with alleles at human leukocyte antigen (HLA)-DQ and the ß peptide of the T-lymphocyte receptor.

These data are consistent with a model of the generation of lupus autoantibodies in general, and of anti-Ro in particular, that requires the participation of HLA-DQ and T cell receptor alleles along with the autoantigen to form a trimolecular complex (Fig. 25-3). Human Ro is suspected to be directly involved, because Ro from other species is less antigenic with human autoantibodies (76). From these data the conclusion that the anti-Ro autoimmune response is Ro autoantigen—driven in lupus is compelling. Similar conclusions have been reached using other experimental strategies for the anti-Sm and anti-DNA responses in lupus (77 ,78). Accordingly, autoantigen—driven autoimmune responses appear to be the general rule in lupus.

Some of the genetic risk factors for the generation of individual autoantibodies have been defined. Associations with clinical manifestations also are known. The question that remains to be determined, however, is how the clinical features, immunogenetics, and autoantibodies are interrelated in lupus, although one would suspect that relationships should flow from the genetic features to the autoantibodies and from autoantibodies to the disease manifestations. This concept of the disease predicts, for example, that HLA alleles generally are related to clinical manifestations through the autoantibodies, and that the relationships of autoantibodies with HLA alleles and of autoantibodies with clinical manifestations will be stronger than those of HLA alleles with clinical manifestations.

This hypothesis was confirmed in a group of 40 patients with lupus in whom the anti-La, anti-Ro, anti-Sm, anti-nRNP, anti-single-stranded DNA (anti-ssDNA), and anti-dsDNA were measured (23). The first four specificities were detected by gel diffusion. Antibodies to ssDNA were detected by radio-immunoassay and anti-dsDNA antibodies were detected by the Crithidia assay. Primary relationships were found between HLA-DQw1/w2 and anti-Ro, and between HLA-B8, DR3, and anti-La. Primary associations were found between anti-Ro and lymphopenia, and between anti-La and the absence of nephritis. Nephritis was present if either proteinuria exceeded 0.5 g per 24 hours or cellular casts were present in the urine. No statistically relevant relationships were present between any of the HLA antigens that were determined and any criterion for the classification of lupus (22). Logistic regression analysis established that the presence of lymphopenia was best explained by considering the combined contributions of anti-Ro and anti-ssDNA (23).

The relationship of anti-La with the absence of nephritis was analyzed by an analogous approach (23). The literature inconsistently shows an association of anti-dsDNA with nephritis, although there is convincing evidence that some anti-dsDNA antibodies are deposited in the kidney (79 ,80 ,81 ,82 ,83). On the other hand, both anti-La and anti-nRNP have been associated with a decreased incidence of nephritis in lupus (28 ,84 ,85). In this group of 40 patients with lupus, there was no simple association between anti-dsDNA and nephritis (23). Logistic regression analysis, however, produces an interesting result (Table 25-2). The association of anti-La with the absence of nephritis is powerful; however, once this effect is incorporated into the logistic model, then anti-dsDNA makes an important contribution. Here, anti-La and anti-dsDNA have opposing effects, which is demonstrated in the resulting logistic equation presented in Figure 25-4 . These data support a mechanism

of disease expression in which the clinical manifestations result from complicated interactions of various kinds of autoantibodies. The autoantibodies in turn are strongly influenced by the particular HLA and T cell receptor alleles that are present. One mechanism to explain these relationships is the recent demonstration that subsets of anti-La antibodies are in fact anti-idiotypes to anti-dsDNA (86).

Table 25-2: Logistic Regression Model of Renal Disease*

		Improvement		Goodness of Fit			Standard
Step	Term	x^2	*p*	x^2	*p*	Coefficient	Error
1	Anti-La(SS-B)	8.7	.003	33	.50	-1.22	0.52
2	Anti-dsDNA	6.9	.008	26	.79	2.75	1.19

*Anti-La (SS-B) is presented as the log10 of the ELISA units of the La (SS-B) solid-phase binding activity (range 2–7.03). Anti-double-stranded DNA (anti-dsDNA) is a dichotomous variable (1 = positive and 0 = negative). From Harley JB, Sestak AL, Willis LG, et al. A model for disease heterogeneity in systemic lupus erythematosus. Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease. *Arthritis Rheum* 1989;32:826–836, with permission.

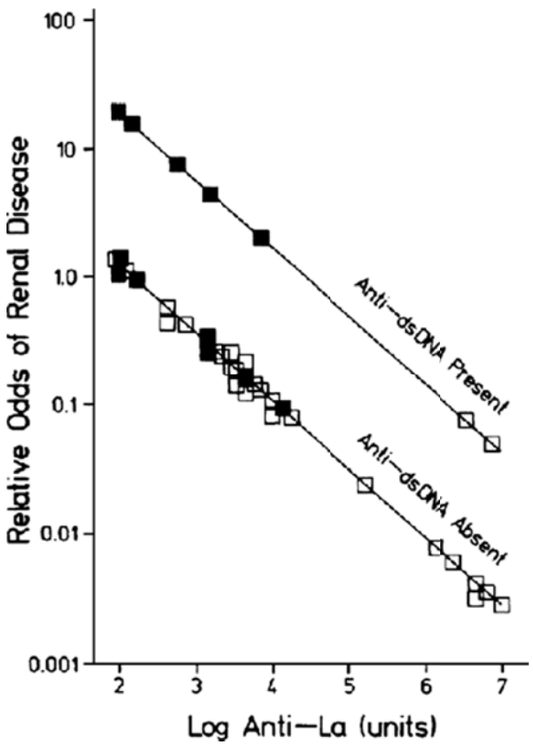

Figure 25-4. Relative odds of lupus nephritis in 40 patients with lupus as modeled from the anti-La and anti-double-stranded DNA (anti-dsDNA) antibody levels. Anti-La titer is expressed as the log10 of the relative binding in a solid-phase assay using purified bovine La. The presence or absence of anti-dsDNA is indicated. The presence (closed squares) or absence (open squares) of renal disease is indicated as well. The relative odds of renal disease are calculated from the logistic regression model presented in Table 25.2 (*n* = 40): ln (relative odds of renal disease) = -1.22 (anti-La) + 2.75 (anti-dsDNA) + 2.67. (From

Harley JB, Sestak AL, Willis LG, et al. A model for disease heterogeneity in systemic lupus erythematosus. Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease. *Arthritis Rheum* 1989;32:826–836, with permission.)

Assays for Detecting Anti-Ro and Anti-La

In clinical practice, Ouchterlony double immunodiffusion is the traditional method of determining whether anti-Ro or anti-La is present (Fig. 25-1). Most data relating these serologies to clinical manifestations have been developed using either this technique or the closely related procedure of counterimmuno-electrophoresis. Double immunodiffusion is specific and sufficiently sensitive for nearly all clinical applications. Unfortunately, the procedure usually requires 2 days to complete, and its performance requires specialized training. Not infrequently, an anti-Ro precipitin is missed, either because poor-quality reagents have been used or because an inexperienced person is performing the test.

These difficulties and inefficiencies have provided an incentive for the development and marketing of alternative methodologies. Immunoprecipitation, immunoblotting, and solid-phase ELISAs are available in research laboratories. Anti-Ro and anti-La ELISAs have been marketed to clinicians and clinical laboratories and these assays have largely supplanted the more traditional methodologies for clinical assessment. These assays appear to provide as much as a 10- to 100-fold increase in sensitivity over double immunodiffusion. There is greater potential for interfering and artifactual binding in ELISA, and consequently the specificity of these assays is reduced relative to double immunodiffusion. The increased sensitivity means that half or more of patients with lupus, and perhaps over three quarters of patients with Sjögren syndrome, are positive for anti-Ro in these assays. The proportion of patients who are positive for anti-La often is doubled, from 10% to 20% in lupus and from 15% to 40% in Sjögren syndrome, by the application of the routinely available assays.

The antigenicity of the Ro antigen is adversely affected by denaturation and often is not detected whether the antigen is fixed in situ (e.g., in a cell line for the antinuclear antibody test) or by another method (e.g., by boiling in sodium dodecyl sulfate for the Western blot test). Indeed, the Ro antigen, as expressed by bacterial systems, produces an antigen that has a similarly profound reduction in antigenicity. Thus, Western blotting or reliance on Ro antigen in Western blots or on expression in a bacterial system from a recombinant complementary DNA (cDNA) will provide misleading results unless special precautions or adjustments are made.

Another ingenious assay is available for the detection of anti-Ro. Three groups have transfected cell lines with the coding sequence of the 60-kd Ro protein behind a promoter that increases the production of Ro protein in the cell (87 ,88 ,89). With the increased Ro antigen available, the problems that are associated with fixation may be overcome (87). Indeed, HEp-2 slides with overexpressed human Ro are now commercially available (HEp-2 2000).

The importance of identifying patients who have anti-Ro or anti-La by an ELISA is problematic for those who do not form precipitins. A significant proportion of normal individuals may fall into this category. At present, this level of anti-Ro does not assist in formulating diagnosis or prognosis. The ready availability and relative ease of performing these solid-phase assays dictates their increasing use. Nevertheless, whatever assay the clinician chooses, he or she should fully understand the meaning of the results and the limitations on their interpretation.

The levels of anti-Ro and anti-La vary over time by as much as 10- to 20-fold during the course of the disease, but the relevance of this to disease expression is not known. Mildly affected patients have been known, or inferred, to have had anti-Ro or anti-La precipitins for decades. It is rare to observe the disappearance of an anti-Ro or anti-La precipitin after disease onset unless the patient has had aggressive cytotoxic therapy, corticosteroids at high doses for an extended period, heavy proteinuria, or renal failure.

Molecular Considerations

Important progress also has been made in defining the molecular properties of the Ro and La antigens. All four human Ro-associated RNAs, known as hY RNAs, have been sequenced (Fig. 25-5). Each is a product of RNA polymerase III and is from 84 to 112 bases in length. The hY RNAs have a triphosphate 5′ terminus and a polyuridine 3′ terminus (90 ,91). Two highly conserved regions of 24 bases, one half from the 3′ end and one half from the 5′ end, are found in each hY RNA sequence (92). Part of this conserved region is thought to bind to the Ro protein by virtue of its being protected from RNAse digestion of the intact ribonuclear particle (91). From two to four Y RNAs have been isolated from every other vertebrate species evaluated (93), leading to the impression of substantial heterogeneity in Y RNAs between species. The Y3 RNA appears to be the most conserved among vertebrate species (94).

The peptide with a molecular weight of approximately 60 kd is the major antigenic peptide in the Ro RNA protein particle (95). Two sequences of this peptide have been obtained, which are essentially identical except for a small region of sequence divergence at the amino terminus (96 ,97). The 60-kd Ro peptide has a putative ribonucleoprotein binding domain and a zinc finger. Itoh et al. (98) have presented evidence for two antigenically related forms of 60-kd Ro. Of the Y RNAs, only hY5 is antigenic (99).

A number of other polypeptides have been related to anti-Ro autoantibodies, including 54-kd Ro and 60-kd Ro, which are both found in red cells; 52-kd Ro, a 57-kd polypeptide; and calreticulin. With La, these constitute a family of autoantigens. Except for calreticulin (100 ,101 ,102), antibodies that bind these autoantigens are found virtually only in sera that contain precipitating levels of anti-Ro autoantibodies.

A 52-kd peptide has been identified from lymphocytes by immunoblot (103), and its cDNA has been cloned and sequenced (104 ,105). Although one study detected a molecular association of the 52-kd Ro with the 60-kd Ro protein (106), this has not been confirmed, and most existing data support there being no stable molecular association between 52-kd Ro and the 60-kd Ro (107 ,108 ,109 ,110). The Ro hY RNAs are immunoprecipitated in association with 60-kd Ro (110 ,111). While some anti-Ro and anti-La sera immunoprecipitate the 52-kd protein, most appear to contain antibodies against the denatured form of 52-kd Ro found in Western blot (110 ,111). The 52-kd Ro gene may be important beyond its being a target of autoimmunity, because an allele of the 52-kd Ro gene is associated with lupus in blacks (112 ,113).

In erythrocytes, 60-kd and 54-kd peptides are variably identified in immunoblot by different sera containing anti-Ro (114). The 54-kd erythrocyte Ro peptide appears to be antigenically related to the 52-kd lymphocyte Ro (104). Interestingly, only hY1 and hY4 RNAs are immunoprecipitated from human erythrocytes, where they are slightly smaller than in other human cell types (92). This difference probably results from the shorter polyuridine 3′ end on the Y RNAs found in erythrocytes. The Y RNAs in human platelets are demonstrated to be restricted to hY3 and hY4 (115).

A 46-kd protein has been identified and sequenced using patient sera that contain anti-Ro activity. This sequence appears to be the human form of calreticulin, which is a calcium-binding protein (116). No clinical associations with this autoantibody have been established.

Maddison et al. (117) defined another specificity found in sera with anti-Ro autoantibodies: anti-p57. These antibodies are found in approximately 10% of patients with lupus and in nearly 40% of mothers of infants with complete congenital heart block or neonatal lupus dermatitis.

Ro particles also have been studied without exposure to denaturing conditions to allow an evaluation of in vivo Ro particle composition. In gel filtration, these particles range from 230 to 350 kd, thus supporting the position that there may be more than one peptide in a Ro particle (118). One of these particles appears to contain the hY5 RNA and not the other hY RNAs (99 ,118).

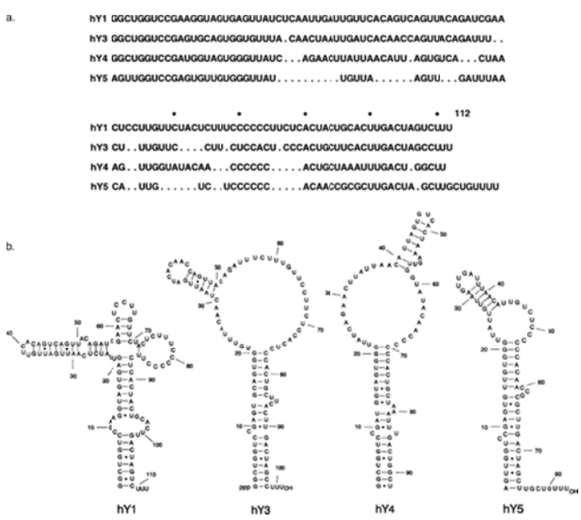

Figure 25-5. hY RNAs: **A,** Sequence comparison of the four Y RNAs. Periods indicate gaps in the sequence arranged to maximize sequence alignment. **B,** Proposed secondary structures of the hY RNAs. (Adapted from Farris AD. *Phylogenetic analysis of Ro ribonucleoprotein associated small RNAs [dissertation].* Oklahoma City, OK; University of Oklahoma Health Sciences Center, 1995:119, for hy1; Farris AD, O'Brien CA, Harley JB. Y3 is the most conserved small RNA component of Ro ribonucleoprotein complexes in vertebrate species. *Gene* 1995;154:193–198, for hY3 and hY4; and O'Brien CA, Margelot K, Wolin SL. Xenopus Ro ribonucleoproteins: members of an evolutionarily conserved class of cytoplasmic ribonucleoproteins. *Proc Natl Aca Sci USA* 1993;90:7250–7254, for hY5.)

At least one of these isolated Ro particles also contains the La peptide, thereby providing a structural basis for the association of anti-Ro and anti-La autoantibodies in patient serum. The La peptide contains 408 amino acids and has a predicted molecular weight of 46.7 kd (119 ,120 ,121). La also has an 80 amino acid conserved element referred to as the RNA recognition motif (122). The carboxyl end of the protein is methionine free and phosphorylated, while the amino end is methionine rich (123). The La peptide appears to bind any RNA with a polyuridine 3′ end (124 ,125). In addition to the Y RNAs, these include the precursors of 7S, 5S, U6, and precursors of transfer RNAs. The bound RNAs generally are the immature transcription products of RNA polymerase III, except for U1-RNA, which is an RNA polymerase II product that binds La (126).

Some virus-encoded RNAs also bind La from adenovirus, Epstein-Barr virus, and vesicular stomatitis virus (127 ,128 ,129). Additionally, La appears to play important roles in the expression of genes from poliovirus and human immunodeficiency virus (130 ,131).

There is evidence for La playing multiple roles in the molecular economy of the cell, while Ro has been implicated in only the discard pathway for 5S RNA (132) and with telomerase (133). La may function as a shuttle protein to carry RNA transcripts from the nucleus to the cytoplasm (134 ,135), and other investigators have obtained evidence that La is a termination factor for RNA polymerase III (136 ,137). La has been shown to melt an RNA-DNA hybrid in a reaction that requires adenosine triphosphate (ATP) hydrolysis (138) and to bind double-stranded RNA, thereby

influencing interferon-inducible protein kinase (139). The most intriguing data show that La increases the efficiency and fidelity of internal translation (131 ,140 ,141).

La proteins are found in widely divergent species, from humans to yeast. In all known instances, the La protein binds polyuridine termini of RNA (142). Despite all the activities found for La and the evolutionary implications of its presence throughout eukaryotic life, yeast are viable despite the destruction of the La protein gene (142 ,143).

Fine Specificity of Anti-Ro and Anti-La

With more detailed structural information now available, attention has turned to the fine specificity of anti-Ro and anti-La autoantibodies. By expressing fragments of the recombinant La cDNA clone, a number of groups have shown multiple epitopes on the La peptide that are distributed throughout the primary structure (119 ,144 ,145 ,146 ,147 ,148 ,149).

The 60-kd Ro peptide also appears to have multiple linear epitopes (150 ,151). In addition, there is evidence of multiple epitopes throughout the 52-kd Ro molecule (149 ,152), suggesting this is a general finding in lupus autoimmunity.

In both anti-Ro and anti-La autoantibodies, IgG1 predominates, with the other subclasses of IgG being variably represented (153 ,154 ,155). Anti-La is composed predominantly of IgG1 antibodies, while anti-Sm is not subclass restricted (153).

Unexpectedly, the antigenic peptides of 60-kd Ro tend to share short sequence homology with the nucleocapsid protein of vesicular stomatitis virus (151 ,150). Humans infected with vesicular stomatitis virus tend to have low levels of anti-Ro (156), and animals immunized with the cross-reactive nucleocapsid from vesicular stomatitis produce antibodies that bind 60-kd Ro (157).

There are many other hints about the origin of anti-Ro and anti-La autoantibodies. Sera with these antibodies and anti-dsDNA cross-react with the denatured Sm D and nRNP A polypeptides (158 ,159), thereby providing some unity for lupus autoimmunity across the major known protein autoantigen specificities. Antibodies that bind to hY5 RNA, but not the other hY RNAs, have been detected (99). Because Ro ribonuclear particles containing hY5 RNA can be isolated as distinct particles, some suspect that this may be the original autoantigen (99).

Very recently, a potentially important relationship has been discovered between anti-Ro and Epstein-Barr nuclear antigen-1 (EBNA-1) (163). The detailed sequence of events was revealed by serial sera from donors who had progressed from no anti-Ro to an anti-Ro precipitin over a few years of observation. (Only the anti-Ro sera alone were studied; none of these developed both anti-Ro and anti-La.)

In all informative sera the first anti-Ro activity detectable bound peptides from the amino acid sequence positions 169 to 180, TKYKQRNGWSHKD (using the single letter code for amino acids). Virtually all of the anti-Ro activity binds this single epitope at this point in anti-Ro development. Astonishingly, the affinity purified anti-TKYQRNGWSHKD from the sera where this is the only detectable anti-Ro activity also bind EBNA-1 at GGSGSGPRHRDGVRR (EBNA-1 amino acids 58 to 72). This presumed cross-reaction assumes a level of structural similarity between the Ro and EBNA-1 peptides, though it is formally possible that the anti-Ro sequence bound (TKYKQRNGWSHKD) and the anti-EBNA-1 sequence bound (GGSGSGPRHRDGVRR) are actually binding different variable parts of the IgG molecule.

In any case, these results provide a second example of a cross reaction between EBNA-1 and the first autoimmune epitope from a lupus autoantigen (162), reinforcing the potential etiologic relevance of EBV infection and SLE (164).

Mechanisms explaining maturation of the autoimmune response are under intensive inquiry. Epitope spreading has been shown for anti-La antibodies (163 ,164) and for the Sm B/B', where this maturation can be induced by immunization with a single peptide. Such a situation is also found in the restricted autoimmune response of the few patients with sera available from early in the disease process (167). Perhaps, defining the initial autoimmune response will provide important clues to etiology and pathogenesis.

Summary

Anti-Ro occurs in nearly half, and anti-La occurs in about one-fifth of patients with SLE. Anti-La always occurs in association with anti-Ro. Anti-Ro is specifically deposited in skin in lupus and is likely involved in the pathogenesis of cutaneous injury. Thrombocytopenia, when it occurs before other clinical signs of lupus, is usually accompanied by anti-Ro. Anti-Ro and anti-La can occur many years before clinical disease of lupus or Sjögren syndrome as evidenced by its association with neonatal lupus. Patients with both anti-Ro and anti-La very infrequently have renal disease. Lupus patients with the clinical findings of subacute cutaneous lupus erythematosus (SCLE) invariably have anti-Ro autoantibodies detected by a sensitive ELISA. There is substantial evidence for the participation of anti-Ro in lupus nephritis.

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Chapter 26

Antibodies to Spliceosomal Components

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Judith A. James

Antispliceosomal autoantibodies are commonly found in systemic lupus erythematosus (SLE) and related systemic autoimmune rheumatic diseases (1 ,2). Anti-Sm and anti-nRNP, which target these spliceosomal proteins, are useful in the diagnosis of SLE and other rheumatic diseases and in spliceosomal characterization. This chapter briefly reviews the structure and function of the targets of these autoantibodies, the methods of detection for these autoimmune specificities, the clinical associations with these autoantibodies and provides a summary of evolving information regarding the epitopes, origins, and potential pathogenic features of spliceosomal autoimmunity.

Structure of the Spliceosome

An important functional component in eukaryotic cells is a variety of discrete complexes consisting of proteins and RNA molecules. Complexes of this theme exist in every compartment of the cell: cytoplasm, mitochondria, nucleolus, and nucleus. Small nuclear ribonucleoprotein complexes (snRNPs) are protein-RNA complexes that are localized to the nucleus and are intimately involved in RNA processing as essential components of the multiprotein splicing machinery known as the spliceosome. Historically, they were identified as autoantigens of patients with autoimmune disorders, particularly SLE (3 ,4 ,5 ,6 ,7 ,8). The U1, U2, U4, U5, and U6 snRNPs are so named after their highly structured RNA counterparts (i.e., U1 snRNA, U2 snRNA, and so forth), and are quite abundant, ranging from thirty thousand to one million copies per cell (9). Each snRNP is composed of a small (i.e., less than 190 nucleotide) RNA molecule that is made by RNA polymerase II and has an unusual trimethylated cap (with U6 an exception on both counts; reviewed in (10)). U1, U2, U4, and U5 snRNPs contain a set of proteins common to all snRNPs; these proteins are called the Sm proteins (B, B', D1, D2, D3, E, F, and G) and range in molecular weight from 29 to 9 kD (reviewed in (11)). The crystal structure of the Sm protein complexes has recently been revealed (12). U1, U2, U4/U6, and U5 snRNPs also contain proteins uniquely associated to each particular complex (reviewed in (13)).

The U1, U2, U4/U6 and U5 snRNPs represent the so-called “major” spliceosome components which are involved in the splicing of approximately 99% of all introns. However, another class of spliceosomes exists in human cells, the “minor” spliceosome, which consists of U11, U12, U4atac, and U6atac (reviewed in (14)). The U5 snRNP is found in both classes. U11, U12, and U4atac snRNPs contain Sm proteins along with other specific protein components and are the U1, U2, and U4 counterparts, respectively (14). Cellular assembly and biogenesis of U snRNPs has been a matter of much investigation recently (reviewed in (15 ,16)). The snRNP assembly pathway is complex, involving export of snRNA from the cell nucleus to the cytoplasm, assembly of the Sm proteins onto the snRNA in the cytoplasm, and re-import of the mature snRNP into the nucleus. Assembly of the Sm complex onto the snRNA is accomplished by the survival of motor neurons (SMN) complex (15 ,16 ,17 ,18). The final steps of snRNP maturation take place in discrete sub-nuclear domains called Cajal bodies (19 ,20). Recently a rare specificity of SLE patient sera with reactivity against Cajal body proteins was noted in a study in Mexico (21).

SnRNPs and Associated SnRNP Factors

SnRNPs, as defined originally, have a set composition of RNA and protein components distinct to that particular snRNP in addition to the Sm, or “core”, complex (11 ,22). The U1snRNP has three unique proteins, called U1 70K, U1A, and U1C. The U2snRNP has eleven unique proteins (23), including two proteins called U2A' and U2B”. This nomenclature is somewhat confusing but historical in nature based on relative size and not actual similarity. The U4/U6 snRNP is formed by extensive base-pairing via intermolecular helices between the two RNA molecules, and has two proteins of 60 and 90 kD uniquely associated (24). The U5 snRNP has at least 9 proteins associated with it uniquely: these are proteins of 220, 200, 116, 110, 102, 100, 52, 40, and 15 kD (25). However, these snRNPs have additional, non-snRNP factors associated with them; these associations will be highlighted here.

The U4/U6 and U5 snRNPs interact with each other in the nucleus, and this association helps to form the mature spliceosome (reviewed in 26). The tri-snRNP complex has

five additional proteins (63, 61, 27, 20, 15.5 kD) that are not part of either the U4/U6 snRNP or the U5 snRNP (25). One of these proteins (20 kD) has been recently identified as a novel cyclophilin, and has been shown to exhibit the cis/trans isomerization of peptidyl-prolyl bonds characteristic of cyclophilins (27). This interaction may possibly reflect a role in the assembly of the tri-snRNP complex or may be involved in conformational changes of proteins required during spliceosome assembly. Another of these proteins, the tri-snRNP-specific 27K protein, has been identified as a member of the SR protein family (see below) (28).

Another notable interaction with snRNPs is a 69kD protein (known as 69KD) that shares structural homologies with human translocated in liposarcoma (TLS) and Ewing sarcoma (EWS) proteins (29 ,30). This protein copurified with snRNPs, particularly U1snRNPs, and associated with the Sm complex of proteins in Xenopus micro-injection experiments as well as in in vitro binding studies. Since TLS and EWS share domains with 69KD that act as transcriptional activators, it is interesting to speculate what role 69KD might also play in mRNA transcription.

SnRNPs and Splicing Factors

Splicing of pre-mRNA is an intricate process involving more than 100 proteins and at least 5 RNA factors (reviewed in (26 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39)). Splicing is a two-step process in which the coding regions (exons) are retained and the noncoding regions (introns) are removed (Fig. 26-1). Catalytic step I results in cleavage of the 5′ splice site, yielding a “free” 5′ exon and a lariat intermediate. Step II involves ligation of the 5′exon to the 3′ exon with displacement of the intron as a lariat product. Cis acting signals present within the mRNA are also involved, such as splice donor and acceptor sites at the 5′ and 3′ ends of the introns, respectively. Recently, exonic sequences have also been identified that promote (exonic splicing enhancer, ESE) or repress (exonic splicing silencer, ESS) utilization of alternative splice sites (reviewed in (40 ,41 ,42 ,43)). These may become more important in the future as alternative splicing is becoming linked to many diseases.

Assembly of the mammalian spliceosome begins with the association of the U1snRNP at the 5′ splice site by base-pairing of U1 RNA with the 5′ splice site, followed by association of the U2 snRNP at the branch site. The U4/U6-U5 tri-snRNP complex then binds, U1 and U4 are destabilized and released, and the spliceosome is activated for catalysis (Fig. 26-1). Changes in intermolecular basepairing between the snRNAs also occur. Recently, cryo-electron microscopy has helped to reveal the three-dimensional structure of individual snRNPs as well as the structure of the native spliceosome (44 ,45 ,46).

Additional, non-snRNP proteins are also required for efficient splicing. In an early step, pre-mRNAs are committed to the splicing pathway not only through association with the U1snRNP, but also with non-snRNP factors, including the U2 auxiliary factors U2AF65 and U2AF35 as well as members of the SR protein family (47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56). SAPs, or spliceosome-associated proteins, are additional non-snRNP proteins that are involved in splicing of pre-mRNA, and include such proteins as polypyrimidine tract binding protein associated splicing factor (PSF) (50 ,57 ,58 ,59). HnRNP proteins are yet another group of highly conserved, non-snRNP RNA binding proteins that participate in pre-mRNA processing (reviewed in (60 ,61)). Interactions of SR proteins and hnRNP proteins with target sequences in the pre-mRNA appear to play important roles in directing alternative splicing, perhaps with additional protein factors in some cases (reviewed in (62)).

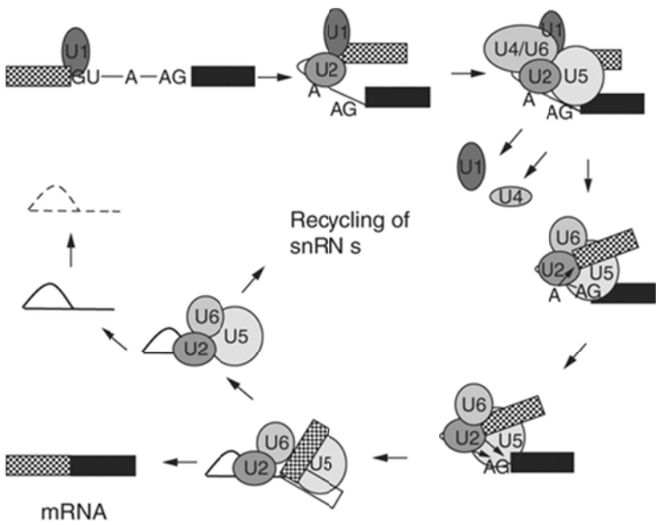

Figure 26-1. Schematic of the splicing cycle. Black and black/white checked boxes represent exons 1 and 2, respectively. The line indicates the intron. Pre-mRNA enters splicing complexes with snRNPs and leaves the cycle as ligated, mature mRNA. Highly conserved GU (5′ splice site), AG (3′ splice site) and A (branch point) residues are noted. Individual snRNPs are depicted as U1, U2 U4, U5, and U6. Other, non-snRNP splicing factors have been omitted for simplicity. The excised intron lariat is degraded after splicing is complete. (Adapted from Moore MJ, Query CC, Sharp PA. Splicing of precursors to messenger RNA by the spliceosome. In: Gesteland RF, Atkins JF, eds. *The RNA World.* Cold Spring Harbor, NY: Cold Spring Harbor Laboraotry Press, 1993:303–358 and Jurica MS, Moore MJ. Pre-mRNA splicing: awash in a sea of proteins. *Mol Cell* 2003;12:5–14.)

Despite all these potential protein-protein interactions of snRNP proteins with the myriad of splicing factors, only a few direct interactions have been characterized. For example, Wu and Maniatis described the interaction between U1 70K and U2AF65 (51).

SnRNPs and Non-SnRNP Complexes

SnRNP-free U1A complexes (SF-A) were discovered using a mouse monoclonal antibody (12E12) (63 ,64). This antibody is specific for U1A as shown by immunoprecipitation followed by Western blotting, but does not immunoprecipitate U1A that is complexed with U1 RNA as shown by Northern blotting. This suggests that SF-A is not complexed with U1 RNA. Additional anti-U1A monoclonal antibodies

that we prepared (1E1, 10E3, and 20F8) precipitated the U1snRNP. These data suggest that monoclonal antibody 12E12 is unable to recognize U1A when it is bound to U1 RNA because the epitope is “masked” (63).

Immunoprecipitation of ^{35}S labeled cell extracts using monoclonal antibody 12E12 revealed additional, non-snRNP proteins associated with U1A. In contrast, monoclonal antibody 10E3 primarily immunoprecipitated the U1snRNP and associated proteins. To demonstrate that the association of U1A with non-snRNP proteins is genuine and not a result of antibody cross-reactivity, 5% to 30% sucrose gradient fractionation of ^{35}S labeled cell extracts was performed, followed by immunoprecipitation (63). The SF-A fraction coprecipitates with at least five other non-snRNP proteins (p105, p65, p63, p59, and p58), and migrates with these proteins during sucrose gradient centrifugation as a novel complex of proteins. The gradient analysis of the SF-A complexes indicates a core complex consisting of U1A, p58, p65, and p105 and suggests that p59 and p63 dynamically associate with the core complex. No RNA species have been detected with the SF-A complex as determined by immunoprecipitation of ^{32}P-orthophosphate labeled cell extracts. Thus, these data establish that U1A protein is not an exclusive component of the U1snRNP. A significant fraction is found in a snRNP-free form in a complex with other cellular proteins (63 ,64) and some SLE patients appear to recognize this novel complex (65).

One of the proteins in the SF-A complex has now been identified as the splicing factor PSF the polypyrimidine-tract binding protein associated splicing factor (57 ,66). Additionally, p68 helicase and p54nrb have been found to be part of the SF-A complex using epitope-tagged U1A (66). Another report has shown that p53 and human MDM2 can be found in association with U1A in the absence of the Sm proteins in leptomycin-treated human primary fibroblasts (67).

SnRNPs and Viral Proteins

Generally, viruses accomplish transcription of their own genes over those of the host cell in a variety of creative ways. Some of these ways include shut-off of host cell splicing by redistribution of splicing factors, strong promoter recruitment of transcription factors, and commandeering the RNA processing machinery. Therefore, opportunities abound for direct interaction with snRNP proteins. Yet these interactions have just begun to be understood. A recent report reveals that human papillomavirus type 5 (HPV-5) E2 transcriptional activator has a hinge region that resembles the SR proteins, and this region interacts in vitro with U1 70K, U5 100 kD, ASF/SF2, and SC35 (68). A two hybrid screen in the same report shows much weaker interaction in vivo of HPV-5 E2 protein with U1 70K and U5 100 kD than that with ASF/SF2 and SC35, but the authors state that this could be due to reduced amounts of the proteins produced in yeast. This report suggests that HPV-5 E2 protein may assist in coupling viral transcription and splicing.

Herpes simplex virus type 1 (HSV-1) accomplishes host cell shut-off by reorganization of snRNPs and inhibition of host cell splicing (reviewed in (69)). Recent studies have shown that the HSV-1 regulatory protein ICP27 is responsible for this effect, and that ICP27 is co-immunoprecipitable with anti-Sm antiserum (70). This suggests that ICP27 interacts with snRNPs during HSV-1 infection but does not demonstrate whether the interaction is direct or indirect.

Protein Targets of Anti-Spliceosomal Autoimmunity

Anti-Sm autoantibodies are directed predominantly against the Sm B, B' (71) and Sm D1 proteins (72) and immunoprecipitate the U1, U2, U4/U6, and U5 RNAs (73). Minor responses are also detected against Sm D2, Sm D3, E, F, and G. As much as 40% of the entire immunoglobulin repertoire of lupus patient sera may bind Sm proteins (74). Nearly all of the SLE patients who have anti-Sm antibodies also have anti-nRNP antibodies. Those who begin with anti-Sm alone nearly universally develop anti-nRNP reactivity over the course of their disease (75). Anti-nRNP autoantibodies immunoprecipitate only U1 RNA and bind the nRNP 70K, nRNP A, and nRNP C proteins (76). Additionally, anti-U2 RNP antibodies recognize A' and B” proteins of the U2 snRNP (77 ,78 ,79) and rare responses specific to U4/U6 (80 ,81), U5 (82), U7 (83), and U11 snRNPs (84) have been reported.

Assays for Measuring Anti-Sm and Anti-U1 RNP Antibodies

The methods for detection of anti-Sm and anti-nRNP autoantibodies have expanded greatly over the past two decades, now to include at a minimum immunofluorescence, immunodiffusion, immunoblotting, immunoprecipitation of proteins or RNAs, and enzyme-linked immunoabsorbent assays (ELISAs) (85 ,86 ,87 ,88 ,89 ,90 ,91 ,92) (Fig. 26-2). Great strides have also been made in the development of detection methods for these autoantibodies by protein microarrays, bead assays and peptide-based methods (93 ,94 ,95 ,96 ,97).

Immunofluorescence remains a dominant screening method for detection of spliceosomal autoantibodies. Anti-Sm and anti-nRNP autoantibodies bind to proteins in the cell nucleus in a nuclear speckled pattern, as do the remaining spliceosomal autoantibodies (2). However, this staining pattern is not specifically unique for spliceosomal antibodies. For many years immunodiffusion has served, and in some locations continues to serve, as the primary method of detection for anti-Sm and anti-nRNP. Indeed, formation of precipitin lines with patient sera against calf thymus extract serves as the basis for many of the clinical diagnostic criteria and clinical association afforded these autoimmune responses (85 ,86). Additional sensitivity may be increased by counterimmunoelectrophoresis (87). Many clinical laboratories, however, are moving to testing by ELISA or perhaps Western blot analysis.

ELISAs for spliceosomal autoantibody detection (Fig. 26-2) have been developed and commercialized (88 ,89). These assays require the binding of a purified or recombinant

antigen to the bottom of a 96-well plate, blocking of nonspecific antibodies and then incubation with various dilutions of patient or control sera. A secondary antibody with an enzymatic tag is developed with substrate to induce a colorimetric change, which is quantified by reading the optical density at given wavelengths. These optical densities (ODs) may be reported directly, or oftentimes are converted to international standardized ratios (ISRs) based upon normalization with positive, negative, and calibrator controls. The major benefits of the ELISA methods are ease of operation, minimized time for results, and increased sensitivity. The primary limitation is the potential for false-positive results.

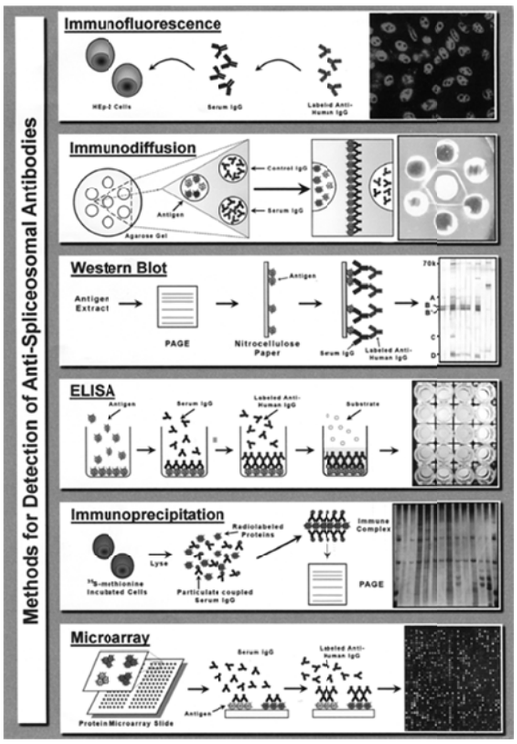

Figure 26-2. (See color plate.) Methods of anti-Sm and anti-nRNP detection. Several of the most common forms of autoantibody detection are presented in this figure with schematic diagrams followed by examples of data produced from each method. Additional detail regarding each method with references is presented within the body of the chapter. The initial approach is still considered the gold-standard screening approach for autoantibody detection, immunofluorescence. However, immunodiffusion is still considered the gold-standard for confirmation of the specific anti-Sm and anti-nRNP immune responses. Western Blot analysis is an immunoblotting technique which allows the determination of antibody binding to specific protein targets. Enzyme-linked immunosorbent assays (ELISAs) are becoming more widely used and have increased sensitivity (with some loss of specificity). Finally, new approaches are being developed to test hundreds of proteins at a time with micro-array analysis.

Additional approaches are available to detect specific protein targets of these autoantibody responses. Two of the most common are immunoprecipitation and immunoblotting. Immunoprecipitation requires the radioactive labeling of cellular proteins that are mixed with patient sera coupled to a particulate carrier, such as protein A-sepharose (90).

Once binding of the antigen to the antibody has occurred, the complexes can be immunoprecipitated with the particulate carrier, run on polyacrylamide gel electrophoresis and identified by autoradiography (Fig. 26-2). The benefits of this approach are the high sensitivity, high specificity and ability to detect U snRNAs. The limitations include use of radioactivity, length of time for results and the highly specialized training required of lab personnel to perform this approach.

Immunoblotting can also identify antibody reactivity with a specific protein of the anti-Sm or anti-nRNP responses (91 ,92). Cellular extracts or purified antigens are separated by gel electrophoresis, transferred to a membrane and then probed with dilutions of patient sera (Fig. 26-2). Using a secondary antibody with enzymatic substrate, color development demonstrates the binding of patient antibodies to specific spliceosomal proteins. Pre-run blots containing spliceosomal proteins are now commercially available. The benefits of this approach include specific protein detection, good sensitivity, and specificity. The potential drawbacks include the preference of this method to detect antibodies directed against denatured proteins and specialized training of personnel.

Several new approaches are emerging to detect spliceosomal autoantibodies. Protein micro-array experiments allow the coupling of recombinant or highly purified spliceosomal autoantigens to glass slides. These protein microarrays are incubated with patient sera and then incubated with a secondary antibody coupled to a fluorescent dye (93 ,94). The intensity of these spots can be measured and compared to standards to determine patient serum reactivity with spliceosomal antigens. Similar approaches using proteins coupled to beads as the antigen source are also becoming available (95). An automated multiparameter line immuno-assay system is a commercialized, standardized, modified immunoblot for which nine individual recombinant or purified antigens are coated as discrete lines on nylon strips, incubated with patient sera followed by a substrate secondary antihuman IgG conjugate and developed colorimetrically (96). Bands are compared to standards run in parallel. This approach appears to have similar sensitivity and specificity to anti-Sm and anti-nRNP but has decreased cost and decreased serum use (96). A modified radioactive method has also been recently reported. Radioligand assays were performed following in vitro transcription and translation from the appropriate labeled cDNA of spliceosomal autoantigens. Patient sera autoantibodies react with this radiolabeled protein and were captured by protein-A Sepharose and quantified by radioactivity counting. This assay showed good specificity and sensitivity and even demonstrated a strong correlation between Sm B' antibody levels and the severity of SLE (measured by <Systemic Lupus Erythematosus Disease Activity Index [SLEDAI]) and showed a correlation between anti-U1A and nephritis (97). These approaches are still primarily used in research settings.

Table 26-1: Prevalence and Association with Anti-Sm and Anti-nRNP in Systemic Lupus Erythematosus and Related Disorders

Specificity	Clinical Association	Prevalence
Anti-Sm	SLE	10%–30%
Anti-nRNP (U1)	Mixed connective tissue disease	100%
	SLE	30%–40%
	Raynaud's phenomenon	60% (SLE)
		<20% (others)
	Myositis	10%
	Scleroderma	10%
	Sjogren syndrome	Rare
Anti-U2 snRNP	MCTD, SLE, scleroderma/myositis	Rare
Anti-U4/U6 snRNP	Sjogren syndrome	Very Rare
	Scleroderma	Very Rare
Anti-U5	SLE (with anti-nRNP)	Very Rare
	MCTD (with anti-nRNP)	Very Rare
	Scleroderma/Polymyostis (alone)	Very Rare
Anti-U7	Scleroderma	Very Rare
Anti-U11	Scleroderma	Very Rare
Anti-U1 RNA	SLE (with anti-nRNP)	15%
	MCTD (with anti-nRNP)	30%
	Scleroderma (pulmonary fibrosis)	5%–10%

Clinical Features Associated with Anti-Sm Antibodies

Sm antibodies are so specific for SLE that they are considered as one of the diagnostic criteria (98). Anti-Sm is detected in approximately 10% to 25% of European-American SLE patients and in higher frequencies in African-American patients (2 ,99 ,100 ,101 ,102) (Table 26-1). Estimates of anti-Sm

prevalence may be even higher with use of the ELISA method of detection without losing the sensitivity for SLE (103 ,104 ,105). SLE, or a disorder closely resembling SLE, is closely associated with anti-Sm in human lupus, inbred mice (lpr/lpr MRL) (106), induced models in mice and rabbits (107 ,108 ,109), two different studies of collections of canine lupus (110 ,111) and macaques fed alfalfa (112). The same disease phenotype found associated with the same autoantibody across these enormous species barriers suggest the fundamental contribution that this autoantibody system may make to SLE.

Patients with both anti-Sm and anti-nRNP precipitating autoantibodies appear to be a distinct subset of SLE patients. These patients have higher morbidity and mortality and more severe renal disease (113 ,114 ,115 ,116 ,117) in some studies. No clear association of SLE disease damage from two lupus clinics in Birmingham, UK with anti-Sm or anti-nRNP reactivity was found (118). Interestingly, patients initially diagnosed with SLE at or older than the age of 50 are less likely to have antibodies to Sm or nRNP proteins and may have a milder form of the disease (119), while childhood-onset SLE may be associated with increased prevalence of anti-Sm and anti-nRNP (120).

Other studies have sought to evaluate the association of anti-Sm specificity with various clinical manifestations. Recent work suggests an enrichment of anti-Sm and anti-nRNP in nephritis; however, only anti-Sm was associated in a multivariate analysis (121). A Japanese study showed that anti-Sm detected by ELISA was associated with a low frequency of progression to end-stage renal disease, but an increased prevalence of late-onset proteinuria and poorer prognosis than patients without anti-Sm (96). In older literature, however, anti-Sm was found less commonly in lupus nephritis (122). In a single center cohort study from Spain of over 600 SLE patients, Sm autoantibodies were associated with cutaneous lupus involvement (123). Sm antibodies are substantially more frequent in patients of African-American heritage (113) and controlling for association based upon ethnic heritage may impact some of the previous studies.

Although quite specific for SLE, anti-Sm reactivity has been reported (especially as detected by ELISA) in several other disorders. These include rheumatoid arthritis after infliximab treatment (124), parvoviral infection presenting as a lupus-like illness (125), pediatric HIV infection (126), monoclonal gammopathies (127), schizophrenia (128), and uveitis (129).

Recently, the American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines published a guidance document summarizing the current literature regarding clinical usefulness of testing for anti-Sm and anti-nRNP reactivity (130). A positive anti-Sm response by a number of methods was found to be very sensitive for SLE; however, based upon the low number of all SLE patients with anti-Sm the specificity was relatively low. Although one study shows that rising anti-Sm titers are predictive of disease flares (131) and another associates anti-Sm with more active disease (132), insufficient data was available in category A publications (based upon a standard Evidence Based literature review approach) (133 ,134) to determine the use of anti-Sm titers to predict disease severity or disease flares (130).

Clinical Features Associated with Anti-U1RNP Antibodies

Anti-nRNP antibodies are found in approximately 30% to 40% of SLE patient sera by immunodiffusion (99 ,100 ,127 ,135 ,136) (Table 26-1). Although sometimes found in combination with anti-Sm as outlined above, oftentimes anti-nRNP will be detected as the sole specificity. The primary clinical association with anti-nRNP antibodies is a clinical entity, mixed connective-tissue disease (MCTD), which is characterized by overlapping symptoms of lupus, scleroderma and myositis. High titers of anti-nRNP antibodies are a hallmark of this disorder and are considered a key feature for diagnosis (103 ,136 ,137). Anti-nRNP antibodies may also be found in other systemic rheumatic diseases such as scleroderma, polymyositis, rheumatoid arthritis, and Sjögren syndrome (103 ,135 ,136) or in patients with Raynaud phenomenon (138) (Table 26-1).

Some studies have focused on the clinical features associated with specific anti-nRNP responses directed specifically against the 70K or U1A proteins. The anti-70K response is found in 75% to 95% of anti-nRNP positive MCTD patient sera (117 ,139 ,140) but may be only found in 20% to 50% of anti-nRNP positive SLE patient sera (117 ,139 ,140). These associations may be significantly influenced by testing methodology used (115 ,127 ,136 ,141 ,142). When anti-nRNP positive individuals are evaluated together (both SLE and MCTD) then anti-nRNP is associated with Raynaud's phenomenon, myositis, esophageal hypomotility, lack of nephritis, and HLA-DR4 (117 ,141 ,143).

Anti-U1A antibodies are also commonly found in individuals with anti-nRNP by immunodiffusion. Overall, the frequency of anti-70K and anti-U1A responses are nearly equivalent in nRNP antibody positive individuals (144), yet this specificity may be present in 75% of anti-nRNP SLE patients and only 23% of all individuals with anti-nRNP (139). Cross-reactive regions between a common lupus U1A epitope have been shown with nRNP C and Sm B' (145 ,146) and this cross-reactive specificity is much more common in lupus than MCTD. Little clinical information regarding the anti-nRNP C response is available.

Again, the American College of Rheumatology Ad Hoc Committee on Immunologic Testing Guidelines has recently published a guidance document summarizing the current literature regarding clinical usefulness of testing anti-nRNP (130). Although with good sensitivity and modest specificity, a positive anti-nRNP response by several different methods was not a strong predictor of SLE over other autoimmune rheumatic diseases. Anti-nRNP did serve as the best clinical surrogate for mixed connective tissue disease (130). Category A publications evaluating the association of this autoimmune response and SLE disease severity or disease flares were not available for assessment (130).

Other Anti-SnRNP and U1RNA Antibodies

Antibodies against the U2, U4/U6, U5, U7, U11 snRNPs, and the U RNAs themselves have been detected infrequently. Significantly less information is available than with the anti-Sm and anti-nRNP response described above; however, all of these specificities appear to be associated with some forms of clinical overlap between SLE and other rheumatic disorders. Anti-U2 snRNP antibodies are found in up to 15% of SLE or MCTD patient sera and are often associated with clinical overlap syndromes, such as scleroderma with myositis, psoriasis, or Raynaud syndrome (2). Anti-U4/U6 snRNP antibodies have been described in a few patients with Sjögren syndrome and scleroderma (80 ,81). Anti-U5 snRNP antibodies are found in anti-nRNP or anti-Sm positive SLE or MCTD patients (2), but as a sole specificity anti-U5 snRNP reactivity has been reported in a single scleroderma/polymyositis overlap patient by two separate groups (91 ,147). Anti-U7 snRNP antibodies have been described in a few SLE patients (83) and anti-U11 snRNP antibodies have been found in a few scleroderma patient sera (84 ,148). Most of these autoantibodies also produce a nuclear speckled immunodiffusion pattern and immunoprecipitation remains the most common method of detection for these specificities.

Anti-U1RNA antibodies have been found in up to 38% of anti-snRNP positive patient sera and again are quite specific for SLE and MCTD based upon an early study by van Venrooij et al. (149). These antibodies were found in anti-nRNP positive but not in anti-Sm positive patient sera. These anti-U1RNA antibodies can bind to the various portions of the U1RNA, including the cap structure and two different hairpin loops (149). Recent work has confirmed the presence of these anti-U1RNA antibodies in SLE and MCTD patients, as well as described their presence in 61% of anti-nRNP positive systemic sclerosis patient sera (150). These anti-U1RNA antibodies were associated with pulmonary fibrosis in this study (150). Interestingly, U1RNA itself has been shown to induce innate immune signaling and may contribute to the immunogenicity of the 70K nRNP autoantigen (151).

Origins and Pathogenic Features of Spliceosomal Autoimmunity

Autoantibodies are a hallmark of SLE and serve as the only classification criterion present in over 98% of patients (99). Therefore, significant investigation has focused on understanding the initiation, perpetuation, evolution, and potential pathogenic roles of these autoimmune responses in SLE. Specific interest has focused on understanding potential genetic risk factors for these responses and considerable work in understanding the fine specificity of the humoral and T cell responses has occurred. This section will briefly summarize some of the areas of current investigation.

Genetics

Familial aggregation of anti-Sm antibodies by ELISA suggest that the anti-Sm response may be governed at least in part by genetic susceptibility (152 ,153). HLA associations have been previously reported between anti-Sm or anti-nRNP and HLA-DR2 and HLA-DR4, as well as with weaker associations with members of the DP, DQ and DR families (3 ,154 ,155). However, these weak associations do not appear to confer all of the risk. The IL-10 high responder haplotype has also been associated with anti-Sm positive SLE in a small cohort of Caucasian German patients (156). The genetic susceptibility to this abnormal immune response is likely multigenic and further information is needed.

Occurrence of Autoantibodies

Autoantibodies are often found years if not decades before clinical onset of SLE (157). Interestingly, the anti-nRNP and anti-Sm responses occur closer to the time of diagnosis than do other common lupus autoantibodies, such as anti-Ro and anti-La (157). nRNP 70K appears to be the initial target of the anti-nRNP response and Sm B/B' appears to be the initial target of the anti-Sm response as detected by immunoblotting of serial patient samples referred for clinical testing (91).

Humoral Responses and Epitope Mapping

Extensive work describing the key humoral antigenic targets of the spliceosomal proteins by SLE patient sera have been performed by recombinant protein mapping, deletion clone mapping, large synthetic peptide mapping, and solid phase overlapping octapeptide mapping
(158 ,159 ,160 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,182 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199). This large body of work is summarized in several reviews (158 ,159 ,160). Briefly, the work in the Sm B', Sm D1, 70K, and U1A systems will be outlined here.

Over the past 10 years, our laboratory has been involved in characterizing the fine specificity of the human lupus autoimmune response against the spliceosome. Mapping the fine specificity by describing the small peptide epitopes of these systems led to identification of the initial anti-Sm B' humoral epitope (107 ,161), descriptions of common epitopes of human SLE (107 ,145 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170), establishment of a new peptide-induced model of lupus autoimmunity (107 ,108 ,200), and identification of a potential etiological trigger for human lupus (201 ,202 ,203 ,204 ,205 ,206 ,207 ,208). We will first briefly discuss the B cell epitopes of anti-Sm and anti-nRNP in naturally arising SLE autoimmune sera. Next, we will briefly present the data from rabbits and mice, which support the theory that peptide immunization results in anti-spliceosome autoimmunity and the association data of human lupus and Epstein-Barr virus (EBV), the candidate etiologic agent identified by our immunochemical approach (145 ,161 ,163).

Characterization of the sequential antigenic regions of the autoantigens Sm B/B', Sm D1, D2, D3, nRNP 70K, nRNP A, and nRNP C have been published by our laboratory (107 ,145 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170) and other groups (158 ,159 ,160 ,161 ,162 ,163 ,164 ,165 ,166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,182 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199). At least

40 common antigenic targets of these responses have been described (158 ,159 ,160).

The anti-Sm B/B' response was the first antispliceosomal autoantigen evaluated extensively for common humoral targets (Fig. 26-3). SLE patient sera, which contain both anti-Sm and anti-nRNP autoantibodies, have a very homogeneous pattern of binding to the overlapping octapeptides of Sm B/B'. All anti-Sm B/B' patient sera bind five very similar regions of B/B'. Four of these epitopes are proline-rich, very similar regions: PPPGMRPP (which is repeated three times in the carboxyl region of the protein) and PPPGMRGP (145).

These antipeptide responses have proven interesting in several ways. First, specific reactivity with these peptides have been confirmed in a large number of patients (compared to controls) using a different peptide methodology, as well as in other research laboratories (145 ,172 ,173 ,174 ,175 ,176 ,177 ,178). Only SLE patient sera with anti-Sm autoantibodies bind these specific proline-rich sequences. Second, these sequences are the targets of two unique anti-Sm monoclonal antibodies developed from MRL lpr/lpr mice suggesting that these are primary targets not only in human but murine lupus (167). Third, this PPPGMRP(G)P structure is the target of human monoclonal antibodies derived from a SLE patient (171). Fourth, these proline-rich, carboxyl terminal regions of Sm B/B' are the first targets of the anti-Sm response in all of the several serial lupus patient sera tested to date (107 ,161). Fifth, some animals immunized with this initial epitope constructed on a branching poly-lysine backbone develop a lupus-like illness (107 ,108 ,200). Finally, antibodies to this repeated antigenic sequence of Sm B' cross-react with a similar sequence of EBV nuclear antigen-1 (EBNA-1) (145). Immunization with this EBNA-1 sequence, PPPGRRP, also can lead to lupus like autoimmunity and features of clinical disease (208). Immunization of inbred strains of mice with EBNA-1 by DNA immunization can lead to anti-Sm and anti-dsDNA antibody production (209). Association studies have shown an increased prevalence of seroconversion to EBV in pediatric and adult SLE patients compared to matched controls (201 ,202 ,203 ,204 ,205 ,206 ,207 ,210 ,211). Additional work has shown intrinsic defects in the control of EBV infection attributable to an abnormal, immune response to EBV in SLE patients compared to matched controls (212 ,213 ,214 ,215). Additional extensive work has mapped the common antigenic regions of the Sm D1, D2, and D3 humoral immune responses in human SLE (163 ,166 ,179 ,180 ,181 ,182 ,183 ,184 ,185). Figure 26-3 partially summarizes the collective work evaluating D1 epitopes. The carboxyl region of Sm D1 contains a common antigenic humoral target, spanning amino acids 83–119 and containing a long glycine-arginine repeat (163 ,166 ,179 ,180 ,181 ,182 ,183 ,184 ,185). This epitope has been found to be a major target of reactivity in nearly all studies completed. This epitope is also bound by the Y12 monoclonal antibody which is derived from MRL lpr/lpr mice (163 ,181). The additional major targets of the Sm D2 and Sm D3 proteins are also quite basic and contain similar glycine-arginine rich regions (166). One study has shown that dimethylarginines in both D1 and D3 are found in these common

antigenic regions and are preferentially bound by patient antibodies (190). SLE patient sera also contain antibodies that cross-react with these antigenic targets of Sm D1 and glycine-arginine regions of EBNA-1 (163 ,186). This common carboxyl-terminal humoral antigenic region of Sm D1 has also been shown to provide T cell help for the production of anti-dsDNA antibodies in an animal model of lupus (216). Recent work has shown that intravenous injection of the parent D1 protein can postpone murine lupus and induce regulatory T cells (217).

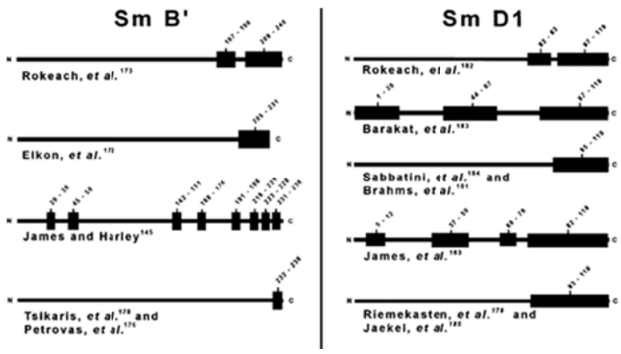

Figure 26-3. Summary of epitope mapping. Multiple groups have used various approaches to identify common humoral antigenic targets of Sm B' (74,172,173,174,175,176,177,178) and Sm D1 proteins (163,179,180,181,182,183,184,185). Antigenic regions of Sm B' identified by each individual study are presented in Panel A and common humoral epitopes of Sm D1 are presented in Panel B. These data are summarized in the following reviews (158,159).

The common humoral antigenic targets of the 70K and U1A responses have been reviewed in more detail in (158 ,159). Briefly, the 70K humoral epitopes have been evaluated by several different methods; however, nearly all studies have found key patient binding to the RNP-80 motif which is essential for binding of U1 snRNA (158 ,162 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196). Fine mapping of this RNP-80 region to key residues and additional 70K epitopes have also been described (158 ,162 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196). The humoral antigenic regions of the U1A response have been somewhat more diverse. Our group has described at least seven different sequential humoral epitopes, two of which are solely recognized by a subset of patient sera (164). One common area of antigenicity recognized by nearly all groups includes the RNP-80 region of U1A (158 ,164 ,197 ,198 ,199). Rabbits immunized with this RNP-80 common antigenic region develop antibodies to multiple different regions of U1A, develop other anti-Sm, anti-Ro, anti-nRNP, and anti-dsDNA antibodies, and develop renal insufficiency and thrombocytopenia (218). Rabbits immunized with another peptide epitope of U1A, which is recognized by various SLE patient sera and the monoclonal antibody which is "masked" by U1 RNA only make antibodies to the peptide of immunization (218). Less work has been performed with the nRNP C system to date.

T Cell Responses

Significant work has been done to characterize the common T cell responses and epitopes of the Sm and nRNP proteins in human SLE. Autoreactive T cells to Sm B', Sm D1, 70K, and U1A have been isolated from SLE patient peripheral blood (219 ,220 ,221 ,222 ,223 ,224 ,225 ,226 ,227 ,228 ,229). These are primarily $CD4^+$, TCR α/β^+ cells that produce substantial amounts of interferon-γ, moderate IL-2, and variable amounts of IL-4 and IL-10 (224). 70KsnRNP specific T cell clones can provide ex vitro help for anti-70K antibody production (228). Limited T cell epitopes have been mapped for these proteins and nearly all are located either in the Sm binding motifs of Sm B' and Sm D1 or the RNA binding domains of 70K. The TCR usage of these clones is quite restricted (226 ,227 ,228 ,229 ,230). Interestingly, recent work has suggested a novel mechanism for autoantigen cross-reactivity. Significant plasticity of TCR usage by autoreactive T cells in SLE showed that a single TCR can recognize two distinct snRNP autoantigen peptides with no primary sequence homology that are located on 70K and Sm B' specifically (223). These findings are nicely reviewed by Robert Hoffman (231).

Pathogenic Mechanisms

Evidence for direct pathogenicity of anti-Sm or anti-nRNP antibodies within SLE has not been clearly demonstrated. However, several evolving areas of investigation suggest pathogenic potential. Animal models that have shown the development of anti-Sm antibodies before clinical onset of disease support a potential pathogenic role for anti-Sm (107 ,108 ,109 ,179 ,200 ,217). Monoclonal anti-Sm antibodies have also been shown to penetrate live cells and localize to the nucleus (232). Finally, anti-Sm antibodies have been associated with the complement-fixing properties of antinuclear antibodies in SLE, which are not found in drug induced lupus antibodies (233). Additional investigation into potential pathogenic mechanisms of these responses is underway.

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Chapter 27
The Lupus Anticoagulant and Antiphospholipid Antibodies

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The antiphospholipid antibody syndrome (APS) is characterized by an ever-widening spectrum of clinical correlates, screening and confirmatory tests detecting diverse antigenic specificities, and both established and evolving models of in vivo pathogenesis (1 ,2 ,3). Chapter 65 considers clinical features of antiphospholipid antibodies (aPLs), specificities detected by enzyme immunoassays (EIAs), and primary/secondary treatment and management. For that set of aPLs that are classically, but ironically, called “lupus anticoagulants” (LACs) defined by coagulation-based tests, their historical development, techniques, new antigenic targets, and novel roles in pathogenesis are considered here.

Historical Perspectives

Circulating anticoagulants (CACs) are ubiquitous inhibitors of coagulation that are detected by abrogation of in vitro coagulation tests and in vivo coagulation. Specific CACs are most often but not always naturally occurring or acquired immunoglobulins (Igs) that either recognize epitopes on various coagulation factors at active sites and thus inhibit functional activity (e.g., an antibody to factor VIII), or do not recognize epitopes associated with the active site and are not always associated with pathology, e.g., the subset of LAC patients who have antibodies to prothrombin (anti-PT), which can be clinically silent, associated with bleeding, or rarely thrombosis. Nonspecific CACs include various LACs, paraproteins, or fibrin split products. LAC is defined here as a mixture of immunoglobulins (most commonly IgG/M/A) that interfere with one or more phospholipid-dependent coagulation tests, originally identified in patients with systemic lupus erythematosus (SLE). The multidisciplinary work on LACs that burgeoned from 1948 to 1983 (Table 27-1) served to explain and interrelate three prior discoveries: (1) reaginic antibodies reactive to ethanol tissue extracts of syphilitic sera in 1906 (i.e., the first aPL recognizing the mixture of the neutral phospholipids choline, cholesterol, and cardiolipin); (2) biologic false-positive serologic tests for syphilis (BFP-STS) in 1938; and (3) cardiolipin (i.e., a negatively charged phospholipid (PL) as its major antigenic component in 1941 (4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20).

From the initial description by Conley et al. (7) in 1948, the chronology of LAC reflects confusion because of limitations of available technology, as well as a lack of recognition of the complex symptomatology that eventually came to be associated with APS. Clinical associations such as bleeding (7), thrombosis (12 ,18 ,20), the BFP-STS (8), and fetal loss (9 ,20) were recognized but incompletely interrelated, even before Laurell and Nilsson (10) demonstrated that the factor responsible for LAC activity was an immunoglobulin, an observation extended to both IgG and IgM isotypes by Yin and Gaston (13). That these clinical features and laboratory components (Table 27-1) occurred in patients with SLE and with non-SLE diseases was shown by Bowie et al. (12) and Schleider et al. (16), and they encompass most major elements of what now is considered to be the spectrum of primary and secondary APS (12 ,16 ,20). Even before the naming of this CAC as “LAC” by Feinstein and Rapaport (14), several major areas reflecting pathophysiology and test variability were known; these include prothrombin-like cofactor activity (11), phospholipid dependence (7 ,11 ,15 ,17 ,18 ,19), and the common occurrence of paradoxic findings in LAC tests (17). Since the 1980s, consensus recommendations in nomenclature and technology have been sought for all LAC tests by the International Society for Thrombosis and Haemostasis, and analogously for aPLs as detected by EIAs by the aPL Standardization Committees and Workshops. Ironically, this chronology foreshadowed controversies about the interrelationships of LAC and aPL (8 ,10 ,13 ,14), necessary and sufficient plasma proteins or cofactors (11), and how understanding the importance of phospholipid-protein interactions, β_2 glycoprotein I(β_2GPI), and prothrombin (PT) has proffered new solutions to old paradoxes (8 ,15 ,19).

That LAC is a misnomer and attempts should be made to simply rename the entity as “a positive coagulation-based test” is supported by several tenets: (a) the majority of “LAC”-associated conditions are not associated with lupus, and represent the primary syndrome, or “lupus-like disease” in patients who do not meet criteria for the diagnosis of SLE, but may be evolving APS patients who will develop thromboses; (b) thrombosis is more common than bleeding, and (c) the number of patients who are being identified as having aPL by LAC or other aPL tests may alert the clinician

to consider special circumstances such as combined clinical conditions.

Table 27-1: Circulating Anticoagulant and Lupus Anticoagulant Chronology

1948	Conley et al. (7)	CAC and bleeding
1952	Conley and Hartmann (8)	CAC and BFP-STS
1954	Beaumont (9)	CAC and fetal loss
1957	Laurell and Nilsson (10)	CAC = immunoglobulin absorbed by cardiolipin
1959	Loeliger (11)	CAC cofactor-prothrombin
1963	Bowie et al. (12)	CAC and thrombosis in SLE
1965	Yin and Gaston (13)	CAC = IgG or IgM
1972	Feinstein and Rapaport (14)	CAC is a LAC
1974	Feltkamp et al. (15)	LAC is phospholipid-dependent
1976	Schleider et al. (16)	LAC and non-SLE diseases
1978	Exner et al. (17)	LAC and paradoxic reactions
1980	Soulier and Boffa (18)	LAC and thrombosis
1983	Triplett et al. (19)	LAC and PNP
1983	Boey et al. (20)	LAC and SLE and thrombosis Thrombosis

BFP-STS, biologic false-positive serologic tests for syphilis; CAC, circulating anticoagulant; Ig, immunoglobulin; LAC, lupus anticoagulant; PNP, platelet neutralization procedure.

Clinical Situations for Testing

Clinical presentations prompting coagulation testing primarily involve patients with unexplained bleeding or thrombosis, asymptomatic or symptomatic prolonged screening tests, or acquired coagulation abnormalities (dysproteinemias, lymphomas, other neoplasia, infections [chronic bacterial], human immunodeficiency virus [HIV], parvovirus, or drugs). The goal of testing is to differentiate the presence of a true phospholipid-dependent LAC from that of primary coagulation factor deficiencies (most commonly, factors VIII, IX, or XI, acquired immunoglobulin inhibitor[s] of these factors, or apparent coagulopathies due to binding of “LAC” to various plasma proteins that bind to phospholipid surfaces) (21). The important differential here is the risk of thrombosis in the LAC group versus that of bleeding, which more commonly is seen with the factor-deficient or factor-inhibited patients (4 ,5 ,6). The astute clinician will already have ruled out historical or age-related comorbidities suggesting causes of venous thrombosis that may exist independently of LAC or aPL positivity (e.g., protein S/C/antithrombin III deficiencies, activated protein C resistance because of the factor V Leiden mutation and activated protein C resistance, paroxysmal nocturnal hemoglobinuria, oral contraceptive use, nephrotic syndrome) or vasoocclusive disease states where vascular damage exteriorizes altered self components that might function as an “antigen driven state” and resultant procoagulant phenotype (e.g., hypertension, diabetes, smoking, intracardiac thrombi, Buerger's disease, hyperhomocysteinemia, or sepsis).

While the presence of LAC in health individuals ranges from 0% to 3.6%, anticardiolipin (aCL) positivity occurs in 1% to 9% of normals. Most studies analyzing LAC positivity show that approximately 45% occur in established or evolving SLE (22 ,23). LAC positivity approximates 15% in the peripartum state (24), and 12% in drug exposures, primarily procainamide (25 ,26), phenothiazines (27 ,28), chlorpromazine (29), and, rarely, procainamide and hydralazine (30 ,31). The remainder occurs in adults and children with viral infections (often transient), HIV or hepatitis C disease (sustained or intermittent), hematogenous or solid malignancies (32 ,33), or they are discovered in normal individuals undergoing preoperative assessment.

An important clinical subset are patients with hemophilia who acquired HIV via blood products and develop aPL or LAC that is uncommonly associated with thrombosis but risk bleeding caused by specific factor deficiencies (34). Rarely, immunoglobulins directed at von Willebrand factor (35), factor VIII (36), factor IX (37), factor XI (38), and fibrin polymerization (39), and protein S/C (40) occur in SLE, and characterization of factor inhibitors that can mimic these states is progressing. Increasing reports of LAC and/or aPL positivity with concurrent antibodies to specific coagulation factors should alert rheumatologists, hematologists, and both hospital and reference laboratory directors, to the importance of multidisciplinary management in these patients (40 ,41 ,42 ,46).

The prevalence of LAC activity in normal individuals as well as in patient populations varies widely because of several factors: (a) the vagaries of methodology and lack of adherence to standardization for performance of the most

commonly used test worldwide, the activated partial thromboplastin time (aPTT), and other screening and confirmatory CAC tests (43 ,44); and (b) the sparseness of studies in which both normal and patient plasma and sera were co-investigated for LAC and aPL, respectively, with sensitive and specific screening as well as confirmatory tests that demonstrated PL-dependency or where a platelet neutralization procedure (PNP) was performed (45 ,46) (Table 27-2). In SLE, the prevalence of LAC varies from less than 10% to approximately 40% relative to the sensitivity and specificity of the individual screening and/or confirmatory test procedures used (21 ,22 ,23 ,44 ,45 ,46) Other studies cumulatively show higher percentages for aCL (69%) versus LAC (48%) positivity in SLE sera analyzed concurrently by multiple methods; when populations are examined based on investigation at the time of a thrombotic event, the percentages are proportionally higher in some studies and in others they are lower, due to aPLs being involved in tissue deposition and/or immune complexes (47 ,48). Of note is that when patients are retested within 2 months of an index event, there is a demonstrable, incremental benefit in diagnosis, especially for LAC testing; performance of a repeat test and a second, different test increases the chance of demonstrating an aPL or a LAC (49) (see Chapter 65 for detailed epidemiology of LAC). That LAC and aPL are heterogeneous antibodies recognizing related but different antigenic determinants is indisputable, and there is a wide range of concordance (0% to 60%) between the functional (e.g., LAC) and the immunologically (e.g., BFP-STS, aPL) derived test procedures.

Table 27-2: Comparative Aspects of Major LAC Tests

	aPTT	KCT	dRVVT	TTI (dPT)	Textarin Time
Test choice	1st	2nd	2nd	2nd	2nd
Sensitivity for LAC	Very	Intermediate	High	Intermediate	High
Screen and confirm LAC	Y/N	Y/N	Y/Y	Y	Y/Y
Phospholipid source	Cephalin	None	Thrombofax	TF/thromoplastin	None/platelets
Initiator	Silica/kaolin	Kaolin	Russell's viper venom	(See text)	Pseudonaja textilis and Ecarin venoms
Normal (secs)	25–35	60–100	25–30	Ratio, <1.3	20–40
Use in pregnancy	N	Y	Y	Y	Y
Resistance to factor deficiency/inhibition	Variable	Variable	Most resistant (except low X, V)	(See text)	Except V
Sensitivity to heparin	Y	Y	N	N	(See text)
Mechanized test	Y	Y	Y	Potential	Potential
Specificity	Low	High	High	High	(See text)

aPTT, activated partial thromboplastin time; KCT, kaolin clot time; dRVVT, dilute Russell's viper venom test; TTI (dPT), tissue thromboplastin inhibition test (dilute prothrombin time), TF tissue factor, Y, yes; N, no.

Laboratory Diagnosis of LAC

Figure 27-1 shows the classic coagulation scheme, which involves dynamic interactions among cellular, protein, phospholipid, and calcium ions that are localized and controlled in space and time. That procoagulant reactions occur faster than fibrinolytic reactions is an important concept to recall as the molecular basis for thrombosis evolves.

Since 1964, coagulation has been thought to be initiated by the intrinsic pathway (i.e., contact factor components)

or the extrinsic pathway (i.e., cell membrane proteins and tissue factor [TF]), with either pathway resulting in factor Xa generation and eventual thrombin formation (50). Testing for the intrinsic pathway involved the aPTT, and for the extrinsic pathway the prothrombin time (PT) (51). In 1996, the accepted scheme involved TF VIIa complexes initiating coagulation via factor X, sustaining the process via factor IXa activation of factor VII, and completing the process by activation of factor XI. Figure 27-2 shows the two classic tenase complexes and the prothrombinase complex; these represent three of the four major phospholipid-dependent coagulation processes.

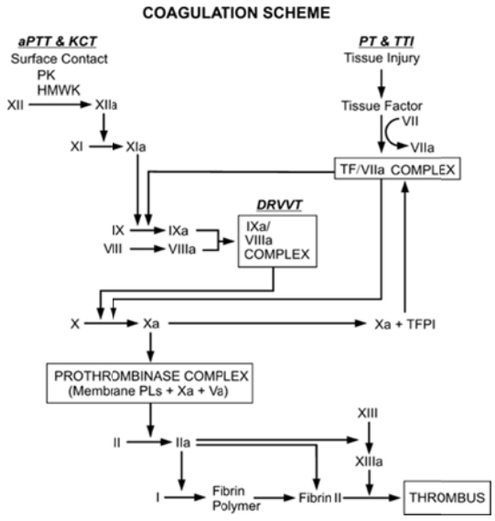

Figure 27-1. The coagulation scheme updated from 1964 to the present, with the three main phospholipid-dependent complexes highlighted in closed boxes (the two classic tenase complexes, tissue factor/factor VIIa and factor IXa/factor VIIIa, and the prothrombinase complex; membrane phospholipids, factor Xa, and factor Va). Relevant coagulation tests are listed in italics for respective sections of the cascade. The end result is thrombosis.

Endothelial cell or platelet damage or activation exposes negatively charged inner membrane leaflets or reorganizes phospholipid arrays. The vitamin K-dependent clotting factors bind to these charged phospholipid surfaces, and platelet receptors allow adherence to subendothelial connective tissue and other sites (Fig. 27-2A-D). The endothelial cell surface itself modulates its procoagulant versus anticoagulant properties via thrombomodulin and the activated protein C/protein S complex (i.e., the fourth phospholipiddependent complex [Fig. 27-2E]). Coagulation therefore is localized to sites of vessel injury and restricted there by binding to negatively charged phospholipid/membrane components that are not normally accessible. Recently, the elucidation of the thrombomodulatory role of annexin V as a regulatory “shield” that clusters on exposed PL-rich surfaces and abrogates the binding of aPL or LAC to PLs in the maternal-fetal unit will likely be shown to be operative at other endothelial cell sites.

Thus, several phospholipid-dependent complexes have the capacity to be influenced by LACs or different aPLs. Additionally, the natural anticoagulant $ß_2$-glycoprotein I, or apolipoprotein H) has affinity for anionic phospholipid as well as other coagulation components; phospholipid flip-flop or loss of membrane asymmetry that exteriorizes or exposes phosphatidylserine (PS), or other phospholipids, via the translocase reaction after membrane perturbation or injury might contribute to immunogenicity by revelation and/or creation of neoepitopes ($ß_2$GPI dimerization, CL/plate irradiation) which might drive LAC/aPL production (52).Thus, there are multifactorial ways in which these antibodies might interact in vivo to generate a procoagulant surface, which might then continue to drive phospholipid-dependent coagulation reactions. Recent vascular injury models in the presence of aPLs or LACs have shown augmentation of cell functions and thrombosis size or frequency (see Chapter 52).

Rationale for Screening Tests

The minimal laboratory assessment for all coagulation abnormalities includes an aPTT, a PT, and a platelet count as a first step (53). The second step is the determination of LAC by demonstrating that the prolongation of the screening test results from an antibody and not a quantitative or qualitative coagulation factor deficit or inhibition. Mixing normal plasma with patient plasma does not correct the aPTT prolongation to normal with most LACs, whereas mixing with normal plasma usually does partially or completely correct aPTT prolongation by the provision of normal clotting factors. This is not infallible, as some aPLs act at multiple levels or on multiple factors in the coagulation scheme.

The definition of a screening test is an assay that is based on a single concentration of phospholipid (54). The major tests compared are the aPTT, the kaolin clot time (KCT), the dilute Russell's viper venom test (dRVVT), the tissue thromboplastin inhibition (TTI) test (otherwise known as the dilute PT), and the Textarin time test (Table 27-3). Other tests include the plasma clotting time (PCT), and new integrated systems where multiple reagents are incorporated in reaction mixtures (PLs or chromogenic substances) (46 ,54).

A source of confusion for nonhematologists interpreting the literature is use of the descriptive phrase that a test component is “sensitive to the presence of the LAC”; often, this is misinterpreted as the test has high sensitivity. When referring to aPTT reagents, reagent sensitivity means that the abnormal aPTT results from the presence of a LAC, while reagent responsiveness means the degree of test prolongation (46 ,53). Thus, reagents often are classified in terms of paired sensitivity/responsiveness, and as laboratories strive to improve tests in different size study populations, inconsistencies in comparative results occur. This is the basis for both past (46) as well as current (55 ,56 ,57) recommendations that even if the screening aPTT is normal, an additional screening test is recommended.

Because LACs are heterogeneous, no single test detects all LAC, and no one test approximates 100% sensitivity or 100% specificity. Caveats and special circumstances are clarified in this text to reflect these methodologic considerations as an evolving area; while a majority of experts now agree that two tests need to be performed (one screening test and a confirmatory test), there are some who espouse that perhaps a third test be added, but they remain in the minority at this time. Figure 27-3 provides an overview of some comparative aspects of LAC screening and confirmatory testing and represents a consensus from clinical experience and the current literature (46 ,54 ,55 ,56 ,57 ,58).

The laboratory criteria for LAC testing, as established by the Scientific and Standardization Committee Subcommittee for the Standardization of Lupus Anticoagulants in 1991 (57), have been simplified by Exner et al. (57) to represent the minimally acceptable criteria to define LAC: (a) an in vitro, phospholipid-dependent coagulation test must be prolonged; (b) an inhibitor (or LAC) must be demonstrable as the cause by mixing studies using appropriate ratios of patient-to-normal plasma; and (c) the inhibitor (or LAC) must be differentiated as being directed at a phospholipid, preferably a hexagonal phase II PL, not at a specific coagulation factor (46 ,54 ,55 ,56 ,58). However, recent data reviewed by Exner (5) show that correction of test abnormality by PL is more specific for LACs than the platelet neutralization procedure that has been “recommended” since 1984. The most recent recommendations from the Subcommittee, summarized by Brandt et al. (21) in late 1995, are similar and state appropriately that despite excellent and careful research, both the ordering physician and the patient must be aware that aPLs are very heterogeneous, and that even well-standardized

LAC assays may have a range of sensitivity for the detection of certain subgroups of LACs.

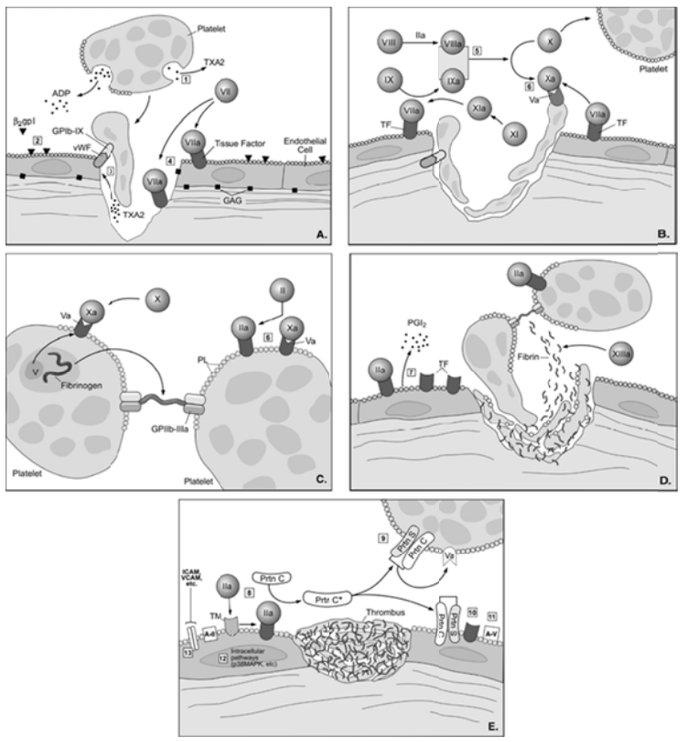

Figure 27-2. The coagulation scheme occurring at a localized site of vascular injury or altered self. The luminal side is up; the basement membrane side is down. For this series, phospholipids (PLs) on cell membranes are designated by open circles on the luminal side; ß glycoprotein I (2GPI) by a black triangle; glycosaminoglycans (GAGs) in subendothelial areas by a black square; coagulation factors by shaded and numbered circles, often bound to tissue factor (TF), with activated coagulation factor fragments as specified and shown by stippled receptors. The boxed numbers 1 to 13 refer to areas where lupus anticoagulants (LACs) and antiphospholipid antibodies (aPLs) have either been proven or suspected to interact with cell surfaces, coagulation components, or PL-dependent binding reactions. *A–D*, The steps in Fig. 27-1 culminate in the formation of a thrombus at the site of a vessel injury or altered self. *E:* The fourth main PL-dependent complex is added, that of protein S/C and thrombomodulin (TM, cross hatch) The annexin V shield has been added (A-V). Thus, LACs and aPLs also may work in effecting the return to an anticoagulant surface after procoagulant effector molecules have resulted in thrombosis. TF, tissue factor; TXA2, thromboxane A2; vWF, von Willebrand factor.

Table 27-3: Antigenic Targets of LAC/aPL

Antigen:	Autoantibody Type:	Test:
$ß_2$GPI	LAC Type 1	Coagulation
	aCL Type 1	aCL ELISA
	anti-$ß_2$GPI	a$ß_2$GPI ELISA
Prothrombin	LAC Type 2	Coagulation
	(Low Affinity)	aPT ELISA
	LAC Type 3	Coagulation
	(High Affinity)	aPT ELISA
Anionic PL	aCL Type 2	aCL ELISA

Additionally, the Subcommittee recommends the following: (a) two or more different screening tests should be performed for LAC, one of which should be a low phospholipid concentration test (KCT, dRVVT, dilute aPTT, daPPT, dPT); (b) the presence of inhibitor activity should be determined by incorporation of a mixing step (i.e., patient plasma's effect on pooled normal plasma) in the initial screening test, using a well-characterized pooled normal plasma; (c) documentation of multiple abnormal screening tests is not presumptive evidence of a LAC until phospholipid dependence is established; (d) confirmatory tests should be based on methodology similar to screening tests (Fig. 27-3); (e) a PT and an aPTT should be performed before screening LAC tests to rule out other coagulation factor disorders that might affect LAC determination, and they should be based on clinical history; (f) heparin presence should be determined by a thrombin time before screening or confirmatory LAC tests if the tests used are sensitive to heparin; (g) concurrent positivity of patient sera for aPL enzyme-linked immunosorbent assay (ELISA) is not considered to be a confirmatory test for the presence of LAC by one method; and (h) the current LAC nomenclature should be retained until a more definitive understanding is available regarding its pathophysiology, although there is universal awareness of the need to consider shedding the

“LAC” designation (58). Lastly, the Subcommittee has not re-addressed the need to consider that some LAC are time dependent, such as factor VIII inhibitors because of the time it takes for factor VIII antigen to dissociate from von Willebrand factor.

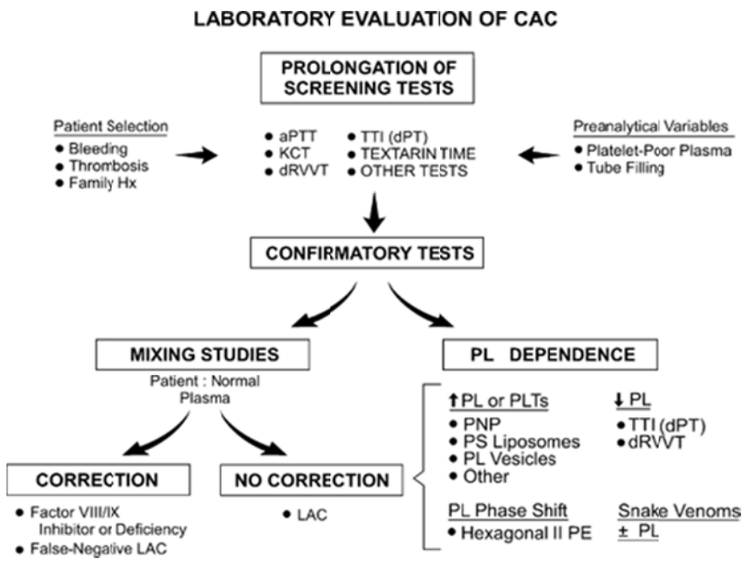

Figure 27-3. The laboratory evaluation of circulating anticoagulants, with the main procedures highlighted in closed boxes (demonstration of a prolonged screening test, performance of a confirmatory test, usually not the same test as the screen, with mixing studies of patient and normal plasma, and demonstration of phospholipid dependence by one of a variety of means). The presence of a LAC often is determined when the mixing study does not correct to normal, thus separating LAC from coagulation factor inhibitors or deficiencies. Phospholipid dependence is now favored over platelet neutralization procedures.

Thus, there are many bases from both past (46) and current (57 ,58) recommendations supporting the premise that even if the screening aPTT is normal, an additional screening test is recommended. Both aPL and aPTT tests appear to be warranted.

Pre-Analytic Variables

Preparation and handling of patient and normal plasma used for both screening and confirmatory studies (e.g., mixing studies or phospholipid dependence) may be affected by the following: (a) less than 50% filling of the anticoagulated tube with patient blood does not afford the proper dilutionary effect; (b) the type of anticoagulant that is used in the tube may be a source of variation; (c) temperature during processing and handling, as membrane microvesicles might be created during a freeze-thaw cycle, gives an in situ PNP and thus a false-negative test result; (d) the pH of the mixture as it changes over incubation time may range from physiologic to 8.2, at which level factor V is likely to be selectively destroyed; and (e) the presence of platelets or platelet fragments (which provide a source of factor V, other procoagulants, and platelet factor 4) that decrease the sensitivity of tests using low phospholipid concentrations because they bind aPLs. Gentle centrifugation (10 minutes, 5,000 to 10,000 g) and/or filtration (pore size 0.22 μm) is recommended to avoid platelet fragmentation and release of platelet factors via activation that can augment coagulation or affect von Willebrand factor assays (5 ,58).

A recent study comparing commercial re-agents for the detection of LA using a photo-optical array versus a mechanical coagulometer showed that considerable variation in sensitivity and specificity was noted for the same reagent used on the different analyzers, as well as between reagents. Thus, there remains a need to address optimization of reagents for specific analyzer types.

aPTT Test

The sensitivity of the aPTT varies proportionally with the nature of the commercial reagents, including the activator, and the source, amount, composition, and physical state of the phospholipid. That excess phospholipid shortens the prolonged aPTT because of LAC is the basis for the development of tests on this model, and the aPTT is more sensitive than the PT to the

presence of LAC. PS concentration and lipid phase (either bilayer or hexagonal phase II conformation) (59), either in the phospholipid in aPTT reagents or the phospholipid in tissue factor reagents, may be operative here. Modifications to increase sensitivity have included using a dilute phospholipid or increasing the dilution of the aPTT reagents (daPTT) (5), but studies have shown that increasing the incubation time rather than the phospholipid concentration may be more important (i.e., dual-incubation aPTT) (58).

That LAC may show time dependency, a feature that previously was relegated to factor deficiency or inhibitor states, has been recently recognized, and extended to include effects related to the pH of the mixture, which varies with time. Addition of 0.05 M HEPES buffer in one study looking at the aPTT over time showed that a stable pH using platelin as the re-agent was achieved (57). Comparative assessments have shown that several reagents (i.e., actin FSL, automated aPTT, and ThromboSol) are sensitive to LAC (60 ,61 ,62). Recent advances such as the Staclot-LA reagent have incorporated a heparin blocker and hexagonal phase II lipids in the incubation mixture for increased specificity, but mechanically this test is more difficult. Some IgM LAC may be missed with this test as well. While rising factor VIII levels have often limited the usefulness of aPTT during pregnancy in prior studies (46 ,53), Blanco et al. (60) showed that the standard aPTT was more useful during pregnancy than the daPTT. False-positive aPTTs occur with contact factor deficiencies, heparin, factor VII inhibitors, and factor IIX/VIII deficiencies (4).

KCT Test

The KCT test is an aPTT without a platelet lipid source, and the activator is provided by kaolin (clay). Current recommendations state that a 2% suspension in water is a more sensitive text mixture, as sodium chloride hastens the precipitation of kaolin out of suspension. Platelet poor plasma is most critical for the KCT (46 ,53 ,58). Its sensitivity is intermediate between that of a sensitive aPTT and the dRVVT, and it is considered to be a test with high specificity. The modified KCT is considered to be useful in the detection of low-titer LACs. Like the aPTT, KCT can have false-positive results under the same conditions as noted earlier. Exner (5) has improved its performance with 4:1 dilutions of plasma, and the test now has been automated because of the ability to decrease the kaolin concentration from 12 to less than 1, thus permitting photoelectric quantification. Additionally, in paired samples with KCT and DRVVT (LA Screen, Gradipore, Sydney, Australia), a platelet count of 1×10^9/L will obviate a positive KCT but will not affect the dRVVT results.

dRVVT Test

Developed by Thiagarajan et al., this test is increasingly used worldwide investigationally and clinically, and it is unique in being a screening as well as a confirmatory test (46 ,53 ,58). Sources of both phospholipid and venom may affect the test, and it now can be performed in a single vial. It requires low phospholipid concentrations. The venom activates factor X, bypasses factors VIII, IX, and contact factors, and eliminates some of the poorly understood effects in LAC testing that can either lengthen or shorten the tests. Polybrene or other heparin inhibitors may be added, which will not give false-positive results, but without these additives the dRVVT may yield false positives with patients who have been on heparin. Sensitive reagents include the LA-Confirm and the DRVV-Confirm.

Like the previous two tests, the dRVVT is sensitive to factor VII inhibitors or factor IX/VII deficiencies, but it is prolonged only in very high-titer factor VIII inhibitors. Therefore, in reality, it is the most resistant (6). The dRVVT is useful during pregnancy as it is independent of the naturally occurring increase in some clotting factors, and does not give false-positive results with contact deficiencies. In the most recent recommendations, it is considered to be the next test after an aPTT (58). In our experience with 392 APS patients followed between 2 and 4 months longitudinally from 1996 to mid-2000, two caveats have emerged: (1) 20% of all patients will only be positive by an LAC test; (2) in paired simultaneously drawn sera/plasma samples, the DRVVT was 3 to 5 times more likely to identify an aPL than the standardized aPTT (McCarty, Indiana APS Database Project, 1996–2000; Univ of Va Database 2000–2003).

TTI (dPT) Test

This test is a dilute PT performed with varying dilutions of tissue factor (TF) or thromboplastin and expressed as a ratio of patient-to-normal PT. A major source of variation is the lack of a standardized normal plasma, which is a common complaint with many of these tests. Because the TTI assesses all three critical phospholipid-dependent coagulation reactions, it seems ideal for a LAC screening test. Like some aPTT variations, however, IgM LAC may be missed. Like the dRVVT, it does not give false-positive results with contact deficiencies, and heparin inhibitors have been added to the reagents to increase the utility of this test. Direct data on use in pregnancy have not been reported, but the test is likely to be useful in pregnancy. Recombinant human TF modifications by Zanon et al. (63) and Arnout et al. (64) are attempts to provide a uniform reagent for the TTI. The TTI because of its use of TF as a reagent may be optimized to more selectively identify anti-β_2-glycoprotein I antibodies, as recent studies have shown that this specific aPL may enhance tissue factor activity on monocytes.

Textarin Time Tests

Venom from *Pseudonaja textilis* and *Echis carinatus* snakes is used in these tests, popularized by Triplett et al. (65), which were developed as a confirmatory test but actually violate the definition of a confirmatory test in that phospholipid is not augmented to demonstrate correction (58). The basis for this test is the difference in the phospholipid requirements of the two venoms. Currently, it is considered to be the

most specific test available for LAC, and interest in its development is based on a high specificity for phospholipid effects on the prothrombinase complex. The Textarin time is compared with the Ecarin time here. These tests are resistant to factor inhibitors or deficiencies, except for factor V.

Other LAC Tests

In contrast to the above functional tests, an ELISA using partial thromboplastin derived from human brain as the antigen was developed for detection of LACs (64). This assay circumvents some of the disadvantages of coagulation assays and is highly sensitive and specific. A synthetic reagent called Synthesil in preliminary tests compared well to rabbit thromboplastin. Additionally, Schjetlein and Wisloff (66) have developed an integrated test system, adapted for computer analysis, that uses aPTT and dRVVT comparison between normal plasma and a mixing test with high PL. Finally, the PCT requires fresh plasma and is unaffected by the presence of platelet membrane fragments in re-agent preparations, but is not generally performed. A new silica clot test appears to be more sensitive, specific, and predictive of thrombosis in APS patients when compared to parallel determinations of DRVVT but remain to be confirmed by others (67).

Rationale for Confirmatory Tests

The observation that excess phospholipid substantially shortens the prolonged aPTT because of LAC has been exploited to distinguish LAC from other CACs. As shown in Fig. 27-3 , the PNP by PS liposomes, and rabbit brain phospholipid are common ways to demonstrate phospholipid dependence. The PNP has been particularly recommended since its inception in the early 1980s as a confirmatory test for aPTT-based tests and the dRVVT. Platelet vesicles are particularly suited for the dRVVT and KCT. Phospholipid dilutions are best matched with the TTI (dPT) and the dRVVT. Hexagonal phase II phospholipid also is used to confirm aPTT results.

Special Cases

Despite appropriate precautions, discrepancies often occur with these tests, and paradoxic results are sometimes the legacy of LAC testing. Several clinical situations may arise to challenge the clinician and the consulting hematologist or laboratory director.

Specific factor inhibitors may cause false-positive results with confirmatory LAC tests. The performance of factor assays using two or more dilutions of patient plasma may be helpful if the clinical history and screening aPTT and PT results are not. LACs often have a dilutional effect on several specific factor activities, whereas specific factor inhibitors are associated with a low level of one factor that does not change with dilution. The LAC can artifactually decrease the values by impairing the reactivity of the PL reagents. One approach is to perform several of the specific, one-stage clotting factor assays based on the aPTT technique (e.g., factors VIII, IX, and XI) (46 ,57 ,58).

Strong factor inhibitors may cause dilutional effects on levels of other factors to which they are not directed specifically. Thus, the absence of a dilutional effect is not presumptive evidence that LAC is not present.

High-titer LACs may artifactually appear as a factor deficiency when a sensitive aPTT is used, without the dilutional effect mentioned earlier. In this setting, specific factor antigenic assays should be performed.

The hypoprothrombinemia-LAC syndrome is an important clinical entity because the prothrombin deficiency resulting from this LAC-like antibody that binds to prothrombin in vivo may result in significant bleeding. Hypoprothrombinemia might also result from direct-acting anti-PT antibodies. Additionally, binding in immune complexes if active aPL antibodies are proximal is possible. This is the modern correlate of the classic LAC misnomer, although its existence was reported in 1959 by Loeliger (11). Prothrombin-LAC immune complexes cause an artifactual hypoprothrombinemia by their normal removal from the circulation, but prothrombin function in the in vitro assays is normal. The clinical clue here is the finding of a substantially prolonged PT, beyond the range that is noted for LAC (e.g., 18 to 20 seconds). Prothrombin antigenic activity is present but the level is decreased and abnormal mobility may be electrophoretically present (68). SLE, viral infections, drugs, and idiopathic etiologies have been associated with this syndrome (69). Prothrombin antibodies have all reacted with epitopes on the carboxy terminal segment of the prothrombin molecule (67). Whether these are distinctive antiprothrombin antibodies or represent part of the antigenic target spectrum of LAC/aPL reactivity with epitopes on prothrombin and anionic phospholipids, however, remains unclear (70).

Caveats on Testing

The innate heterogeneity of aPLs and the features of APS are mirrored by the necessity to perform several levels of screening and confirmatory testing, and the vagaries of LAC screening and confirmatory tests remain protean despite significant new knowledge regarding multifactorial mechanisms by which aPLs interfere with coagulation. Large meta-analyses are difficult because of the rare performance of simultaneous or longitudinal standardized studies, so summary statements regarding overall sensitivity and specificity remain incorrect and misleading if taken out of the context of the individual test. As has been noted for the EIA-based aPL tests since the publication of the Sapporo criteria, the trend is toward the performance of two tests for LAC (aPTT and DRVVT), coupled with the caveats for addressing pre-analytical variables such as sample obtainment and reagent matching for type of analyzer. Appropriate studies regarding the positive predictive values of individual as well as panels of tests are needed. A synthesis of current laboratory approaches to diagnosis LACs has been used to generate Fig. 27-3 . Table 27-2 addresses specific caveats regarding individual tests.

The 1995 update from the Scientific and Standardization Subcommittee (21) supports performing two screening tests (i.e., an aPTT plus another test, such as dRVVT) and further evaluating those tests results either sequentially or concurrently. If both an aPTT and dRVVT are abnormal, a mixing study of patient and normal plasma is done. If the mixing study is abnormal, then progression to demonstration of phospholipid dependence is performed, and if positive, LAC is confirmed. If the initial mixing study corrects to normal, then an incubated mixing study is performed. If the incubated mixing study fails to correct to normal, then the demonstration of phospholipid dependence is next. If it corrects to normal, then specific factor assays are done. Although no specific protocols are as yet agreed on for mixing studies, these tests are relatively easy to perform in most laboratories and may be helpful cost-savers as standards and methods for LAC indices or calibrations evolve.

Mechanisms of LAC Action

A consideration of proven and putative mechanisms of action for LAC supports the contention that they are separable activities from other aPLs in some ways (13) (see Chapter 52). The reader is referred to Fig. 27-3 , in which both proven and putative mechanisms of action that are relevant to coagulation components and vascular injury models are indicated by numbered squares. Although there exist murine induction and vascular injury models that produce with some fidelity the major features of APS and a fulfillment (to some extent) of Koch's postulates for their contributions to pathogenicity, the full in vivo correlates of these likely mechanisms are still not fully understood. Recent reviews examined the hypotheses that concepts regarding the relevant antigens should be extended beyond phospholipids only (i.e., the antiphospholipid antibody protein syndromes) (71 ,72 ,73 ,74).

Phospholipid Binding

Anionic phospholipid binding initially was demonstrated for an IgM monoclonal LAC (75) and, since then, for sera and plasma samples using affinity purification (76 ,77 ,78 ,79). Studies using affinity-purified patient IgGs and improvements in column chromatography of LAC plasma have fostered the reexamination of anionic phospholipid binding. The discovery of $ß_2$GPI as a major antigenic target of aPLs has prompted this re-evaluation. The presumed form of the phospholipid was a lamellar array but subsequently was shown to be a different phase form changed by calcium and PS. When critically examined, the data for direct cardiolipin binding in the absence of $ß_2$GPI or other lipid-protein interactions have been based primarily on the initial IgM monoclonal LAC (75 ,78 ,79). Perhaps other anionic phospholipids such as PS will prove to be more important or that the true epitopes represent phospholipid–protein-lipid combinations that are recognized differentially by LAC and aPLs (Fig. 27-2A).

$ß_2$GPI, Prothrombin Interactions

LACs were shown to be chromatographically separable into two different populations using phospholipid liposome preparations (79 ,80 ,81 ,82 ,83). The identification of $ß_2$GPI, a natural anticoagulant as the cofactor responsible for a prolonged PTT or KCT of patient plasma lengthening further with the addition of normal plasma (i.e., the source of ßGPI) has allowed the profiling of aPLs into different sets (Table 27-2) (many aPLs and some LACs also have been shown to require ßGPI for binding (82 ,83). These (Table 27-3) enhance the inhibition of prothrombin conversion by $ß_2$GPI; thus, the LAC effect in plasma results from enhancement of $ß_2$GPI binding (80 ,81 ,82 ,83). Although this research has opened several new areas of mechanistic consideration, there are still some areas of controversy (71 ,72 ,73 ,74 ,80 ,84). However, the irony that is intrinsically associated with LACs continues in that although $ß_2$GPI is a natural anticoagulant, patients who are genetically deficient in $ß_2$GPI paradoxically do not appear to be at risk for thrombosis (71 ,72). Recent studies by Hwang et al. have shown multiple patient-derived monoclonal antibodies react to prothrombin and/or thrombin, and thus inhibit feedback regulation of thrombin (84).

Prothrombinase Complex and Other Phospholipid-Dependent Coagulation Reactions

LACs have been shown to interfere with phospholipid-dependent coagulation reactions in the presence of human plasma (79 ,80 ,81 ,82 ,83). Three potential sites exist where LAC might bind and shift the balance from an anticoagulant to a procoagulant surface on endothelial cells or platelets (Fig. 27-2B). Because LAC affects all phospholipid-dependent reactions, its site of action is at least at the level of the prothrombinase complex. The inhibition is specific for human, but not for bovine, prothrombin and required phospholipid vesicles (83). Thus, this population of LAC is different from the antiprothrombin LAC described later. The anticoagulant effect of LAC likely results from binding to the phospholipid-bound human prothrombin complex once calcium has also bound. That this reaction could occur on platelet surfaces was shown by the substitution of activated platelets or platelet-derived vesicles as phospholipid sources (79 ,84 ,85). An IgM monoclonal LAC was shown to inhibit factor X activation by the intrinsic tenase complex, thus confirming previous direct LAC binding to anionic phospholipids.

Endothelial Cell/Platelet-Protein S/Protein C and Thrombomodulin Interactions

Prostaglandins are major products of endothelial cells and platelets, and their production is important in maintaining the neutrality of a coagulant surface. LACs (and aPLs) have

been shown to stimulate prostacyclin release from endothelial cells in both patients with and without thrombosis (71 ,72 ,79). Thromboxane A2 has been shown to inhibit endothelial cell production of procoagulant molecules and thus plays a role via its balance with prostacyclin (PGI_2) (Fig. 27-2A). Once tissue injury occurs, TF on the cell surface, and glycosaminoglycans (GAGs) from the subendothelium may become exposed, and GAGs are important in determining the anticoagulant properties of the endothelial cell surface (Fig. 27-2A,E). Platelets are thought to bind LAC (as well as aPL), but most LACs may require only platelet activation to bind, independently of $ß_2$GPI, although some LAC may be dependent (84 ,85). It currently is unclear whether this binding generates procoagulant effects. When thrombin binds to thrombomodulin on the surface of endothelial cells, an anticoagulant milieu is generated, protein C is activated, and, once complexed with protein S, is capable of inhibiting factors Va and VIIIa. Currently, LACs are thought by some investigators to inhibit thrombomodulin-mediated activation of protein C, although this has been questioned (71 ,72 ,79 ,84 ,86 ,87) (Fig. 27-2E).

Other Antiphospholipid Antibody ELISA Tests and Their Phospholipid Targets

A consensus statement on the preliminary classification criteria for definite antiphospholipid antibody syndrome in 1999 now referred to as the Sapporo Criteria represented the extensive work of an international panel of physician-investigators and laboratory researchers to put forth general guidelines based on the most widely used tests (the IgG/M aCL enzyme-linked immunoabsorbent assay [ELISAs] as described in Chapter 55 , and one or more LAC tests as detailed in this chapter and meeting specifications (55 ,56 ,57 ,58 ,88). There are some caveats: (1) as with most clinical guidelines, the criteria may not be applicable in an individual patient, who may not make these particular antibodies or have only these manifestations of APS; (2) that proven thrombotic events were required to be temporally relevant to positive tests, and (3) strict exclusions were refined for the diagnosis of APS-related fetal loss (88). Despite the widely known studies since 1990 showing that $ß_2$GPI binding to CL and dimerizing molecules forming a neoepitope that is the most frequent antigenic target of aPLs, anti-$ß_2$GPI tests were not considered in the Sapporo Criteria, because very low percentages of APS patients exhibit solely this antibody. The updated International Consensus Statement by Miyakis et al. now includes this test (89). Despite use of the Harris standards for IgG/M ELISAs in second- and third-generation kits, various commercial and hospital labs use different criteria for the definition of aPL positivity. Some of the variability in aPL assays has been shown to be related to the provision of adequate $ß_2$GPI in the diluents in the ELISA tests, which initially used fetal or newborn bovine serum as blocking agents or patient sera diluents (88 ,89 ,90 ,91 ,92). Most of the negatively charged PLs (CL and PS) require $ß_2$GPI as a cofactor. Buffers containing only $ß_2$GPI do not detect aPL that are dependent on other PL-binding proteins such as prothrombin or protein C, or those such as aPE that require the low or high molecular weight kininogens (LMWK-HMWK), and are clinically relevant to patients, being associated with the same APS clinical criteria as cited above. Recent characterization of patient-derived monoclonals show that additional targets of aPLs include tissue plasminogen activator (91). The incremental value of adding IgA aPS and IgA aPE testing to IgG and IgM aCL ELISA testing in 5632 patients with APS-associated events has been well demonstrated to additionally identify patients who would otherwise be considered “seronegative” and therefore not be identified for treatment by McIntyre et al. (92). IgA isotype testing and anti-$ß_2$GPI testing has been suggested by Harris as the next steps to consider in IgG/M aCL/LAC negative patients with APS symptoms in a recent review; the prevalence of IgA aCL responses may also be related to ethnicity rather than methodology (90).

Animal Models

Most work on APS has involved the passive or active immunization of purified human IgG aCL or purified PL antigens into nonautoimmune prone or naive mice. aPLs are not exclusively produced by autoimmune mice such as the MRL/lpr and the (NZW × BXSB)F1 strains, but develop in the nonobese diabetic (NOD) mice; several recent reviews address this subject in detail (93 ,94). Demonstration of thrombocytopenia, autoimmune hemolytic anemia, decreased fertility due to involution of murine pregnancies, and placental thrombotic micro-angiopathy have been found for CL and PS antibodies by active induction (93 ,94). Active induction studies with $ß_2$GPI show decreased fecundity, elevated APTTs, and hemolytic anemia, but no clinical thromboses: some mouse models develop aCL, aPS, aPI (phosphatidylinositol) in response. In most experiments, CL alone is not immunogenic by itself in most of the early studies but the response is enhanced by its cofactor. The spontaneously developing aPL mice produce functional antibodies but may not faithfully reproduce human pathology-only male NZB/W F1 mice develop arterial thrombosis, and aCL have been detected in mouse strains without clinical APS features. Another approach has been to induce an endothelial cell pinch injury by clamping femoral or cremasteric veins, infuse aCL or other aPLs, and evaluate the kinetics of thrombus formation, size, and platelet content, showing aPLs do induce thrombosis at sites of vascular injury in vivo (95).

When immunized with $ß_2$GPI, mice made antibodies to this antigen and to aCL; when immunized with human IgG, murine aCL and anti-human IgG were produced, and mean thrombus area was significantly greater in these groups than controls immunized with human serum albumin. These murine aCLs were thrombogenic analogously to the passively immunized mice in other models, showing thrombogenicity is related to aCL specificity rather than to the source of the aCL. Recently, this group has shown that some human IgG monoclonal aCL and aPS antibodies

recognize 15 amino acid sequence GDKV binding site epitope of $ß_2$GPI in the pinch model activates endothelium as measured by the upregulation of vascular endothelial cell adhesion molecule 1 (VCAM-1) on cultured endothelia (96). A recent analysis of hybridomas from a (NZW × BXSB) F1 male showed binding to three supramolecular complexes of anionic PLs and protein: CL-$ß_2$GPI, PS-annexin V, and nucleosomes, and H and L chain analyses showed recurrent H and/or L chain rearrangements (97). That aCL monoclonal antibodies cross react with nucleosomes may explain the frequent coincidence of APS and SLE (secondary APS) and primary APS patients with antinuclear antibodies. In a rat mesenteric model, platelet aggregate and thrombus formation induced by anti-$ß_2$GPI was related to pro-inflammatory events involving terminal complement components (98). These models are important in both pathogenesis and evaluation of treatment modalities for APS.

Summary

Lupus anticoagulants are a heterogeneous set of aPLs with the potential to reflect in vitro actions with in vivo consequences. Endothelial cell or platelet membrane injury or activation could result in an enhanced procoagulant surface that is permissive for the binding of prothrombin, $ß_2$GPI, or protein S and activated protein C to exposed anionic phospholipids. In a manner analogous to the creation of a neoepitope or altered reactivity of other domains of $ß_2$GPI, these components then might be recognized as immunogenic and continue to drive the production of LACs/aPLs, which likely are part of the natural repertoire already. This scenario would explain the protean nature of LAC effects, in that the shift to the procoagulant state would then drive phospholipid-dependent coagulation reactions and cause thrombosis. That qualitative and quantitative differences in regional vascular beds may explain why thrombosis (while sometimes mimetic in site) can range in scope from trivial and localized to catastrophic and generalized remains under investigation. A proposed sequential evaluation flow diagram for LAC/aPL that represents opinion synthesized from extensive academic practice and recent literature comprises Fig. 27-4 (2 ,4 ,21 ,23 ,71 ,88 ,90 ,92). Although cost containment is important, consideration of the cost of morbidity, and possibly mortality, is balanced only by that which might be prevented by appropriate therapy (see Chapter 65). The emerging knowledge that $ß_2$GPI is a control protein in fibrinolysis (99) may offer new therapeutic options, address positive predictive values that will help to identify at-risk patients for prophylactic treatment. Further elucidation of surface-based LAC binding to cell surfaces such as the monocyte with subsequent activation of p38 MAPK resulting in the upregulation of tissue factor (100) and other surface proteins in endothelial cells may open pathways for specific targeted therapies for patients.

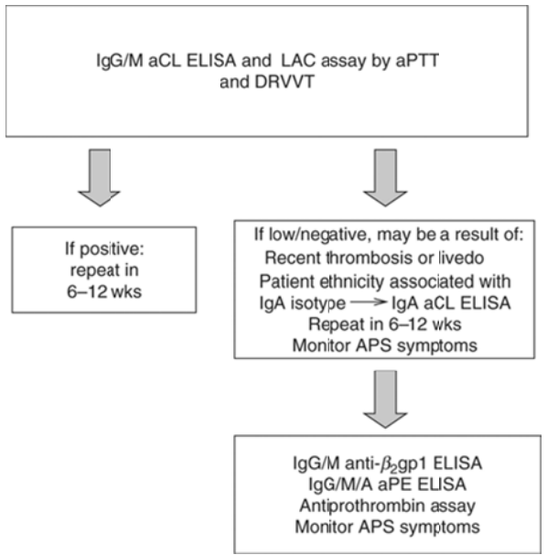

Figure 27-4. A proposal for the sequential evaluation of LAC/aPL is presented.

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Chapter 28

Other Serologic Abnormalities in Systemic Lupus Erythematosus

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Antierythrocyte Antibodies

Antibodies to red blood cells (RBCs) are detected by the antiglobulin (i.e., Coombs) test, of which there are two variations. The direct Coombs test, also known as direct antiglobulin test (DAT), measures the presence of antibodies that are bound to the surface of circulating RBCs. The antiglobulin reagent, usually rabbit or goat antibody to human gamma globulin, is added to a saline suspension of washed erythrocytes of the patient, and if the erythrocytes are coated with antibodies, cell agglutination ensues. The indirect Coombs test measures for free anti-RBC antibody in the serum of the patient. Test serum is incubated with suspension of a mixture of washed normal group O red cells that express most of the known RBC antigens; thereafter, the cells are allowed to react with antihuman gamma globulin. Cell agglutination indicates the presence of free anti-RBC antibodies in the patient's serum.

Antiwhole human gamma globulin usually is employed as the antiglobulin reagent, but monospecific antisera to human IgG and other Ig classes, IgG subclasses, C3, and C4 also are being used. In certain situations, immune complexes that are unrelated to RBC antigens may bind to erythrocytes, giving rise to a positive direct Coombs test.

Washed RBC from healthy nonanemic subjects has less than 14 fg IgG per 10,000 cells. Hypergammaglobulinemia of various etiology results in an increase in surface IgG, causing a positive direct Coombs test, but with no evidence of significant hemolysis. Immunoglobulin eluted from the RBCs of patients with hypergammaglobulinemia and the corresponding serum is negative for specific anti-RBC antibody activity (1). On the other hand, RBC eluates of patients with SLE and polyclonal hypergammaglobulinemia and a positive direct Coombs test contain anti-RBC autoantibodies. This has been interpreted to mean that the net effect of hypergammaglobulinemia on the Coombs test is obscured by the bound anti-RBC antibodies (2).

The limited sensitivity of the antiglobulin test has led to the development of other methods with improved sensitivity, such as the enzyme-linked immunosorbent assay (ELISA) and radioassay (3 ,4 ,5); however, in most clinical laboratories, the Coombs test remains the standard test for anti-RBC antibodies.

Characteristics of Anti-Red Blood Cell Antibodies in SLE

Autoantibodies to RBCs are classified into two major types (5) according to their thermal requirements: warm antibodies react optimally with surface membrane antigens at 37°C (6 ,7), and cold-type autoantibodies react more avidly with RBC antigens at 0°C to 5°C than at higher temperatures (8 ,9).

Autoantibodies to RBC in SLE as well as in idiopathic autoimmune hemolytic anemia (AIHA) are predominantly warm antibodies. Warm antibodies most commonly belong to the IgG class (8). All four subclasses of IgG are represented, although IgG1 is the predominant IgG subclass, and IgG2 and IgG3 are found less frequently. Warm anti-RBC antibodies belonging to IgG4 subclass are uncommon. Cold-reacting antibodies to RBCs are mostly IgM antibodies and rarely of the IgG class. A few cases of patients with SLE and hemolytic anemia associated with cold agglutinins have been reported (9 ,10). The cold agglutinin has specificity for “I” antigens.

Specificity of Anti-RBC Antibodies

Warm antibodies that are bound to the surface of RBCs in vivo can be eluted and examined for biologic properties.

In patients with idiopathic AIHA, the warm antibodies react with antigenic determinants of the Rh complex (11 ,12). The specificity of warm antibodies in SLE is not completely known; however, it has been noted that warm antibodies eluted from erythrocytes containing both IgG and complement on their surface (including that from patients with idiopathic AIHA) react with Rh null cells (13 ,14).

Investigations employing more sensitive immunochemical techniques have shown that warm antibodies (including those seen in SLE) are heterogeneous, reacting with a variety of antigens on the RBC membrane. Two minor RBC proteins that appear to be members of the Rh family as well as two major integral membrane glycoproteins (band 3, an anion transporter and glycophorin A) have been identified as target antigens of warm anti-RBC antibodies (15 ,16).

Several studies have demonstrated a significant association between anticardiolipin (aCL) antibodies and AIHA

in SLE (17 ,18). Sthoeger et al. (19) reported that immunoglobulin eluted from the RBCs of patients with lupus contained aCL antibody activity, suggesting that in some patients, aCL antibodies may act as anti-RBC autoantibodies, causing hemolysis. IgG aCL antibodies may bind to phospholipids of the Rh system or to the phosphatidylcholine moiety of RBC membranes.

Prevalence of Anti-RBC Antibodies in SLE

Mongan et al. (20) examined the frequency of a positive direct Coombs test in patients with various types of systemic rheumatic diseases. Five of 103 (5%) patients with rheumatoid arthritis (RA) and 15 (65%) of 23 patients with SLE had a positive test. None of six patients with systemic sclerosis and two with polyarteritis nodosa were positive. Additionally, two of three cases of thrombotic thrombocytopenic purpura and only one of 103 subjects with nonrheumatic conditions were positive. Worlledge (21) found positive antiglobulin tests in 16 (44%) of 35 patients with SLE. Among normal blood donors, the frequency of a positive Coombs test was estimated to be 1 in 14,000 (22).

Despite the high frequency of positive direct antiglobulin tests among patients with SLE and RA, Mongan et al. (20) found no clinical evidence of active hemolysis. This observation illustrates the frequent dissociation between abnormal serologic findings and the occurrence of tissue injury. A positive direct Coombs test in the absence of hemolysis should be regarded as one of the multiple serologic abnormalities that frequently are seen in SLE.

Pattern of Reaction with Antiglobulin Serum

With the use of antisera of different specificities, three patterns of reactivity are commonly identified in the direct Coombs test: (a) type I: IgG, IgM, IgA, either singly or in combination is present on the RBC surface; (b) type II: both immunoglobulin and complement components are bound on the RBC surface; and (c) type III: RBCs are coated with complement components (C3, C4) alone. Type I is the pattern most commonly found in patients with idiopathic AIHA, whereas types II and III are the patterns generally associated with SLE (21 ,23 ,24).

Of 180 patients with warm-type AIHA, Worlledge (25) found that 83 patients (46%) had IgG coating alone on their RBCs, 64 (36%) had both IgG and complement, and 33 (18%) had complement coating alone. Of the 17 patients with SLE who were included in her series, none had bound IgG alone, 12 showed bound IgG and complement, and the remaining 5 had complement reactivity alone. Chaplin and Avioli (8) suggested that a diagnosis of SLE is unlikely in a patient with immune hemolytic anemia if complement components are not detectable on the RBC surface.

Among patients with SLE who have a positive direct Coombs test but no evidence of overt hemolysis, 12 of 13 showed complement reactivity alone, 3 had both IgG and complement, and none showed IgG alone (25). Of 103 patients with RA tested, 5 had a positive direct Coombs test, and all showed complement reactivity alone (20). The pattern of red-cell autosensitization in RA was confirmed by Gilliland and Turner (26), who found that 12 of 75 consecutive patients with RA who were tested reacted with anti-C antiserum exclusively. Two independent investigations established that RBCs coated with complement components alone, as determined by the standard direct Coombs test, contain IgG antibody as well, suggesting that complement deposition is in fact antibody mediated (27 ,28). Gilliland et al. (27) devised a sensitive, complement-fixing antibody consumption test, based on the principle of the antiglobulin test that detected as few as 20 IgG molecules on the red-cell surface. On the other hand, MacKenzie and Creevy (28) detected IgG antibody on the surface of complement-coated RBCs when the standard Coombs test was performed at 4°C, but not at 37°C. The IgG antibody was not eluted from RBCs at 37°C; however, it apparently underwent a thermal-dependent conformational change so that agglutination with the Coombs antiglobulin reagent did not occur. Patients with SLE and combined warm-reacting IgG and cold-reacting IgM anti-RBC antibodies have been reported (29 ,30).

Pathophysiology of Immune Hemolytic Anemia

The pathogenesis of RBC damage by anti-RBC autoantibodies has been extensively investigated (31 ,32). Erythrocytes that are sensitized with warm-reactive IgG antibodies are cleared from the circulation by macrophages in the splenic sinusoids. Macrophages express surface receptors for the Fc portion of the IgG molecule and C3b fragment of complement. The macrophage Fc receptors bind erythrocytes with bound IgG anti-RBC antibody, causing membrane damage, spherocytosis, and phagocytosis of some RBCs. Microspherocytes have a shortened life span, because of their increased rigidity and increased osmotic fragility. As the amount of surface-bound antibody increases, splenic trapping becomes more efficient, and red-cell survival shortens significantly. When the density of bound IgG antibody is substantial, complement activation occurs. RBCs coated with IgG and complement are cleared by two distinct macrophage receptors, the C3b and Fc receptors, causing an accelerated clearance of RBCs from the circulation that results in extravascular hemolysis. Sequestration of sensitized RBCs by hepatic macrophages with complement, but no Fc receptors also may occur at this stage.

The IgG subclass of the anti-RBC antibody is an important determinant in RBC destruction, because splenic macrophages have IgG Fc receptors for IgG1 and IgG3 subclasses. The macrophage FcR avidity for IgG3 is greater than that for IgG2 antibodies. RBCs with critical quantities

of IgG1 and/or IgG3 antibodies on their surface are destroyed. It has been calculated that RBCs coated with IgG1 antibody alone or with additional IgG2 and IgG4 antibodies require at least 2,000 molecules per each RBC to initiate phagocytosis or rosette formation with monocytes in vitro. In contrast, as few as 230 molecules of IgG3 anti-RBC antibodies per each RBC are required for binding to monocytes (33). Moreover, the clearance of RBCs that are sensitized with IgG antibody and complement is accelerated, and IgG1 and IgG3 antibodies fix complement efficiently, whereas IgG2 antibodies are less efficient and IgG4 antibodies do not activate complement.

Table 28-1: Antierythrocyte Antibodies in Systemic Lupus Erythematosus (SLE)

1. A positive direct Coombs test (direct antiglobulin test) in the absence of active hemolysis is a frequent serologic finding.
2. The direct Coombs test frequently shows reactivity with complement alone or with immunoglobulin plus complement.
3. Antierythrocyte antibodies in SLE are IgG antibodies, warm type, and react with a variety of antigens on the RBC surface.
4. These antibodies can be associated with significant hemolysis in some patients.

Erythrocytes coated with increased amounts of IgM and IgA in addition to warm IgG anti-RBC antibodies are more predisposed to hemolysis than are RBCs coated with IgG antibodies alone (34). Erythrocytes coated with IgM anti-RBC antibody, as in the case of cold hemagglutinin disease, are cleared by a mechanism that is dependent on complement activation. IgM-coated RBCs bind to C3b receptors of macrophages and are cleared rapidly in the liver (35 ,36). When the amount of IgM antibody on the RBC surface is high, complement activation is rapid and extensive so that the terminal components of complement become activated, resulting in intravascular hemolysis (Table 28-1).

Platelet Antibodies

A special relationship exists between SLE and chronic immune thrombocytopenic purpura (ITP), both of which primarily afflict young women. Some patients with thrombocytopenic purpura that is labeled as idiopathic at the onset later develop classic SLE (37 ,38), suggesting that ITP may be an early manifestation of the disease. Further, a thrombocytopenic purpura that is indistinguishable from chronic ITP may develop during the course of SLE. Thrombocytopenia in SLE, as in chronic ITP, is caused by increased peripheral destruction of platelets brought about by autoimmune mechanisms. Platelet survival studies in SLE with 51Cr-labeled platelets have demonstrated shortened life span (39).

In 1953, Harrington et al. (40) transfused normal human volunteers with plasma from patients with chronic ITP and noted a significant and prompt drop in platelet counts. Autologous plasma from chronic ITP subjects, obtained during disease exacerbations and then stored, produced thrombocytopenia when reinfused into the same patients during periods of disease remission (41). The humoral antibody nature of the antiplatelet factor in this disorder has been established, and Shulman et al. (41) showed that the factor was a 7S gamma globulin, reactive to autologous as well as to allogeneic platelets, and produced in vivo effects both quantitatively and qualitatively similar to those exhibited by known antiplatelet antibodies. These findings indicate that the platelet-depressing factor in the plasma is an antiplatelet antibody.

Similar plasma transfusion experiments have not been performed in patients with SLE and thrombocytopenia. However, Nathan and Snapper (42) reported an analogous situation in a premature infant born of a mother with SLE who at the time of delivery had thrombocytopenia. At birth, the infant had low platelet counts, which persisted until 3 weeks of age. Both mother and infant had platelet agglutinins and positive lupus erythematosus (LE)-cell tests. It was suggested that transplacental transfer of both platelet antibody and antinuclear antibody (ANA) had occurred from the mother to the baby.

Tests for Antiplatelet Antibodies in SLE

Although the transfusion experiments provided a strong argument for the autoimmune nature of chronic ITP, some investigators remain unconvinced, because reliable in vitro tests for the detection of antiplatelet antibodies are not available. Over the years, many in vitro tests have been introduced, indicating that a test of reasonable specificity, reproducibility, and sensitivity has yet to become widely accepted. Of the many in vitro tests for antiplatelet antibodies, the following few have been employed in SLE: platelet agglutination (43 ,44), direct antiglobulin consumption test (45 ,46), dextran agglutination test (47), platelet factor III method (48), the indirect immunofluorescence method (49 ,50 ,51), direct platelet immunofluorescence test, immunobead (52), and more recently the monoclonal antibody-specific immobilization of platelet antigens (MAIPA) (53 ,54).

Karpatkin and Siskind (55) introduced the platelet factor III immunoinjury technique to detect antiplatelet antibodies in SLE and chronic ITP. The method is based on the property of antiplatelet antibodies to damage normal platelets, releasing factor III. In turn, this factor is made available to the coagulation cascade, and its effect is measured as a shortening of the clotting time. The gamma globulin fraction of serum isolated by ammonium sulfate precipitation rather than whole serum was tested for antiplatelet antibodies, and with this sensitive method, they found platelet antibodies in 65% of patients with chronic

ITP (of whom 96% were thrombocytopenic at the time of testing). The antiplatelet antibody was removed with prior incubation of the serum using rabbit antihuman IgG or absorption with normal human platelets. Further, the antiplatelet activity can be eluted from normal platelets after prior incubation with positive, but not with negative, serum (48). Karpatkin and Lackner (56) suggested that patients with SLE who test positive for antiplatelet antibodies, but have normal platelet counts represent a subset of patients with a compensated thrombocytolytic state, in which an increased turnover of platelets is compensated for by increased platelet production. On the other hand, Kutti et al. (57) showed that the abnormal values of platelet factor III assay in nonthrombopenic patients with SLE correlated with the amount of circulating immune complexes, suggesting that the assay measured not only antiplatelet antibodies, but also immune complexes that presumably bind to surface Fc receptors.

The direct antiglobulin consumption test detects the presence of gamma globulin that is fixed onto the surface of platelets. Used extensively by early workers, this test appears to be sensitive, but it is technically complex and may yield false-positive results (58). Van de Wiel et al. (46) found that 13 of 23 patients with chronic ITP and all of 6 patients with SLE and thrombocytopenia were positive by this test. Dausett et al. (45) reported that 46 of 93 patients with chronic ITP and 23 of 24 patients with SLE were positive.

Pujol et al. (50) found a high prevalence (62%) of antiplatelet antibodies in 90 consecutive patients with SLE, especially in those with active disease, using a platelet immunofluorescence method. The antibodies were predominantly IgG, although IgM and IgA isotypes also were detected in some patients. Except for thrombocytopenia, the presence of platelet antibodies was not associated with other disease manifestations.

Platelet-Bound IgG in SLE

In 1975, Dixon et al. (59) introduced a quantitative method of measuring the IgG bound on the surface of platelets that is based on the inhibition of complement lysis. All of 17 patients with chronic ITP showed an elevated level of platelet-associated IgG when compared with that of healthy controls. Moreover, an inverse relationship between platelet count and the concentration of platelet-associated IgG was observed both before and during drug therapy. These observations were soon confirmed by several investigators using other methods of measuring platelet-bound IgG (60 ,61).

Platelet-associated IgG has been shown to be increased in practically all patients with SLE and thrombocytopenia (62 ,63 ,64 ,65). Kelton et al. (64) reported an inverse correlation between platelet count and platelet-associated IgG in 10 thrombocytopenic patients with SLE. Mulshine et al. (65) confirmed this inverse relationship and further observed that patients with SLE and normal platelet counts had platelet-associated IgG levels even lower than those seen in normal controls. Conversely, Bonacossa et al. (66) found an elevated level of platelet-bound IgG in patients with SLE and normal platelet counts. Subjects with high amounts of platelet-associated IgG had significantly more anti-DNA antibodies than those with normal or slightly elevated levels of platelet-bound IgG. However, they found no correlation between disease activity and platelet-bound IgG.

The IgG antiplatelet antibodies in the sera of patients with chronic ITP appear to be restricted to the IgG3 subclass (67), whereas in SLE sera, all four IgG subclasses are represented (68). Conversely, all four IgG subclasses are bound in vivo to platelets of patients with chronic ITP, suggesting that circulating antiplatelet IgG and platelet-associated IgG may represent different populations of platelet antibodies (69 ,70). The nature of the platelet-associated IgG is not completely known. It may represent IgG antibody bound to platelet-specific surface antigens (i.e., autoantigens), IgG antibody bound to HLA or blood group antigens or to exogenous antigens absorbed on the surface of platelets, IgG nonspecifically fixed to damaged platelets, or circulating immune complexes attached to platelet surface Fc receptors (60 ,71).

McMillan (72) showed that IgG platelet antibodies that are synthesized by splenic lymphocyte cultures of patients with chronic ITP bind to platelets through their Fab terminus, indicating a specific antibody reaction. Moreover, eluates from platelets of the same patients contain IgG that bind to normal allogeneic platelets. Kelton et al. (73) reported that the increased amounts of platelet-associated IgG in malaria were partly caused by the binding of IgG-specific antibody to malarial antigens absorbed on the surface of platelets. Thrombocytopenic purpura in patients infected with human immunodeficiency virus is associated with increased platelet-associated IgG. Walsh et al. (74) presented evidence to show that the platelet-associated IgG in these patients does not result from bound antiplatelet antibodies, but rather from the deposition of complement and immune complexes onto the surface of platelets. Samuel et al. (75) reported higher amounts of platelet-associated IgG, C3 and C4 in thrombocytopenia-associated immune complex disease including SLE, human immunodeficiency virus (HIV) infection, and chronic liver disease than in classic ITP.

The nature of platelet-associated IgG in SLE has not been fully studied, but it may in part represent bound immune complexes (60). This is supported by the observation that the antiplatelet antibody found in SLE sera fixes complement, unlike that found in the sera of patients with chronic ITP, which is noncomplement fixing (68). Moreover, preformed complexes of DNAanti-DNA antibodies bind to the surface of platelets (76). Puram et al. (77) noted a positive relationship between platelet counts in SLE with immune complex-like material in serum, as measured by polyethylene glycol precipitation, but not with platelet-associated IgG. On the other hand, no correlation was evident between the level of circulating immune complexes as measured by C1q binding (66) or Raji-cell assay (56) and the amount of platelet-bound IgG in SLE, indicating

that platelet-bound IgG is not entirely caused by antigenantibody complexes. Kurata et al. (78) reported an ether elution method of differentiating between platelet-specific antibodies and bound immune complexes, and they showed that eluates from platelets of patients with SLE contain specific antiplatelet antibodies.

Elevated levels of platelet-associated IgG are not necessarily diagnostic of chronic ITP or thrombocytopenia as a result of SLE. High levels can be seen in patients with thrombocytopenia that is considered to be nonimmune in origin (60) as well as in some patients with normal platelet counts (66). Conversely, a diagnosis of ITP is unlikely in a patient with thrombocytopenia if the platelet-associated IgG level is low or normal.

Specificity of Antiplatelet Antibodies in SLE

A limited number of studies have examined the antigenic specificity of autoantibodies to platelets in SLE (79 ,80 ,81). Platelet autoantibodies are directed mainly to GpIIb/IIIa complex, although multiple platelet antigens, including surface membrane and cytoplasmic proteins, have been identified, implying heterogeneity of these antibodies in SLE.

Howe and Lynch (80) examined the binding specificities of circulating antiplatelet antibodies in SLE by Western blotting. All patients with SLE who were thrombocytopenic and had increased amounts of platelet-bound IgG had serum antibodies that reacted with platelet protein fractions having molecular weights of 120 and 80 kd. Patients with SLE and normal platelet counts but with elevated platelet-associated IgG also were positive for serum antiplatelet antibodies. Absorption of sera with whole platelets or platelet lysates removed the antibody activity. The binding pattern was found to be relatively specific for SLE and was not seen in healthy controls. Sera from patients with chronic ITP reacted with multiple platelet fractions, but with no consistent pattern, unlike that seen in SLE, suggesting that the specificities of platelet antibodies in the two conditions are different.

Using a similar methodology, Kaplan et al. (81) confirmed the presence of serum antibodies to platelets in three of ten thrombocytopenic patients with SLE. The antigens involved were cytoplasmic proteins from normal platelets with molecular weights of 108 and 66 kd, respectively. Tomiyama et al. (82) identified the 120-kd antigen to be vinculin, a cytoplasmic platelet protein. Antivinculin antibodies were found to be prevalent not only in patients with immune thrombocytopenia (67%), but also in healthy subjects (40%), suggesting that these are naturally occurring antibodies. The pathogenetic role of these antibodies remains to be determined.

In chronic ITP, target antigens of circulating antiplatelet antibodies as well as platelet-bound IgG have been identified by immunoblotting and by using monoclonal antibodies. Antigenic epitopes on the GPIIb-IIIa complex (CD41/CD61) have been the most frequently observed, whereas GP Ib/IX and GPIa/IIa antigens also have been reported (83 ,84 ,85). These glycoproteins belong to a complex of membrane proteins on the surface of platelets, termed integrins, which function as receptors for fibrinogen, fibronectin, collagen, and other extracellular matrix components that are important in hemostasis.

Berchtold et al. (79) examined the specificity of antiplatelet antibodies in patients with disease-related immune thrombocytopenia, including SLE, other connective tissue diseases, and lymphomas. Autoantibodies against platelet GPIIb-IIIa complex were found in patients with SLE and in mixed connective tissue disease and Sjögren syndrome, showing that the specificity of the antiplatelet antibodies in some patients with systemic rheumatic diseases is similar to that seen in patients with chronic ITP (Table 28-2). The presence of specific antibodies against GP IIb-IIIa and to other platelet glycoproteins in SLE has been confirmed by other investigators (52 ,53 ,86). Serum antibodies to platelet membrane antigens (GP IIb-IIa, GP Ib-ICX, GP Ia-IIa, and GP IV) were found to more prevalent in thrombocytopenic SLE patients than in those patients with normal platelet counts (86), however, other investigators have failed to confirm this association (53).

In a more detailed study, Michel et al. (87) confirmed that the specificity of antiplatelet antibodies in SLE is directed mainly against GpIIb/IIIa complex. Using an indirect MAIPA assay, they found these serum antibodies in 36% of thrombocytopenic SLE patients and in only 5% of SLE patients with normal platelet counts. Antibodies to other platelet antigens including GpIbIX, GpIaIIa, CD9, and others were also found in smaller number of patients, implying the wide heterogeneity of antiplatelet antibodies. More importantly, when platelet-bound immunoglobulin and platelet eluates from thrombocytopenic patients were tested, 69% showed the presence of specific antiplatelet antibodies.

Table 28-2: Platelet Antibodies in Systemic Lupus Erythematosus (SLE)

1. In vitro tests for platelet antibodies, such as immunofluorescence, direct antiglobulin consumption test, and monoclonal antibody-specific immobilization of platelet antigens frequently are positive in SLE. These tests are of limited clinical application.
2. Tests that measure platelet-associated IgG show elevated values in practically all patients with SLE and thrombocytopenia as well as in some patients with normal platelet count.
3. The nature of platelet-associated IgG is not completely known, but it probably represents specific antiplatelet antibodies and bound immune complexes.
4. Platelet antibodies in SLE react mainly with platelet membrane glycoproteins GpIIb/IIIa complex that function as receptors.

Antigen-Specific Platelet Autoantibody Assays

The clinical application for antigen-specific platelet autoantibody assay for the laboratory diagnosis of immune thrombocytopenia in SLE remains to be established. McMillan (88) opines that a positive antigen-specific platelet autoantibody assay provides strong evidence of immune thrombocytopenia in chronic adult ITP and SLE. However, in prospective studies a significant number of patients have tested negative, so that a negative result does not exclude the presence of immune thrombocytopenia (89).

Antiphospholipid Antibodies and Antiplatelet Antibodies

The presence of antiphospholipid antibodies, including aCL, and lupus anticoagulant in SLE is strongly associated with thrombocytopenia (see Chapter 29 , “Pathomechanisms of Cutaneous Lupus Erythematosus”). For this reason, it has been hypothesized that aCL may cross-react with platelet phospholipids, resulting in inactivation and/or subsequent sequestration in the reticuloendothelial system. Rupin et al. (90) examined the significance of specific platelet antibodies and aCL in patients with SLE and thrombocytopenia, and although one half of their patients with low platelet counts tested positive for aCL, the thrombocytopenia correlated better with the presence of serum IgG antibody to an 80-kd platelet antigen. Moreover, absorption of the serum with platelets removed the aCL activity in only one half of the sera with antiplatelet antibodies. Jouhikainen et al. (91) examined 71 consecutive patients with SLE for platelet antibodies by immunoblotting. The most common antibody found reacted with a 65-kd platelet antigen, and its presence was significantly associated with lupus anticoagulant, a history of thrombocytopenia, and thrombosis, especially arterial occlusions. Out et al. (92) observed that IgG eluted from the platelets of patients with SLE had antibody activity against negatively charged phospholipids. Nevertheless, there was no evidence of the in vivo activation of platelets, and platelet aggregation was not impaired. By adsorption experiments, Lipp et al. (52) have shown that antiphospholipid antibodies and antiglycoprotein antibodies (GPIIb-IIA and GPIb-IX) in chronic ITP and in SLE with thrombocytopenia have distinct specificities and do not cross-react. Moreover, in patients with primary antiphospholipid antibody syndrome and associated immune thrombocytopenia, Godeau et al. (93) reported that specific antiplatelet glycoprotein antibodies, but not anticardiolipin antibodies were found in the platelet eluates. This finding suggests that the absence of cross-reactivity between anticardiolipin and antigen-specific platlet autoantibodies.

These data indicate a heterogeneity of platelet antibodies in SLE, some of which cross-react with phospholipids and some with specific platelet glycoproteins, as well as other membrane and cytoplasmic antigens. Further studies to characterize the antigens and to clarify the relative importance of the different antibodies in the pathogenesis of the thrombocytopenia are needed.

Antineutrophil Antibodies in SLE

The frequent occurrence of leukopenia in SLE, possibly mediated by immunologic processes similar to those described in autoimmune hemolytic anemia or autoimmune thrombocytopenia, led to investigations on the presence of antileukocyte antibodies. Various conventional serologic methods, such as agglutination, complement fixation, antiglobulin consumption test, and cytotoxicity, have been used (94). Early studies employed whole leukocyte preparations rather than purified fractions (e.g., lymphocyte subsets) as substrate, and differences in their specificity and sensitivity make it difficult to compare results of the various tests. Further, the presence of isoantibodies against leukocytes, which may be a consequence of multiple pregnancies and/or blood transfusions, must be differentiated from genuine leukocyte antibodies when interpreting the results.

Technical improvements in the fractionation of peripheral blood leukocytes led to the development of new procedures to detect antibodies to lymphocytes. In 1970, Mittal et al. (95) employed the lymphocyte microcytotoxicity test, which was developed for histocompatibility testing, for the detection of cytotoxic antibodies to lymphocytes in SLE. They found a high prevalence of specific lymphocytotoxic antibodies in SLE, and this observation soon was confirmed independently by other workers (96 ,97 ,98) (see Chapter 65 , “Clinical and Management Aspects of the Antiphospholipid Antibody Syndrome”). In contrast, techniques to detect specific immune reactions to granulocytes have been more difficult to standardize (99).

Tests for antineutrophil antibodies fall into two major types: immunochemical and functional (99). The former detects immunoglobulins that are bound to the surface of the patient's neutrophils (i.e., direct test) or free antibodies in the serum (i.e., indirect test) using normal allogeneic neutrophils as substrate. When interpreting the results of these tests, immune complexes binding via Fc and complement receptors on leukocytes should be excluded from the binding of specific antineutrophil antibodies. The latter type measures in vitro sequelae of granulocyte antibodies such as lysis of sensitized cells, phagocytosis, and so on. Flourescence flow cytometry has been adapted for measuring IgG antineutrophil antibodies, and a study using this method found that sera from nonneutropenic patients with SLE as well as patients with other connective tissue diseases show significant binding (100).

Both IgM- and IgG-specific antineutrophil autoantibodies have been found in SLE. Drew and Terasaki (101) described cytotoxic granulocyte-specific antibodies in 53% of 57 patients with SLE using a panel of 70 granulocytes from random normal persons. The antibodies were of IgM class, complement fixing, exhibited optimum activity at 4°C, and were present in 10% healthy, nonimmunized individuals. The clinical significance of these antibodies in

SLE was not examined. Starkebaum et al. (102) studied the mechanism of neutropenia in a patient with SLE and found increased amounts of IgG bound on the surface of polymorphonuclear neutrophils (PMNs). The patient's serum caused opsonization of normal neutrophils for ingestion by other neutrophils. Additionally, fractionation of the serum showed that both immune complexes and monomeric IgG antineutrophil bound to PMNs; however, only the latter caused opsonization of neutrophils. The F(ab)2 fragment of the IgG reacted to the PMNs, confirming the true antibody activity (103). Although IgG neutrophil-binding activity of serum was found to be common in SLE, there was no association between the level and neutropenia (103). Two independent groups of investigators confirmed the absence of correlation between neutrophil count in SLE and the titer of PMN-binding IgG in the serum (104 ,105). In contrast, there is some correlation between antilymphocyte antibody titers and lymphopenia (see Chapter 29 , Cutaneous Manifestations of Lupus Erythematosus). However, the ability of SLE sera to opsonize normal PMNs to be phagocytosed by monocytes (104), as well as the capacity of serum antineutrophil antibodies to fix C3 on allogeneic normal PMNs, were both found to be correlated inversely with neutrophil count in SLE.

Specificity of Antineutrophil Antibodies

Studies on the specificity of antineutrophil antibodies in SLE have revealed multiple antigens, however the molecular structure and biological functions of these antigens remain to be elucidated. Ro(SSA) and La(SSB) antigens have been reported to be target antigens of antineutrophil antibodies in some SLE patients.

Using Western immunoblots, Sipos et al. (106) found antineutrophil antibodies in SLE reacted with two membrane antigens with molecular weights of 50 to 60 kd and of 30 kd, respectively. Moreover, the antineutrophil antibodies inhibited the binding of mouse monoclonal antibodies to CD15 (granulocyte antigen) and CD16 (FcR) to normal neutrophils. Whether the antigens seen in the immunoblots are identical to CD15 or CD16, however, remains to be clarified. Chen et al. (107) described two antigens in mature neutrophils and five antigens in precursor neutrophils as target antigens of antineutrophil antibodies in SLE. The structure and identity of these antigens are presently unknown.

Kurien et al. (108) found a correlation between neutropenia and the presence of anti-Ro antibodies in SLE. Anti-Ro antibodies were shown to bind to neutrophils and thus, can potentially induce cell injury via complement activation. However, they found that the antigen bound on the surface of neutrophils was not the 60-kd Ro but instead a 64-kd membrane protein called “D1,” an antigen associated with autoimmune thyroid disease. Inhibition studies showed serologic cross-reactivity between the two antigens, implying that anti-Ro antibodies may be important in the pathogenesis of granulocytopenia.

Table 28-3: Antineutrophil Antibodies in Systemic Lupus Erythematosus (SLE)

1. Circulating antineutrophil antibodies are prevalent in SLE; however, the antibody titer does not correlate with the neutrophil count.
2. Ability of SLE sera to opsonize as well as to fix C3 on normal allogeneic PMNs is inversely correlated with the neutrophil count.
3. Most antineutrophil antibodies are directed to surface antigens on polymorphonuclear cells; however, some react with cytoplasm of PMNs. P-ANCAs may be found, but not C-ANCAs.

ANCA, antigen(s) of human neutrophils and monocytes; PMN, polymorphonuclear neutrophils.

Hsieh et al. (109) reported that 20% of SLE sera contain antineutrophil antibodies that reacted with a 50 kDa membrane protein. Further analysis revealed that the antigen was surface-expressed La(SSB) autoantigen on neutrophils. Functional studies revealed that purified anti-La(SSB) antbodies bind and penetrate neutrophils and impair phagocytosis, accelerate apoptosis and enhance interleukin-8 production in vitrol.

The lack of correlation between titer of antineutrophil antibodies and neutrophil count suggests that factors other than antineutrophil antibodies are important in the pathogenesis of neutropenia in SLE. Antibody-coated PMNs may remain in the circulation longer because of the defective reticuloendothelial function in SLE. The role of antibody avidity, specificity, and the density of membrane antigens may be important. A study of neutrophil kinetics is needed to determine whether peripheral destruction of neutrophils in SLE is compensated for by increased production, such that the net result is a normal peripheral neutrophil count (Table 28-3).

Antineutrophil Cytoplasmic Antibodies

Autoantibodies directed against cytoplasmic antigen(s) of human neutrophils and monocytes (ANCA) are associated with Wegener granulomatosis, microscopic polyangiitis, and other primary systemic small-vessel vasculitides (110 ,111). These antibodies are detected by the indirect immunofluorescent test using ethanol-fixed normal neutrophils as substrate. Four fluorescent patterns are seen: a classic cytoplasmic (C-ANCA), atypical C-ANCA, a perinuclear with or without nuclear extension (P-ANCA), and atypical ANCA (112). C-ANCA is detected in most patients with Wegener granulomatosis and reacts with proteinase 3, although other antigens also are involved. P-ANCAs are found in patients with idiopathic, necrotizing, crescentic glomerulonephritis, and polyarteritis nodosa and react predominantly with myeloperoxidase, a lysosomal enzyme,

although other antigens such as elastase, cathepsin G, lactoferrin, and azurocidin are involved as well (111 ,113).

ANA may interfere with interpretation of the immunofluorescent test for P-ANCA (114). All serum samples containing ANCA should be tested for specific antibodies to proteinase-3 and myeloperoxidase by ELISA. Antimyeloperoxidase antibodies may be overestimated in SLE serum containing anti-DNA-DNA immune complexes. The DNA antigen in the complex binds to the cationic myeloperoxidase used in the ELISA test giving rise to a false-positive result (115).

None of 96 patients with SLE studied by Nassberger et al. (116) using the immunofluorescent test had C-ANCA, whereas 93 had ANA. Antimyeloperoxidase antibodies were found in 21% of the patients by a specific ELISA test. Serum titers generally were low, and presence of the antibody did not correlate with any particular disease feature. Other investigators have reported serum titer of antimyeloperoxidase antibodies in 9% of patients with lupus (117 ,118). Both antibodies to elastase and to myeloperoxidase were found to be prevalent in hydralazine-induced LE (116).

Lactoferrin is a single-chain glycoprotein that is present in many body secretions and derived primarily from neutrophils; it is located in the secondary granules of these cells. IgG antilactoferrin antibodies are reported in five to 39 of patients with SLE (119 ,120), and IgM antilactoferrin antibodies are present in 10% of these patients (120). IgG antilactoferrin antibodies are more prevalent in patients with active disease and appear to be associated with adenopathy and crescentic glomerulonephritis (119).

Schnabel et al. (121) found ANCA in 40 of 157 (25%) patients with SLE. Only P-ANCA was found. The specificities of the SLE antibodies were directed to lactoferrin, elastase, and lyzozyme. No reactivity to myeloperoxidase or to proteinase 3 was detected. More importantly, there was no correlation between P-ANCA with organ system involvement, including lupus vasculitis. Other investigators confirmed the lack of correlation between ANCA, disease activity, and clinical features of SLE (114 ,122). In contrast, a study of a large cohort of European patients with SLE showed correlation of ANCA and antilactoferrin with certain clinical manifestations including serositis, livedo reticualris, thrombosis, and arthritis. However, anticardiolipin and anti-Ro antibodies were more closely correlated than ANCA with these features (123). Antibodies to lysozyme were found in one third of 44 patients with SLE in another study (124).

A 5-year prospective controlled study of ANCA in a large number of patients with various connective-tissue diseases including SLE and healthy controls showed a high prevalence of P-ANCA and atypical ANCA. P-ANCA was associated with the presence of antinuclear antibodies. None had C-ANCA and specific antibodies to proteinase-3 rarely were found (125).

A recent analysis of 13 published studies on ANCA in SLE concluded that 15% to 20% of SLE patients have pANCA and in some patients there may be an association with lupus disease activity (126).

Thus, the presence of C-ANCA and antibodies to proteinase-3 suggests a systemic vasculitic disease other than SLE. P-ANCA, however, can be found in patients with SLE as well as in patients with other conditions.

Rheumatoid Factors

Rheumatoid factors (RFs) comprise a heterogenous group of antibodies that are reactive with antigenic determinants on the Fc portion of human or animal IgG. Although RFs belonging to the IgM class are the most commonly measured isotype by clinical tests, RFs belonging to the IgA, IgG, IgD, and IgE classes have been identified (127 ,128). RFs can react with autologous and isologous as well as homologous IgG.

Serum RFs are measured by a variety of serologic methods, including agglutination, ELISA, radioimmunoassay, and nephelometry. Agglutination tests such as the latex fixation test preferentially measure IgM RFs that are reactive with human IgG. The sheep-cell agglutination test (SCAT) measures IgM RFs using rabbit IgG as an antigen. Clinical laboratories prefer nephelometry over the latex fixation test, because the former is automated and less labor intensive.

Prevalence of RFs in SLE

The prevalence of RFs in large series of patients with SLE as measured by the latex fixation test varies from 20 to 60% (mean, 33%). Singer (129) reviewed several earlier reports and found that 20.5 of tested patients with SLE were positive. Estes and Christian (130) found a positive latex fixation test in 21% of their 150 patients. The serum antibody titer was relatively low, and unlike RA, in which the titer persists, most of their patients did not have a sustained titer. Lee et al. (131) reported positive tests for RFs in 36.7% of 110 patients with SLE. In 31.2% of their patients, the titer was equal to or greater than 1:160, and in 28.8%, the serum titer was greater than 1:320. On the other hand, Feinglass et al. (132) described a higher frequency of RFs among their patients with SLE; 61% of their 122 patients had a positive latex fixation test. In agreement with other studies, the RF titers were modest, with a titer of 1:80 seen in one half of the patients. Further, the serum titer fluctuated intermittently in patients in whom serial determinations were performed.

The SCAT for RFs is less sensitive than the latex fixation test, but a positive SCAT is considered to be more characteristic of RA (128). None of 25 patients with SLE studied by Cathcart et al. (133) had a positive SCAT, and only three patients had borderline titers of less than 1:32.

RFs belonging to isotypes other than IgM are not commonly seen in SLE. If present, they tend to have lower serum titers than those observed in patients with RA. IgG RFs, which are implicated in the pathogenesis of synovitis and extraarticular lesions of RA such as vasculitis, generally are absent in SLE (134 ,135). IgE RFs that also are associated with the extraarticular manifestations of RA are not seen in SLE (136 ,137). Dunne et al. (138) found elevated levels of

IgA RFs in the sera of patients with RA, Sjögren syndrome, and SLE. The serum level of IgA RFs in SLE was lower than that in RA.

The major RF cross-reactive idiotype (RF-CRI) is a public idiotype defined by human IgM RF paraproteins. RF-CRI is expressed in adult and juvenile RA. In SLE, Bonagura et al. (139) found RF-CRI to correlate with the presence of anti-double-stranded DNA (anti-dsDNA) antibodies and disease activity.

Potential Significance of Rheumatoid Factors in SLE

In vitro experiments as well as studies in experimental animal models point to a dual effect of RFs on immune-mediated tissue injury. On one hand, RFs have been shown to exert protective effects by competing with complement for binding to immune complexes (140 ,141). RFs binding to antigenantibody complexes may result in more efficient removal from the circulation by the reticuloendothelial system (142 ,143). Bolton et al. (144) showed that RFs blocked the attachment of C3 to aggregated IgG and the formation of C3b capable of reacting with the C3b receptors of glomeruli in vitro. This phenomenon potentially can shield the glomerulus from deposition of pathogenic immune complexes.

Conversely, others have found RFs to enhance immune-mediated tissue injury in different experimental animal models (145). Floyd and Tesar (145) showed that the administration of IgM RFs aggravated cutaneous Arthus reaction in animals. RFs and immune complexes injected into the mesenteric arteries of rats induced thrombosis and hemorrhage (146). Another series of experiments (147 ,148 ,149) showed that RFs bind in situ to immune complexes that are bound to renal glomeruli in experimental glomerulonephritis. These investigators postulated that bound RFs subsequently act as an immunosorbent, trapping circulating antigenantibody complexes that may be unrelated to the initial renal insult and, by fixing complement, contribute to the chronicity of the renal disease. Birchmore et al. (150) noted that RFs enhanced the binding of DNAanti-DNA immune complexes to C3b receptors on RBCs in vitro by fixing complement by way of its own Fc region. Their finding suggests that RFs may potentiate renal injury in SLE.

Miyazaki et al. (151) found IgM, IgA, and IgG RFs in the serum of five patients with diffuse lupus nephritis, but not in two patients with membranous lupus nephritis. More importantly, they observed the binding of fluorescein-labeled normal human IgG and Fc fragment, but not F(ab)2 fragment, to the renal glomeruli in diffuse lupus glomerulonephritis. No binding was observed in membranous lupus nephritis or in IgA nephropathy. They interpreted this to mean RF activity was present in the glomerular deposits that bind the labeled IgG, suggesting that RFs may be important in the development of diffuse lupus nephritis. This study confirms earlier observations by Agnello et al. (152), who showed glomerular deposits of IgM RFs in lupus nephritis by reacting fluorescein-labeled aggregated human IgG and antidiotypic antibody to RFs with renal biopsy specimens.

Clinical Correlates of Rheumatoid Factors in Systemic Lupus Erythematosus

Which of the many and varied biologic effects of RFs in vitro or in experimental models are important in the pathogenesis of lesions in SLE remains to be seen. Nevertheless, several investigators have examined clinical correlates of RFs in SLE. In 1966, Davis and Bollet (153) noted that nephritis was less prevalent in a group of patients with SLE who were RF positive by the latex fixation test. They suggested that RFs exerted a protective role in vivo in SLE and other diseases of immune complex deposition. A retrospective analysis of their patients confirmed their initial observation, and additionally, they found that patients with SLE who are RF negative and cryoglobulin positive are likely to develop renal disease, whereas those who are RF positive and cryoglobulin negative are unlikely to do so (154). Hill et al. (155) found that patients with SLE and nephritis who were RF positive (by latex fixation test) had milder morphologic renal lesions compared with RF-negative patients with SLE and nephritis. This protective effect of RFs on lupus nephritis was likewise observed in a study using the SCAT for measuring RFs (156), and Corke (157) found that in patients with SLE, proteinuria was less frequent in RF-positive than in RF-negative patients. Mustakallio et al. (158) described a negative correlation between RFs and anemia, skin disease, and the LE-cell test. Moreover, the RF-positive patients with SLE tended to have a more benign and chronic clinical course. A multicenter European study of 1,000 patients with SLE found RF in 180 patients (16) and was associated with higher prevalence of discoid skin rash, sicca syndrome, and a lower prevalence of nephropathy (28 vs. 41) (159).

In the foregoing studies, the tests used for detecting RFs preferentially measured IgM RFs. Tarkowski and Westerberg (160) used an ELISA assay to measure the different isotypes of RFs in SLE and found that the presence and serum level of IgG RFs, IgA RFs, and IgM RFs correlated significantly with the absence of renal disease.

Witte et al. (161) confirmed this negative association and in addition found that the presence of IgA RF defined a clinical subset characterized by sicca syndrome, anti-Ro and anti-La antibodies, and HLA DR3.

Other investigators, however, have failed to confirm a protective role of RFs in SLE nephritis or in other organ involvement (162). Kantor et al. (163) measured RFs in 51 consecutive patients with SLE and found that the frequency as well as the antibody titer of RFs in those with nephritis did not differ from those without clinical renal disease. Baldwin et al. (164) confirmed these observations and found that the presence of RFs was not associated with histologic type of nephritis. Estes and Christian (130)

reported that the frequency of renal disease as well as the 5-year survival rate of RF-positive patients with SLE did not differ from those of the general SLE population. Two other studies using the SCAT for RFs (165) and radioimmunoassay for IgM, IgA, and IgG RF isotypes (166) failed to find a protective effect from RFs on the development of nephritis.

Certain observations not only refute the protective role of RFs but in fact point to their participation in the pathogenesis of tissue lesions in SLE. Cryoglobulins, which represent a subset of circulating immune complexes in SLE, often contain RF activity. Agnello et al. (152) identified RFs in the glomerular immune deposits of patients with SLE and nephritis, hypocomplementemia, and cryoglobulinemia. Deposition of antiglobulins (including RFs) in the renal glomeruli was observed in lupus nephritis and in other types of glomerulonephritis, and their presence appeared to be associated with a relatively severe renal injury (167 ,168). My own group has found that immune deposits in the walls of pulmonary arteries in patients with SLE and pulmonary hypertension were eluted when incubated with aggregated human IgG, suggesting the presence of RFs in the vascular immune deposits (169).

In addition to renal disease, a correlation between RFs and other clinical features of SLE has been examined. RFs were more prevalent in late-onset SLE, defined as onset in individuals above 50 years of age, than in younger patients (170) Moutsopoulos et al. (171) described a high frequency of RFs in patients with SLE and histologic evidence of sicca syndrome on lip biopsy. The prevalence of RFs in 35 patients with SLE positive for anti-Ro/SSA antibody (63%) was significantly higher than that in 77 such patients negative for anti-Ro/SSA antibody (7%). Zizic et al. (172) reported a high frequency of RFs among patients with SLE presenting with acute abdomen secondary to vasculitis and/or serositis. My own group, as well as other investigators have found a high prevalence of RFs in patients with SLE presenting with pulmonary hypertension (169 ,173).

Conversely, Feinglass et al. (132) observed no correlation between RFs and neuropsychiatric involvement in SLE. Patients with SLE and a persistently positive latex fixation test for RFs tended to have less severe clinical manifestations of the disease, were less likely to have received high-dose steroids or cytotoxic drugs for treatment, and were less likely to have had an episode of herpes zoster than patients with SLE who had persistently negative or inconsistently positive tests for RFs (174).

The discrepancy in the results of various investigations on the relationship between RFs and lupus nephritis or SLE in general probably is caused by several factors such as differences in the methods used to measure RFs, selection of patients, ascertainment of activity of the renal disease, and the retrospective design of most of the studies. In addition, the titer of the RFs may fluctuate or even disappear along the course of the illness, thus the timing of the test will affect the result of the study. Moreover, it now is well recognized that RFs are heterogenous with respect to the immunoglobulin class, reactivity with IgG of different species, complement-fixing property, avidity, and other characteristics. Conceivably, varying types of RFs may differ in their effects on renal disease and other tissue lesions. My own group's observations, showing that IgM RFs specific for rabbit IgG correlate positively with arthritis and negatively with other clinical manifestations, suggest a dual effect of RF (175).

Rheumatoid Factors Cross-Reactive with Nuclear Antigens

In 1963, McCormick and Day (176) observed that exhaustive absorption of certain sera containing both RF and ANA activities with aggregated gamma globulin removed the RF activity and was accompanied by a substantial loss of ANA titer. They suspected that the phenomenon was caused by the presence of IgG ANAs associated with the IgM RFs as an immune complex. Subsequently, Hannestad (177) and Hannestad and Johannessen (178) established that this was not caused by complexed IgG ANAs, but rather by the dual reactivity of certain polyclonal IgM RFs with both IgG and nuclear antigens. Other investigators have confirmed these findings (179) and showed that isolated IgM RFs reacts with DNAhistone complex (180 ,181), with histones (182), and/or with nonhistone nuclear polypeptides (183). Cross-reactive IgM RFs have been found most frequently in patients with RA and in some cases of overlap syndromes and mixed connective-tissue disease (180). Kinoshita et al. (184) found IgM RFs that are cross-reactive with single-stranded DNA to be prevalent in a variety of rheumatic diseases, including SLE; however, the serum titer was highest among patients with RA and extraarticular features. Johnson (179) suggested that the cross-reactive IgM RFs are of limited immunopathogenic significance, because such antibodies are noncomplement fixing, react optimally at pH 8 or 9, and fail to bind at pH 6.5. The explanation for the reactivity of human RF with histones is not entirely clear, but recent evidence suggests multifunctional combining regions on RF for human IgG and histones with distinct binding sites located in the variable regions (185) (Table 28-4).

Table 28-4: Rheumatoid Factors (RFs) and Anti-CCP Antibodies in SLE

1. The latex fixation test for IgM RFs is positive in 33% of patients with SLE. Serum titers generally are lower than those seen in patients with RA, tend to fluctuate, and may become negative.
2. RFs have been identified in serum cryoglobulins and glomerular deposits of some patients with SLE.
3. The hypothesis that renal disease tends to be less frequent and less severe in RF-positive than in RF-negative patients with SLE has not been confirmed consistently by other investigators and remains controversial.
4. Anti-CCP antibodies may be seen in a small number of SLE patients. The presence of anti-CCP antibodies alone does not exclude a diagnosis of SLE.

Anticitrullinated Protein Antibodies in SLE

Anticitrullinated protein antibodies are found in RA and are considered diagnostic marker for the disease with high sensitivity and specificity. Commonly measured by an ELISA using fillagrin epitopes and most recently citrullinated cyclic peptides (anti-CCP) as antigens, the test is now widely available as a surrogate marker for the diagnosis and prognosis of RA. However, a few patients with other diagnoses including SLE, psoriatic arthritis, Sjogren syndrome, and juvenile idiopathic arthritis may test positive for anti-CCP antibodies (186).

Anti-CCP antibodies are uncommon in SLE (187) but may be useful in differentiating rheumatoid arthritis from SLE patients with erosive arthritis. Mediwake et al. (188) found erosive arthritis in 10 of 231 SLE patients. Rheumatoid factor was positive in 6 of the 10, whereas only 1 patient had anti-CCP antibodies. Eleven of 201 (5.5%) SLE patients studied by Hoffman et al. (189) tested positive for anti-CCP antibodies. Six fulfilled classification criteria for both RA and SLE and 3 had erosive arthritis. Sauerland et al. (190) found anti-CCP antibodies in 9 of 71 (12.7%) SLE patients tested in a cross-sectional study. Thus, the presence of anti-CCP antibodies alone does not necessarily exclude a diagnosis of SLE. Whether the presence of anti-CCP antibodies in SLE patients predisposes to the development of erosive arthritis is not known.

Cryoglobulins in Systemic Lupus Erythematosus

It has long been recognized that serum specimens from certain groups of patients develop a precipitate spontaneously when they are allowed to incubate in a test tube at low temperatures. Lerner et al. (191) described this phenomenon in patients with leukemia, pneumonia, bacterial endocarditis, and other diagnoses. Having identified gamma globulin as the serum protein fraction that reversibly precipitates at 5°C, the term cryoglobulin was introduced to refer to the cold-insoluble precipitates.

Frequency of Cryoglobulins in SLE

Barr et al. (192) examined sera from patients with various diagnoses as well as from normal subjects for cryoprecipitation. Sera from 8 of 121 patients, but none from 57 healthy controls, had significant amounts of cryoglobulins. Three of the 6 patients with SLE in this early series had cryoglobulins. Christian et al. (193) reported the presence of cryoglobulins in 10 of 12 patients with SLE, with protein concentrations ranging from 7 to 38 mg/dL. In a larger series, Stastny and Ziff (194) studied 137 sera from 31 patients with SLE; 37 sera from 11 patients had cryoglobulins. Lee and Rivero (195) observed cryoglobulinemia in 9 of 57 SLE sera, whereas Garcia-Carrasco et al. (196) reported cryoglobulins in 31 of 122 (26%) consecutive SLE patients.

Components of Cryoglobulins

Immunochemical analysis of the cryoprecipitate (197) has revealed three major types of cryoglobulins. Type I cryoglobulins consist of a single monoclonal immunoglobulin: IgG, IgM, IgA, or Bence Jones protein. Type I cryoglobulins are associated with lymphoproliferative disorders. Type II cryoglobulins are mixed cryoglobulins with one of the components being a monoclonal immunoglobulin. Monoclonal IgM with polyclonal IgG is the most common combination, and frequently, the monoclonal component has antiimmunoglobulin (i.e., RF) activity. Type II cryoglobulins are found in patients with chronic hepatitis C infection, lymphoproliferative diseases, Sjogren syndrome, and autoimmune disorders. Type III cryoglobulins are mixed cryoglobulins with polyclonal components. This type is the most common and is associated with infections and autoimmune disorders such as SLE, RA, and systemic sclerosis. Types II and III may contain RF, other autoantibodies, and complement components C1q, C3, and C4.

Hanauer and Christian (198) analyzed isolated serum cryoprecipitates from six subjects with SLE and observed that these consisted largely of IgG, IgM, and C1q. When the washed cryoglobulins were used as immunogens in rabbits, the resulting antiserum reacted with IgG, IgM, C1q, and 2-macroglobulin. Some of the antisera also reacted with C4, C3, and IgA.

Barnett et al. (199) found IgG and IgM in all 156 SLE cryoprecipitates they studied. Eleven contained IgA and C3. Stastny and Ziff (194) reported that IgM was not a prominent component in SLE cryoprecipitates and that the complex consisted mainly of IgG and C1q. Although IgG was the predominant immunoglobulin, relatively more IgM than IgG was concentrated when compared with the corresponding immunoglobulin levels (200).

The formation of cryoprecipitates in SLE sera requires the presence of C1q (193). Prior incubation of SLE serum at 56°C for 30 minutes to inactivate complement resulted in the loss of cryoprecipitability, which was restored when either fresh human serum or purified C1q was added to the test serum.

Fibronectin, which is a normal plasma protein and a major cell surface membrane protein of fibroblasts, is a component of cryoglobulins from patients with connective tissue diseases and other illnesses (201 ,202 ,203). Plasma fibronectin can bind to other molecules, such as collagen, fibrin, and heparin, and the binding may result in formation of a precipitate at low temperatures. Kono et al. (204) showed that fibronectin is capable of binding to C1q, including C1q that is fixed to immune complexes. Because fibronectin and C1q frequently coprecipitate in SLE cryoglobulins, this reaction and/or binding of fibronectin to other serum proteins may be important in the formation of cryoglobulins.

Clinical Correlates of Cryoglobulinemia in SLE

Cryoglobulinemia is associated with clinical disease activity. Eight of 11 patients with cryoglobulins reported by Stastny and Ziff (194) had active nephritis, and the remaining

3 had evidence of extrarenal involvement. Nine of the 12 patients with cryoglobulins studied by Christian et al. (193) had active nephritis. Cryoglobulinemia in SLE also has been associated with reduced serum levels of C3 and C1q (205). Cryoglobulins and RF have independent and opposite association with lupus myelitis (154).

Cryoglobulinemia is a common finding in hepatitis C infection. Garcia-Carrasco (196) reported correlation between cryoglobulinemia in SLE and cutaneous vasculitis, rheumatoid factor, hypocomplementemia and evidence of hepatitis C infection. Other investigators have confirmed the high prevalence of cryoglobulinemia in SLE patients with concomitant hepatitis C infection (206 ,207).

Cryoglobulins as Circulating Immune Complexes

The association between cryoglobulinemia, disease activity, and hypocomplementemia in SLE led to investigations on the potential pathogenicity of the cryoprecipitates. Mixed cryoglobulins are considered to represent circulating immune complexes for several reasons. In certain conditions, such as essential mixed cryoglobulinemia, the property of cryoprecipitability does not reside on either moiety of the cryoglobulin; it requires combination of the separated components (208). Complement components are required for cryoprecipitability, and further, isolated cryoglobulins have the property of activating the complement system (209 ,210). Cryoglobulins isolated from the sera of patients with lupus nephritis activate the complement system in vitro either through the classic or alternative pathway (211).

Despite similarities in immunoglobulin content, SLE cryoglobulins differ from RA cryoglobulins in their complement-binding property. Although SLE cryoglobulins frequently bind C1q in vitro, isolated RA cryoglobulins do not (200). This difference may reflect varying properties of the antigen-antibody systems that involved in cryoglobulin formation in the two conditions.

Antibody Activity of SLE Cryoglobulins

The deposition of ANAs and their corresponding antigens in target organs has been implicated in the pathogenesis of organ injury in SLE. Cryoprecipitates in patients with SLE during periods of disease activity may represent circulating immune complexes of ANAs and nuclear antigens. Lee and Rivero (195) found no ANA activity in the cryoprecipitates of nine SLE sera that contained high titers of ANAs. Stastny and Ziff (194) reported ANA IgG component in two out of three SLE cryoglobulins tested; however, they failed to find a preferential concentration of ANAs in the cryoglobulins. Conversely, Winfield et al. (205) found that SLE cryoglobulins were highly enriched with antibodies to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) and, less frequently, with antiribonucleoprotein antibodies relative to the concentration of these autoantibodies in the corresponding sera. Erhart et al. (200) confirmed these findings and reported that 95 of isolated SLE cryoglobulins studied contained antibodies to dsDNA.

Hanauer and Christian (198) found anti-IgG antibody (i.e., RF) in SLE cryoglobulins, and this finding has been confirmed by several investigators (152 ,212 ,213 ,214). In addition, RF activity was found to be preferentially enriched in the cryoprecipitates when compared with the antibody activity of the matching serum specimen (212 ,213). Similarly, cold-reactive IgM antilymphocyte antibodies were found to be selectively concentrated in SLE cryoglobulins (212 ,213). Lymphocytotoxic activity of SLE cryoglobulins did not correlate with clinical severity of the disease, serum complement level, or serum titer of anti-DNA antibody (214).

Specific Antigens in SLE Cryoglobuinns

To identify specific antigens that may be complexed with corresponding antibody in the cryoprecipitate, experimental animals were immunized with cryoprecipitates isolated from patients with SLE (198). After absorption with pooled normal human serum, anti-SLE cryoglobulin antisera did not recognize any unique antigens, except for two antisera that contained antibody activity against intrinsic determinants in IgM molecules. Using a similar approach, Klippel et al. (215) detected reactivity of anti-SLE cryoglobulin antiserum against lymphocytes, suggesting that lymphocyte membrane antigens or antigens that cross-react with cell surface determinants were present in SLE cryoprecipitates. McPhaul and Montgomery (216) found that anti-SLE cryoglobulin antisera reacted not only against nuclear antigens but also with reticulin and idiotypic determinants of immunoglobulin deposits in the renal glomeruli. These observations indicate the multiplicity of antigenantibody systems that are involved in the formation of cryoglobulins in SLE.

Lee and Rivero (195) reported the presence of DNA in SLE cryoprecipitates in only one of nine specimens using ultraviolet light absorption, diphenylamine reaction, and immunoprecipitation. Davis et al. (217) identified DNA in most SLE cryoglobulins they tested, but only after digestion of the precipitate with pronase, suggesting the DNA was bound to anti-DNA antibodies and thus was inaccessible to biochemical or immunologic detection. The major portion of the DNAanti-DNA antibody system in cryoglobulin from patients with lupus nephritis consists predominantly of low molecular weight complexes (218).

The presence of DNA in cryoprecipitates is not specific for SLE. DNA also has been identified in cryoglobulins isolated from patients with bacterial endocarditis, Sjogren syndrome, and non-SLE glomerulonephritis (219 ,220). Free DNA has been demonstrated in the sera of normal individuals (219), patients receiving high doses of corticosteroids

for a variety of medical conditions, and patients undergoing cardiac surgery (221 ,222). Not only was DNA found in non-SLE cryoglobulins, but Roberts and Lewis (223) identified anti-DNA activity in cryoglobulins isolated from patients with nonlupus glomerulonephritis, bacterial infections, and essential cryoglobulinemia. IgG anti-DNA antibody was demonstrable in cryoglobulins after preincubation of the precipitate in acid buffer or after digestion with deoxyribonuclease, suggesting that anti-DNA antibody in the cryoglobulin was bound to antigen.

It now is recognized that the syndrome of mixed cryoglobulinemia is strongly associated with chronic hepatitis C infection (225 ,226). Hepatitis C virus, polyclonal IgG, and monoclonal RF have been identified in isolated cryoprecipitates, and these represent pathogenic immune complexes that become deposited in the blood vessels of target organs. Studies have shown no association between hepatitis C infection and SLE, and hepatitis C infection is not the primary etiology of cryoglobulinemia in lupus patients (227).

Pathogenetic Significance of Cryoglobulins

In addition to complement activation, mixed cryoglobulins possess other biologic properties that suggest a potential pathogenic role. Intradermal injection of redissolved cryoglobulins in unsensitized animals caused localized skin edema, erythema, and hemorrhage within 24 hours, and intravenous administration caused either anaphylaxis or a glomerulitis (228). Whitsed and Penny (229) described the development of a cutaneous vasculitis following intradermal injection of autologous cryoglobulin into the clinically normal skin of a patient with mixed cryoglobulinemia. Deposition of cryoglobulin in target organs in vivo is supported by the demonstration of a distinctive crystalline fibrillar structure in renal glomeruli that was identical to that found in the serum cryoglobulins of the same patient with essential mixed cryoglobulinemia. Using an anti-idiotypic antibody, Agnello et al. (152) demonstrated the deposition of IgM RF moiety of cryoglobulin in the renal glomeruli of a patient with SLE nephritis. Su et al. (230) described an identical “fingerprint” morphology between glomerular deposits and serum cryoglobulins by electron microscopy in diffuse proliferative lupus nephritis.

Cryoglobulins may contribute to the susceptibility of patients with SLE to bacterial infections. Nivend et al. (231) found that the impairment of opsonization of *Staphylococcus aureus* by SLE serum was associated with cryoglobulinemia. When the cryoglobulin fraction of the immune complexes was removed from the SLE serum, normal opsonic capacity was observed. Moreover, reduction of opsonic property was transferred with SLE cryoglobulins to normal serum. The binding of cryoglobulins to protein A of *Staphylococcus* species probably blocked contact between surface receptors of phagocytic cells and opsonized organisms, resulting in defective phagocytosis and killing (Table 28-5).

Table 28-5: Cryoglobulins in Systemic Lupus Erythematosus (SLE)

1. Serum cryoglobulins in SLE usually are type III (mixed polyclonal) consisting of immunoglobulins, complement components, and fibronectin.
2. Elevated levels of serum cryoglobulins are associated with hypocomplementemia and clinical disease activity, especially active nephritis.
3. Cryoglobulins represent cold-precipitable, circulating immune complexes. ANAs (including anti-DNA), antilymphocyte antibodies, RFs, as well as DNA and lymphocyte antigens have been identified in SLE cryoglobulins.

ANA, antinuclear antibody; RF, rheumatoid factor.

The Lupus Erythematosus Cell

In February 1946, Dr. Malcolm M. Hargraves, a hematologist at the Mayo Clinic, examined bone marrow aspirate from a boy with an obscure medical problem. Part of his report (136) read: “The outstanding thing in this bone marrow is the phagocytic reticuloendothelial cells which contain a blue-staining material which we have not previously observed. Some cells are markedly filled with this material gathered together in round vacuoles or droplets. An occasional cell has been ruptured, with the material in discrete globules scattered out among the other cells. This material stains from a light blue to a very dark, almost indigo blue. There is an occasional reticuloendothelial cell that has other phagocytized material as well as that noted above, but most of the reticuloendothelial cells involved seem to be specifically concerned with this material and do not show other phagocytic activities.”

On learning that the patient probably had SLE, Dr. Hargraves went on to examine bone-marrow specimens in the next 4 days from two other patients with definite SLE. He observed that the striking feature is the marked phagocytic activity of neutrophils containing a muddy purple homogenous material. Some of the cells are so filled with the material that the nucleus is crowded to the periphery.

This is the initial description of the LE cell, and 2 years later, Hargraves et al. (232) reported their experience in 25 patients with SLE and noted the frequent appearance of the LE cell in acute cases. The inclusion body of the LE cells as well as the extracellular material stained with Feulgen stain showed that both contained DNA and were presumed to be nuclear in origin. Hargraves et al. postulated that phagocytosis of the material resulted in the formation of LE cells.

The LE-cell phenomenon occurs in vitro during the incubation of peripheral blood or bone marrow aspirate. It is completed in two distinct stages (Fig. 28-1), with the initial phase involving the immunologic reaction of the LE-cell factor that is present in the serum of patients with the nuclear material of damaged or traumatized leukocytes.

Trauma allows the nuclear penetration of the LE-cell factor, and the reaction leads to nuclear swelling accompanied by the disintegration of the normal chromatin pattern and basophilia. The altered nucleus then detaches itself from the cytoplasm and appears as a free extracellular LE body. In the second stage, the LE body is engulfed by a neutrophil (or occasionally a monocyte) in the presence of complement. The cytoplasm remains outside and is not taken up by the phagocyte (233). When stained with Wright's stain, the globular inclusion body appears as a homogenous, pale blue to deep purplish material, pushing the nucleus of the phagocyte to one side of the cell (Fig. 28-2).

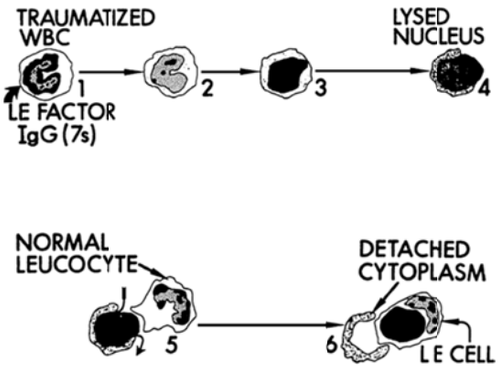

Figure 28-1. Scheme of the lupus erythematosus cell formation.

In 1951, Lee et al. (234) observed a striking morphologic resemblance of the LE-cell inclusion body to the hematoxylin bodies found in the tissues of patients with SLE at autopsy. The latter, described earlier by Klemperer et al. (235), consisted of altered nuclear material containing DNA in a depolymerized state. The LE-cell inclusion body and the tissue-bound hematoxylin body were found to have diminished affinity for methyl green, which is a dye that binds stoichiometrically with DNA. Studies by Godman and Deitch (236) have established that the diminished affinity is not a result of DNA polymerization but rather of interference by proteins that are bound to the DNA moiety of the inclusion body.

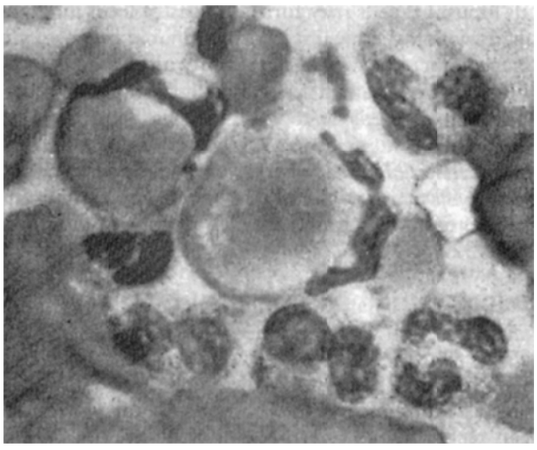

Figure 28-2. Two typical lupus erythematosus cells (× 1,700).

Lupus Erythematosus-Cell Factor

Haserick et al. (237) fractionated SLE plasma by electrophoresis and identified the serum factor that participated in the LE-cell phenomenon as gamma globulin, implying that it is an antibody. Other investigators, using ultracentrifugation and chromatography separation methods, confirmed the LE factor to be a serum 7S gamma globulin (238 ,239), whereas the 19S was inactive. Subsequent studies established that IgG but not IgM antibody to deoxyribonucleoprotein induces LE-cell formation in vitro (240).

Lupus Erythematosus-Cell Antigen

In 1960, Miescher and Fauconnet (241) noted that the capacity of SLE serum to induce LE cells in vitro diminished when the serum was preabsorbed with nuclei isolated from human leukocytes. This observation as well as the information that the LE-cell factor is a gamma globulin led to definitive investigations that established the ANA activity of the LE-cell factor. Application of the fluorescent antibody technique by Friou et al. (242) contributed significantly to our understanding the nature of the LE-cell factor and other ANAs. In a series of elegant studies, Holman et al. (243 ,244 ,245) established that the LE-cell factor is an antibody to deoxyribonucleoprotein of cell nuclei, requiring both DNA and histone for the reaction.

More than 50 years after its description, Schett et al. (246), using highly purified antigens and a series of immunoprecipitation and immunoblotting and blocking experiments, recently has identified histone H1 as the major antigen involved in the LE-cell phenomenon. In addition, they have shown that a positive LE-cell prep correlated not only with antihistone 1 antibody measured by ELISA test, but also with active disease with major organ involvement (246 ,247).

Apoptotic Bodies

An alternative mechanism in the formation of LE cells in vitro proposed by Schmidt-Acevedo et al. (248) involves the phagocytosis of apoptotic bodies induced by antinuclear antibodies that can penetrate cells. They found that the nuclear material engulfed by LE cells contains DNA strand breaks that corresponds to apoptotic bodies. Lee et al.

(249) described in vivo LE cells in the biopsy of the skin lesions of a patient with an overlap syndrome. The material engulfed by the LE cells seen in the papillary dermis appeared to be apoptotic bodies rather than opsonized bare nuclei released from dying cells.

Role of Complement in Lupus Erythematosus-Cell Formation

In 1959, Aisenberg (250) reported that the incubation of SLE serum at 56°C for 30 minutes diminished its capacity to induce LE-cell formation in an indirect LE-cell test. Instead of classical LE cells, the preparation instead showed homogenous, globular, purplish extracellular material. Addition of fresh human serum to isolated cell nuclei complexed with LE-cell factor caused formation of LE cells in the presence of viable phagocytes. This observation suggested that a heat-labile factor in normal serum is required for completion of the LE cell. It remained for Golden and McDuffie (240) and MacDuffie et al. (251) to establish the importance of complement in the LE-cell phenomenon. Frozen and thawed human leukocytes were incubated with gamma-globulin fraction of SLE serum containing antideoxyribonucleoprotein antibody. Viable leukocytes then were added to the system to complete the second, or phagocytic, stage of the LE-cell phenomenon. Typical LE cells formed even after thorough washing of the phagocytes and the leukocyte nuclei with saline. These investigators assumed that small amounts of complement remained adherent to the viable phagocytes. Nevertheless, when antiserum to human C3 was added to the system, there was inhibition of LE-cell formation; addition of excess fresh human serum abrogated the inhibitory effect of anti-C3 antiserum. Thus, complement clearly is required for the second stage of the LE-cell phenomenon, and the amount that is required probably is relatively small.

Extracellular Nuclear Material

Extracellular basophilic aggregates of amorphous or ovoid shape sometimes are seen in LE-cell preparations. This extracellular material (ECM), sometimes referred to as a hematoxylin body, may occur either alone or accompanied by typical LE cells. It has been assumed that ECMs represent products of the initial stage of the LE-cell phenomenon (i.e., complex of the LE-cell factor and nucleoprotein) left unphagocytosed. Arterberry et al. (252) found hematoxylin bodies without typical LE cells in 358 out of 3,000 patients with various diagnoses who underwent an LE-cell test. Three morphologic types were identified: (a) homogenous round hematoxylin body, (b) a lacy type, and (c) an amorphous variety. The homogenous round body was the most common, being found in 259 patients. Forty-five percent of patients in this group had definite SLE, and another 46% had RA or some disease variant.

By block titration of serum, Golden and McDuffie (240) have shown that the number of LE cells produced by lupus serum decreases as the number of ECMs increases, whereas the amount of complement and number of viable phagocytes stay constant in the indirect LE-cell test. IgG and IgM antideoxyribonucleoprotein antibodies were purified, and both antibody preparations produced ECMs. In contrast, only IgG antibody had the property to induce the classic LE cell. McDuffie et al. (251) suggest that the inability to induce typical LE cells may be related to the lack of complement-fixing property of IgM antideoxyribonucleoprotein antibody.

In Vivo Occurrence of Lupus Erythematosus Cells

The LE-cell factor and most other ANAs are not capable of penetrating intact and viable cells to react with their corresponding antigens in the nuclei. Lachmann (253) observed no morphologic changes in actively dividing HeLa cells when grown in tissue culture medium containing lupus serum. Rapp (254) showed that ANAs reacted with the nuclei of air-dried, but not viable, HeLa cells. Direct smears of the peripheral blood of patients with SLE showed absence of in vivo binding of immunoglobulin with the nuclei of leukocytes (255). However, in vivo LE cells occasionally have been described in direct smears of pericardial fluid (256), pleural fluid (257), joint fluid (258), ascitic fluid (259), and cerebrospinal fluid (260) of patients with lupus. LE cells may form in areas of local inflammation, as within the pleural cavity, probably because of the presence of leukocytes that are subtly altered or damaged to allow nuclear penetration by the IgG antideoxyribonucleoprotein antibody to form LE bodies. These damaged leukocytes may appear to be active and viable by morphologic criteria (261). Phagocytosis of the LE bodies by polymorphonuclear leukocytes or by macrophages will complete the reaction to form classic, and sometimes atypical, LE cells (262).

Clinical Significance of the Lupus Erythematosus Cell

The LE-cell test has largely been abandoned as a routine clinical test and supplanted by the fluorescent test for ANAs. The LE-cell test is labor intensive, and it requires a skilled technician to interpret the cytology. Atypical LE cells are seen not infrequently in clot preparations, and some observers may report this as a positive test while others will not unless classic LE cells also are present. Among the many modifications that were developed to increase the yield and/or to improve the cytology, the heparinized rotary glass bead method, the 2-hour clot test, and the combined rotated and washed clot technique were the major procedures used for several years. The American Society of Clinical Pathologists recently has endorsed that the LE-cell

test (CPT No. 85544) be abandoned in favor of more definitive, quantitative immunologic tests for SLE (263).

The frequency of a positive LE-cell test in SLE varies with the method of testing used, the frequency of performing the test, and the duration of time that the patient is studied. At some point during the course of the illness, the rotary glass bead LE-cell test was positive in 75.7 of 520 patients with SLE who were studied by Dubois and Tuffanelli (264). With combined rotary glass-bead and washed-clot methods, LE cells were found in approximately 90 of the patients.

Despite the high frequency in SLE, it now is well recognized that a positive LE-cell test is not entirely specific for the disease. Positive LE-cell tests have been reported in five to ten of adult patients with RA and in a smaller percentage of patients with systemic sclerosis, polymyositis, polyarteritis nodosa, and mixed connective-tissue disease (94 ,265 ,266 ,267 ,268). LE cells have been described in drug reactions secondary to penicillin (269 ,270 ,271 ,272), tetracycline (273), chlorpromazine (274), anticonvulsants (268), hydralazine (268), and procainamide (275) (see Chapter 47 , The Mother in Systemic Lupus Erythematosus). LE cells have been found in a case of intermittent hydrarthrosis with positive ANAs (276), in lupoid hepatitis (263 ,264), in two cases of lymphoma with no autopsy evidence of SLE (277), and in DiGuglielmo disease (acute erythroleukemia) (278).

Positive Lupus Erythematosus-Cell Test with Negative ANAS

There have been occasional reports of cases in which the LE-cell test is positive, despite a negative fluorescent test for ANAs (278). Koller et al. (279) found 20 cases among a large group of patients tested for LE cells. Five met the criteria for SLE, seven had RA, and three had drug reactions. Wallace and Metzger (280) described two patients with biopsy-proven, cutaneous mild SLE with major organ involvement who had positive LE-cell and negative ANA tests using both rat liver and Hep-2 cells.

The explanation for this discrepancy in most of the cases that have been studied is not entirely clear (278). Nevertheless, when the clinical picture of a patient is compatible with or highly suspicious for SLE and the standard fluorescent test for ANA (FANA) is negative (after using multiple substrates), an LE-cell test should be ordered to corroborate the diagnosis (281). In addition, other tests that may be of value in this situation include the lupus band test on nonlesional skin and serologic tests for anti-Ro/SSA antibody and ELISA test for antihistone 1 antibody.

Table 28-6 summarizes information about the LE cells. Because positive LE cell tests are rare in patients without rheumatic disease, its presence in a patient with a low-titer, positive ANA may be confirmatory of lupus. (The reader is referred to pages 211 to 226 of the third edition of this textbook for a detailed description of LE-cell methodologies.)

Table 28-6: The Lupus Erythematosus (LE) Cell

1. The LE cell is induced in vitro by an IgG antibody to deoxyribonucleoprotein (LE-cell factor) and has specificity for histone H1.
2. The LE-cell factor reacts with the nuclear material of traumatized white blood cells to form a hematoxylin body. Phagocytosis of the hematoxylin body by a viable phagocyte in the presence of complement leads to the formation of classic LE cells.
3. In vivo LE cells may be seen in pleural, pericardial, synovial, ascitic, blister fluid, and the cerebrospinal fluid of patients with systemic lupus erythematosus.
4. The LE-cell test is positive in 90% of all patients with SLE at some time during the disease course. A positive LE-cell test may be seen in other conditions, including rheumatoid arthritis, scleroderma, mixed connective-tissue disease, lupoid hepatitis, and drug-induced LE.

Antiendothelial Cell Antibodies

Antiendothelial cell antibodies (AECAs) were first reported in 1971 by Linqvist and Osterland (282) using an indirect immunofluorescent test with mouse kidney sections as substrate. Since then, AECAs have been reported in SLE, systemic vasculitis, other connective tissue diseases and inflammatory conditions (283). Several different techniques are used to measure AECAs, including cellular ELISA, cytofluorometry, microcytotoxicity, Western blot analysis using cell extracts, and immunoprecipitation of radiolabelled endothelial proteins. Human umbilical vein endothelial cells commonly are used as substrate.

AECAs are a heterogenous group of antibodies that bind to a number of vascular endothelium cell antigens including surface-membrane proteins, nuclear and cytoplasmic antigens. However, nuclear and cytoplasmic components may represent contaminants released or exposed when endothelial cells are disrupted or fixed to prepare the antigen preparation in certain test systems for AECAs. Westphal et al. (284) showed that an ELISA test using fixed cultured endothelial cells detected antibodies not only to cell surface antigens but to intracellular components including DNA, histones, and cytoskeletelal proteins. On the other hand, flow cytometry analysis using unfixed endothelial cells detected antibodies to endothelial cell surface antigens.

AECAs bind to endothelial cells from arteries, veins, and human and murine endothelial cell lines, and cross-react with fibroblasts and peripheral blood mononuclear cells (285 ,286). The specific target antigens in endothelial cells recognized by AECAs are not known. Del Papa et al. (287) found AECAs in SLE sera reacted with a heterogenous series of endothelial-cell surface antigens including four proteins with molecular weights of 200, 180, 155, and 25 kd. Other candidate autoantigens that have been identified

include heat shock protein 60, ribosomal P protein P0, endothelial-specific plasminogen activator inhibitor, $ß_2$-glycoprotein I and proteins that have not previously been identified as autoantigens including ribosomal protein L6, elongation factor, profilin II, heparin sulfate, and other novel proteins (288 ,289 ,290). The target antigen(s) recognized by SLE AECAs may differ from one patient to another.

Clinical Association of AECAs in Systemic Lupus Erythematosus

AECAs are prevalent in SLE and have been reported by various investigators in 39% to 93% of patients (291). The wide range of prevalence rate is in part a result of the difference in the test method used and in the selection of patients. D'Cruz et al. (292) showed that AECAs were associated with active lupus nephritis and the highest serum antibody titers were found in patients with both nephritis and vasculitis. There was no correlation between AECAs levels and anti-dsDNA, antineutrophil cytoplasmic antibodies, and other antinuclear antibodies. The association of AECAs and lupus nephritis has been confirmed by other investigators (286). Conti et al. (293) reported a high prevalence and elevated serum titers of AECAs in SLE patients with psychosis and mood disorders.

Arterial and venous thrombosis and anticardiolipin antibodies also have been reported to be associated with AECAs in SLE (283), however, other investigators have failed to observe correlation with anticardiolipin antibodies (292). Yoshio et al. (294) found elevated titers of AECAs in SLE patients with pulmonary hypertension, Raynaud phenomenon, and digital vasculitis.

A prospective study in a small group of SLE patients for 25 months showed that the serum level of AECAs can serve as a marker of disease activity. In some patients, a rise in the serum titer of AECAs was the only serologic marker of disease exacerbation when the serum C3 and anti-dsDNA levels remained unchanged (295).

Pathogenic Signficance of AECAs in SLE

The clinical association between AECAs, active nephritis, and vasculitis led investigators to propose a role of AECAs in the pathogenesis of vascular damage in SLE. Carvalho et al. (296) have shown that purified IgG isolated from SLE sera containing AECAs upregulated the expression of adhesion molecules and leukocyte adhesion to endothelial cells. AECAs induced the release of interleukin-1 and another mediator that stimulates endothelial cells in an autocrine manner. AECAs in patients with Wegener granulomatosis also have been shown to exhibit similar in vitro activity and in addition induced the secretion of IL-1ß, IL-6, IL-8, and MCP-1 (297).

AECAs that recognize heat shock protein 60 can induce apoptosis of endothelial cells providing a target for antiphospholipid antibodies leading to vascular thrombosis (288). A monoclonal IgG AECA derived from SLE was shown to bind and activate endothelial cells in vitro through the NF-κB pathway (298).

Based on these observations, it is proposed that AECAs induce vascular damage in SLE and other systemic vasculitides by activating endothelial cells, inducing the secretion of cytokines and chemokines, and facilitating leukocyte recruitment and adhesion and local production of tissue actor leading to thrombosis.

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Section IV
The Skin in Lupus

Chapter 29
Pathomechanisms of Cutaneous Lupus Erythematosus

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Abnormal cutaneous reactivity to sunlight is such a seminal clinical feature of lupus erythematosus (LE) that it is one of the 11 classification criteria proposed by the American Rheumatism Association in 1982 for a case definition of systemic lupus erythematosus (SLE) (1). Photosensitivity also is a cardinal feature of the cutaneous and neonatal forms of lupus erythematosus. This strong clinical association has led to the postulate that abnormal photoreactivity participates in the pathogenesis of cutaneous lesions in lupus erythematosus. In this review we will discuss the evidence for abnormal photoreactivity in lupus erythematosus and speculate on the cellular, molecular, and genetic factors that may underlie this abnormality. Most of our current understanding of cutaneous photoreactivity is derived from animal studies. As there is yet no convincing animal model of cutaneous lupus erythematosus, many studies remain descriptive in nature. To arrive at an understanding of the potential mechanisms underlying the development of cutaneous lupus, we will discuss the possible interrelated roles of ultraviolet light-mediated induction of apoptosis and inflammation as well as immunomodulation. Additionally, we will consider the role and importance of humoral and cellular factors and also discuss the roles of blood vessels as targets and participants in the disease process. Finally, we will comment on the participation of soluble cytokines and cofactors of inflammation in lesion induction. An incorporation of recent advances in the fields of photobiology, immunology, cell biology, and genetics then will allow the construction of a current model of the pathophysiology of cutaneous lupus.

Clinical Photosensitivity in Lupus

Skin lesions are common in SLE and are found in up to 90% of patients (2). Lupus-specific cutaneous findings such as malar rash (acute cutaneous lupus erythematosus [ACLE]) and discoid lupus (chronic cutaneous lupus erythematosus [CCLE]) were found in 64% and 31% of patients in a large cohort (2), respectively. Skin disease is the first symptom of disease in 23% to 28% of patients with SLE (3). There is a clear relationship between sunlight exposure and the manifestations of cutaneous LE and cutaneous lesions tend to occur in sun-exposed skin. Cazenave, in the original 1851 description of LE stated that outdoor workers were predisposed (4). Isolated case reports suggested that lesions could be induced by light (5). In 1965, Epstein used a repeated light exposure technique to demonstrate that ultraviolet (UV) radiation could induce skin lesions in patients with LE (6). This observation was confirmed quickly by two other groups (7 ,8). Lesion induction was often delayed by up to 2 weeks, and patients with either systemic lupus or cutaneous lupus were shown to develop more prolonged skin redness than normal controls (9 ,10). Recent studies have confirmed that clinical aberrant photosensitivity manifested as prolonged and delayed erythema is present in almost all patients with cutaneous or systemic disease; however, the minimal doses of UV light required to induce erythema in standard testing protocols appears to be within the normal range of the general population (336).

Action Spectrum of Cutaneous Lupus Erythematosus

Ultraviolet light is commonly divided into germicidal UV light (UVC), midrange UV light or sunburn UV light (UVB) and long-wave UV light (UVA) also termed near UV or black light (Fig. 29-1). This separation is important as the differing wavelengths have varying biologic effects (vide infra). Although UVC has been used in many in vitro studies of the cellular response to UV irradiation, this spectrum of UV light is completely blocked by the earth's atmosphere and is of dubious pathophysiologic relevance. Early investigators (6 ,7 ,8 ,11 ,12), defined an action spectrum in the UVB range (290 to 320 nm) for the cutaneous forms of LE. More recent studies have demonstrated that UVA (320 to 400 nm) also can contribute to the induction of skin lesions. Lehmann et al. performed extensive photoprovocation studies (13). They were able to induce lesions in 63% of patients with subacute cutaneous lupus erythematosus (SCLE), in 72% of tumid LE cases, in 60% of SLE cases and 45% of CCLE cases (14). Of those with UV-induced lesions, 53% were induced by a combination of UVB and UVA, 34% by UVA alone and 42% by UVB alone. Abnormally prolonged erythema was also noted in SLE patients after exposure to

UVA (15). Although UVA-induced erythema in normal skin requires 1,000 times more energy than from UVB (12), daily exposure to UVA is much greater than UVB, and at the level of the dermal capillaries, the UVA effect, as a result of greater penetrance, is much stronger that UVB (Fig. 29-2). Thus UV light of varying wavelengths can induce abnormal skin responses in lupus patients and can induce cutaneous lesions.

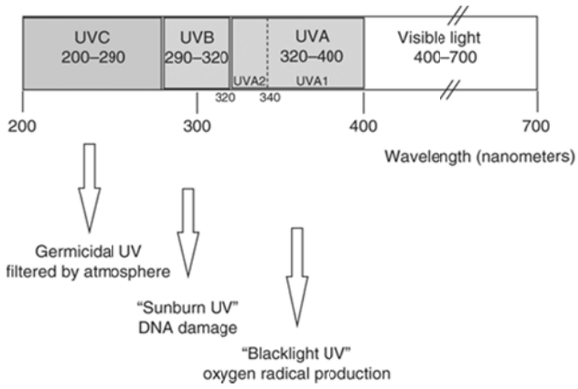

Figure 29-1. The spectrum of ultraviolet light irradiation by wavelength. Ultraviolet light is commonly divided into germicidal UV light (UVC), midrange UV light or sunburn light (UVB) and long-wave UV light (UVA) also termed near UV or black light. Both UVB and UVA can induce skin lesions in photosensitive lupus erythematosus. UVA-1 is light limited to the longer wavelength spectrum of UVA and has been used therapeutically in systemic lupus erythematosus.

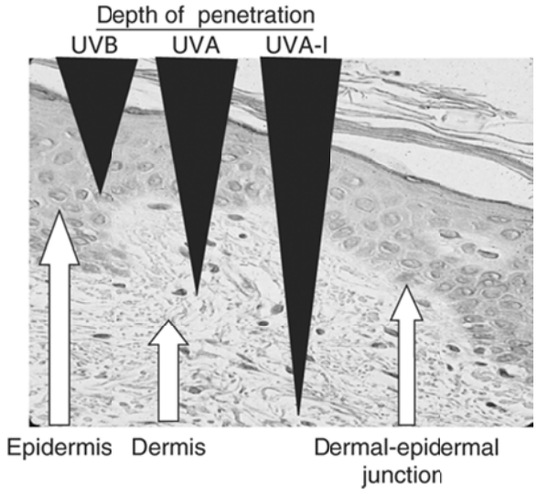

Figure 29-2. Photomicrograph of normal skin depicting the depth of penetration of the various forms of ultraviolet radiation (UVR). The skin is formed by an epidermal compartment that includes the stratum corneum (horny layer), the epidermis proper, and a basement membrane zone. Keratinocytes (skin cells), melanocytes (pigment cells), and Langerhans cells (dendritic cells) are found in this compartment. The dermal compartment includes the vasculature of the skin and connective tissue. Penetration of UVR is directly proportional to the wavelength of the radiation. UVB is absorbed primarily in the epidermis. UVA penetrates the dermis and can affect the skin vasculature. UVA-1 has the potential to penetrate the skin more deeply that UVA of shorter wavelength.

Role of Ultraviolet in Exacerbation of SLE

It often is stated that sunlight cannot only aggravate cutaneous LE but can potentiate systemic features of disease. Up to 73% of patients with SLE report photosensitivity (16). However, phototesting with standardized protocols correlates poorly with patient-reported photosensitivity (17). This may be because of the delayed nature of the phenomena observed on phototesting (14). Although patients report that several disease symptoms (including weakness, fatigue, and joint pain) are increased by sun exposure (16), variation in disease activity related to sun exposure using objective variables has not been shown in large cohort studies. The available evidence is largely anecdotal. Repeated single-patient observations indicate that sunlight may precipitate disease de novo or aggravate existing disease. Phototherapy for incorrectly presumed psoriasis (using UVB) has led to aggravation of the lupus lesions (18). Tanning bed use (a source of predominant UVA) also has been reported to either induce (19) or exacerbate SLE (20). Two recent studies show that although cutaneous manifestations are more common in the summer months, systemic disease activity is increased in the 3 to 6 months following maximal potential sun exposure. This has led these authors to suggest that summer UV light exposure may lead to systemic flares several months later (21 ,22). Interestingly, a pale, sun-reactive skin type was independently associated with increased risk (OR = 2.3) of developing SLE in a Swedish case-control study (23).

Polymorphous Light Eruption as a Predisposing Feature of LE Photosensitivity

Is there direct clinical evidence of a predisposing sensitivity to UV irradiation in patients with LE? Polymorphous light eruption (PLE) is a common photodermatosis affecting up to 10% of the population (24). There is a lag time of 2 hours to 5 days between sun exposure and the development of a pruritic, erythematous eruption on the skin. Selected patients with PLE have been noted to have severe photosensitivity as defined by the persistence of photo-induced lesions. These patients were noted to have positive antinuclear antibodies in significant titers and have been proposed to be a “forme fruste” of photosensitive LE (25). Nyberg recently assessed 337 consecutive lupus patients (with either cutaneous or systemic LE) recruited from dermatology departments in Finland and Sweden and found that almost 50% gave a history of photosensitivity consistent with PLE (26) and this predated the clinical onset of LE by many years. In a separate study, they went on to photoprovoke lesions in their photosensitive LE patients and produced lesions of clinical PLE with histology consistent with PLE in

about half of these patients (27). Immunohistochemical analysis shows upregulated intercellular adhesion molecule-1 (ICAM-1) staining on basilar keratinocytes in both PLE (28) and cutaneous LE (29). This molecule can enhance inflammatory cell infiltration. It is thus possible that there is a mechanistic link between abnormal photosensitivity (as manifest by a PLE-like eruption) and susceptibility to LE. A similar association was subsequently noted in British families of patients with photosensitive cutaneous lupus where first-degree relatives had a significantly higher risk of PLE (30). It should, however, be pointed out that studies of long-term cohorts of patients with PLE have not documented an increased risk of LE in these populations (31 ,32). Thus a less common disease (LE) may be preceded by an increased incidence of a more common photosensitive condition (PLE) but the presence of PLE-like photosensitivity alone does not predict lupus.

A Selective Sensitivity to UV Light in LE?

Clinical observations suggestive of a role for UV light in the pathogenesis of systemic lupus erythematosus and lupus skin disease have been supported by mechanistic studies. Autoantibodies to DNA and DNA-associated proteins characterize systemic LE. In early studies, UV-irradiated DNA but not native DNA was shown to induce a humoral immune response in animals. Repeated injections of UV-irradiated DNA (UV-DNA) into rabbits resulted in renal disease characterized by proteinuria and renal immunoglobulin deposition (33). Similar results were obtained in mice and some of the immunopathologic and histopathologic changes associated with cutaneous LE were then reproduced by exposing the skin of UV-DNA immunized mice to UV radiation (34). One of the changes induced in DNA by UV is the formation of thymidine dimers. Thymidine dimers were found in the skin of UV-irradiated mice and UV-DNA antibodies reacted to these thymidine dimers (35 ,36). Such immune responses to UV-altered DNA have also been found in LE patients (37). From this concept of an immune response to UV-DNA evolved the idea that patients with LE may have an impaired ability to repair UV-damaged DNA with subsequent persistence of potentially immunogenic UV-damaged DNA. Studies using fibroblasts from patients with LE showed that these cells were more susceptible to the cytotoxic activity of UVB (38) as well as UVA (39) than fibroblasts from controls. This susceptibility was manifested as an increase in cellular lethality following UV exposure. This enhanced cellular toxicity to UV was not from defects in DNA repair and has not yet been adequately explained (40).

Table 29-1: Biologic Effects of Ultraviolet Radiation

Characteristic	UVB	UVA
Absorption by molecules	DNA, amino acids, melanin, urocanic acid	Melanin
Direct DNA damage	Increased	Minimal
Free-radical production	Minimal	Increased
Depth of penetration	Epidermal	Dermal
Epidermal effects	Stratum corneum thickening, intermediate and delayed apoptosis, keratinocyte cytokine transcription and release	Immediate apoptosis
Langerhans cell effects	Inactivation, emigration	Minimal

UVA, ultraviolet A; UVB, ultraviolet B.

UV-altered DNA can induce lupus-like disease in animals and LE patients may exhibit increased levels of UV-altered DNA. Does UV light also accelerate disease in animal models? Repeated exposure to UV light can accelerate the spontaneous systemic lupus of certain murine strains. Exposure of BXSB autoimmune lupus mice to UVB has been shown to induce the release of autoantigens, to promote antibody production and to promote early death (41). This could not be reproduced in other lupus strains such as the NZB/NZW model (42), the MRL/lpr mouse (41), or a C1q-deficient mouse (43), suggesting that UV light may have a variable role in the genesis and acceleration of lupus depending on the genetic background (44). Nevertheless, these observations, both clinical and mechanistic, have been used to justify continued photo-protection for patients with systemic lupus (45 ,46).

Biologic Responses to Ultraviolet Light

Ultraviolet light has multiple effects on living tissue. Potential molecular targets of ultraviolet light include not only DNA, but RNA, proteins, and lipids. Table 29-1 summarizes the biologic effects of UV light on the skin. In addition to alteration of DNA, cytoskeletal reorganization was noted in keratinocytes (skin cells) after UV irradiation (47). An early study by LeFeber revealed that UV light can induce the binding of antibodies to selected nuclear antigens on cultured human keratinocytes (48). The specificity

of these antibodies was not defined, but it is now known that they are commonly directed against Ro/SSA, La/SSB, ribonucleoprotein, and Smith (Sm) antigens, and are the antibodies associated with LE and photosensitivity. Norris later noted increased antibody binding to keratinocytes following in vivo UV irradiation of human skin (49). This was confirmed independently by Golan, who observed binding of anti-Ro/SSA–positive sera to cultured keratinocytes (50). These results could be explained by UV-induced translocation of antigens to the cell surface with or without the death of the cell, or by other alterations in the antigens allowing the binding of autoantibodies taken up by the living cell (51). In 1994, Casciola-Rosen et al. demonstrated that when keratinocytes grown in cell cultures are irradiated with UVB, they actively cleave their DNA and die by a process termed apoptosis (52). During this process, the antigens recognized by autoantibodies such as Ro/SSA, and calreticulin are concentrated in structures termed blebs or apoptotic bodies found at the cell surface. Larger blebs arise from the nucleus and harbor Ro/SSA, La/SSB, and other nuclear material. These investigators (53) and others (54) have proposed that these bleb-associated antigens may then be phagocytosed, packaged, and presented to lymphocytes, thereby stimulating autoimmune responses.

Ultraviolet Light, Apoptosis, and the Skin

Apoptosis and necrosis are the two major mechanisms of cell death. Apoptosis is an ordered means of noninflammatory cell removal in which a central biochemical program initiates the dismantling of cells by nuclear fragmentation, formation of an apoptotic envelope, and shrinking of the cell into fragments leading to phagocytosis by parenchymal cells as well as phagocytes (55 ,56 ,57). In necrosis, cells are passive targets of extensive membrane damage leading to cell lysis and release of contents. Apoptosis can be further categorized into “immediate” apoptosis or preprogrammed cell death, and “intermediate” and “delayed” apoptosis or programmed cell death (reviewed in (58)). Immediate apoptosis is protein synthesis–independent, occurs rapidly after triggering (59), and is the result of singlet-oxygen damage to mitochondrial membranes. Intermediate apoptosis is commonly the result of activation of a membrane receptor with a death domain such as Fas (reviewed in (60)). Delayed apoptosis requires several hours for execution and is protein synthesis–dependent. This can be the result of DNA damage (61) or from lack of essential survival signals (62).

Keratinocyte Apoptosis and Ultraviolet Light

Keratinocytes die by programmed cell death as part of their normal program of differentiation (63 ,64 ,65). This occurs normally in the granular cell layer of the epidermis, at the interface with the stratum corneum. The molecular machinery controlling this programmed cell death in keratinocytes is complex and still poorly understood (66). Basilar keratinocytes have been found to be relatively resistant to apoptosis induced by a variety of stimuli (67). This may be as a result of the expression of proteins that specifically inhibit apoptosis such as bcl-2, survivin (68), and other inhibitors of apoptosis (IAPs) (reviewed in (69)). Ultraviolet light has long been known to induce apoptotic death in suprabasilar keratinocytes; such cells were called “sunburn cells” by morphologists (70). UV light now is known to induce apoptosis by multiple mechanisms. Long-wave ultraviolet light (UVA1; 380 to 400 nm) can induce “immediate” apoptotic death through singlet-oxygen damage to mitochondrial membranes (71). UVB can induce direct, ligand-independent activation of membrane death receptors such as Fas (72) as well as FasL (Fas ligand) upregulation and subsequent Fas-FasL binding (73). UVB also sensitizes keratinocytes to TNF-related apoptosis inducing ligand (CD253) (TRAIL)-induced apoptosis by downregulating the level of TRAIL decoy receptors (74). Tumor necrosis factor-α (TNF-α) release and consequent ligation of the TNF receptor p55 (TNFR1) also has been shown to be an important mediator of UVB-induced keratinocyte apoptosis (75 ,76). Finally, UVB can induce keratinocyte apoptosis secondary to DNA damage (77). Once the signal for apoptosis is triggered, specific enzymes within the cell begin the dismantling process. These enzymes now are collectively called caspases, an acronym for cysteine asparate proteinases (78). These enzymes are known to be important in UV-induced cell death because specific inhibitors of these enzymes prevent the UV-induced death of keratinocytes (79).

Apoptosis in Cutaneous Lupus Erythematosus

The potential importance of apoptosis in the pathogenesis of cutaneous lupus is underscored by a number of recent observations. Using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining to detect nuclei with DNA damage, Norris demonstrated the presence of an increased number of apoptotic keratinocytes in the basal zone of CCLE lesions and in the suprabasal zone of SCLE lesions (67). An increase in the number of apoptotic cells in lesional skin from patients with cutaneous LE since then has been confirmed and has been associated with increased p53 protein expression as determined by immunohistochemistry (80 ,81). The nuclear phosphoprotein p53 is a tumor suppressor that is upregulated in response to UV-induced DNA damage (82) and in response to the cytokines TNF-α (68) and interferon-γ (IFN-γ) (83). Upregulation of p53 in suprabasilar keratinocytes can initiate cell death by apoptosis (84). Therefore, the increased number of apoptotic cells could be a result of an increased rate of apoptosis induction mediated directly by UV light or as a consequence of UV-induced cytokine release. Apoptosis also can be induced by cellular cytotoxic mechanisms. Cytotoxic T lymphocytes (CTL) and natural killer (NK) cells can induce apoptosis through multiple mechanisms (reviewed in (85)),

including the release of perforin and granzymes (83); cytokine release (IFN-γ, TNF-α, TNF-α, interleukin (IL)-1) (86); and triggering of Fas by FasL (87). The presence of leukocytes in proximity to the apoptotic cells (67) and the presence of FasL-positive macrophages in proximity to apoptotic cells in lesional hair follicles (88) suggest a role for such cellular apoptotic mechanisms in established lesions.

An increased number of apoptotic cells are noted in lesional LE skin. Can this have systemic as well as local consequences? There is evidence that the biochemical processes of apoptosis generate novel antigens that are uniquely targeted by autoantibodies. Casciola-Rosen et al. have shown that the caspases activated during apoptosis cleave intracellular proteins into fragments that are bound by autoantibodies from some patients with LE (89). Further, proteins specifically phosphorylated by stress-induced apoptosis are targeted by antibodies from LE patient sera (90 ,91). From these observations, it has been inferred that the process of apoptosis is important in the initiation of autoimmune responses. Recently, it has been shown that patients with LE skin disease have autoantibodies that preferentially recognize apoptotic-modified U1–70-kd RNP antigen when compared to patients without skin disease (92). This provides further in vivo evidence that immune recognition of modified forms of self-antigen occurs in cutaneous LE and suggests that this immune recognition and the processing of apoptotic-derived antigens may participate in the pathogenesis of the disease.

Granzyme B, a serine protease found principally in the cytotoxic granules of CTL and NK cells, also can induce cell death by apoptosis in susceptible target cells. Granzyme B can cleave cellular proteins into unique fragments not detected in other forms of apoptosis. Such cleavage products, specific for cytotoxic-granule–induced death, also are bound by antibodies present in LE sera (93). Interestingly, expression of granzyme B has been detected in keratinocytes, suggesting that these molecules may participate in cutaneous defense mechanisms (94) and perhaps in keratinocyte death. In this case, specific correlation of autoantibodies to granzyme B–generated epitopes with cutaneous LE has not yet been made. Novel autoantigens can be generated by apoptosis that is either stress-induced (UV light, viral infection, or other trigger) or secondary to cellular immune mechanisms. The generation and concentration of such neo-antigens could pose a challenge to self-tolerance (95). Whether the increased keratinocyte apoptosis noted in cutaneous LE leads directly to the formation of autoantibodies specific to apoptosis-derived byproducts is still speculation.

Abnormalities of Ultraviolet-Induced Keratinocyte Apoptosis as a Predisposing Factor in Cutaneous Lupus Erythematosus

Although detection of an increased number of apoptotic cells in LE epidermis may underlie an increase in apoptosis, either an increase in the rate of apoptotic death or a decrease in the rate of clearance of apoptotic debris could lead to the observed increase in apoptotic cell numbers. Phagocytosis by macrophages or parenchymal cells is the final event in the clearing of cells undergoing apoptosis (56 ,57). A number of observations suggest that clearance of apoptotic debris may be impaired in LE. Systemic autoimmunity has been noted in mice deficient for molecules potentially involved in the clearance of apoptotic cells including serum amyloid P (SAP), c-Mer, C4, IgM or C1q (reviewed in (96)). SAP is a member of a family of proteins termed pentraxins that bind to apoptotic cells and then interact directly with phagocyte receptors or with C1q. C1q and a related protein, mannose binding lectin (MBL) are collectins or proteins with globular lectin–like heads and collagen-like tails that also flag late-apoptotic cells for disposal by phagocytosis. Interestingly, the surface blebs of apoptotic keratinocytes bind C1q, an early component of the complement cascade (97). The major C1q-binding protein that has been identified in apoptotic blebs to date is calreticulin (52), and autoantibodies to calreticulin can interfere with this binding (98). The binding of C1q to apoptotic cells has been postulated to facilitate the clearance of these cells by macrophages that express a C1q cell surface receptor (99). A potential role for C1q in the clearance of apoptotic debris and in the genesis of cutaneous LE is suggested by two observations. First, patients with C1q deficiency develop LE-like photosensitive eruptions (100). Second, mice with C1q deficiency develop an SLE-like disease associated with an accumulation of apoptotic cells in the kidney (101). Moreover, a recent study in humans has demonstrated that the clearance of apoptotic lymphocytes by macrophages is indeed impaired in some patients with SLE (102) and impaired uptake of apoptotic cells by macrophages has been noted in germinal centers of patients (103). While a number of cellular signals and receptors for the phagocytosis of apoptotic debris has been identified, the magnitude of a clearance defect in patients with cutaneous lupus remains unclear. Further it is unknown if impaired clearance is secondary to a defect in the recognition and the binding of apoptotic particles or in macrophage phagocytosis. It is also unknown if this defect extends to the phagocytosis of other cell types such as keratinocytes. Sunburn cells normally are cleared rapidly and disappear within 24 to 48 hours in murine skin (104 ,105). Whether this is the result of shedding or of phagocytosis by either neighboring keratinocytes or macrophages has not yet been clarified.

Macrophages are the organism's primary remover of cellular debris. Macrophages that have ingested apoptotic cells in vitro secrete factors such as transforming growth factor-β and prostaglandin E-2 (PGE2). These factors can inhibit the release of pro-inflammatory cytokines such as TNF-α by neighboring cells. In addition to removal of cellular debris, macrophages may therefore actively promote tolerance and inhibit pro-inflammatory cytokine production (106). Acquisition of apoptotic cells material by immature dendritic cells (DC), similar to macrophages, is

enhanced by C1q and MBL, and promotes the production of immunomodulatory cytokines such as IL-10 (107). Mature DC, in contrast, are professional antigen-presenting cells present in the skin that have been shown both to acquire antigen from apoptotic cells and then to prime naive T cells in an antigen-specific fashion (108). Thus, depending on the nature of the phagocytic cell (macrophage and immature DC versus mature DC) with which the apoptotic cell interacts, autoimmunity or tolerance may ensue. High numbers of apoptotic cells have been shown to act as a trigger for local DC maturation and to promote antigen presentation to class I and class II MHC-restricted T cells in a murine system (109). Necrosis is a cell-death process characterized by the rapid depletion of ATP stores and subsequent loss of cell membrane integrity that can also result from UV light injury. For example, high doses on UVB preferentially induce keratinocyte necrosis (110). Necrotic cells release potent pro-inflammatory mediators such as high mobility group 1 protein (111) and uric acid (112). The incidence of necrotic cells following UV insult has not been accurately determined. An abundance of apoptotic cells and possibly necrotic cells, either from excessive amount of death induction by UV or other mechanisms or from a defect in clearance could permit tolerance to self antigens to be broken. Figure 29-3 summarizes the potential role of apoptotic mechanisms in the initiation and perpetuation of photosensitive LE.

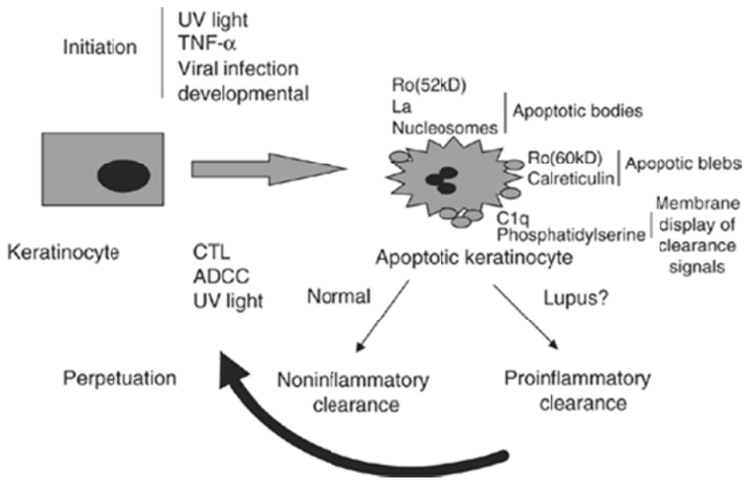

Figure 29-3. Potential role of keratinocyte apoptosis in the pathogenesis of photosensitive lupus erythematosus. Apoptosis is an ordered means of cell death. Apoptosis can be initiated in keratinocytes by ultraviolet (UV) radiation (UVB as well as UVA), by viruses, by cytokines (TNF-α), by growth-factor withdrawal, by differentiation, and by cytotoxic cellular assault. Apoptosis leads to small bleb formation in which Ro antigen and calreticulin are concentrated. Larger apoptotic bodies contain other potential autoantigens including Ro antigen (60 kd), La, nucleosomes, and 70-kd RNP antigen. Apoptosis leads to the exposure of phosphtidylserine on the cell surface and to the binding of C1q. The presence of apoptotic cell in a proinflammatory environment may lead to uptake and processing by antigen-presenting cells leading the priming and boosting of T cells and B cells to self-antigen.

Ultraviolet Light as Inflammatory Stimulus

Erythema (redness) is a normal response to UV light and is mediated by multiple eicosanoids, vasoactive mediators, neuropeptides, and cytokines released from keratinocytes, mast cells, endothelial cells, and fibroblasts (113 ,114). Table 29-2 lists the wide range of mediators released by UV light in the skin. As discussed, UV light can induce prolonged erythema and cutaneous lesions in patients with LE. UV light is not only an executioner, killing keratinocytes by apoptosis, it also is a generator of neo-antigens (such as UV-DNA).

Cytokine Release

UV light can induce cutaneous inflammation by promoting the release of inflammatory mediators and cytokines by inducing adhesion molecule display and by releasing chemokines to attract inflammatory cells into the skin (reviewed in (115)). Both UVB and UVA can participate in lesion induction and act by differing mechanisms. UVB induces the release of the primary cytokines IL-1α and TNF-α from the epidermis, initiating a cascade of inflammatory events. UVB induces IL-1α gene transcription in keratinocytes (116) and elevates circulating levels of IL-1α

bioactivity (117). Likewise, UVB induces the release of TNF-α from keratinocytes (118). This may be partly dependent on the photo-isomerisation of trans- to cis-urocanic acid in the differentiated epidermis (119). TNF-α release has been noted to be greatest in terminally differentiated keratinocytes in culture (120) and is thus proposed to be maximal in the superior layers of the epidermis. IL-1α and TNF-α are “primary cytokines” that induce the release of a number of other pro-inflammatory cytokines from the epidermis (reviewed in (121)). For example, IL-1α and TNF-α induce the secondary release of IL-6, PGE2, IL-8, and granulocyte-monocyte colony-stimulating factor (GM-CSF) by keratinocytes (122 ,123). These molecules are costimulatory factors for lymphocyte activation by antigens or superantigens, stimulate Langerhans cell function (the Langerhans cell is the resident DC of the skin), stimulate collagenase production, and act as pyrogens and stimulators of acute-phase reactants. Additionally, IL-8 and GM-CSF are chemotactic and induce inflammatory cell migration into the skin (124). Both IL-1α and TNF-α also induce adhesion molecule expression such as ICAM-1. Importantly, TNF-α can induce activation of Langerhans cells, the professional antigen-presenting cells of the epidermis, via binding of the TNF p75 receptor (TNFR2) on these cells (125). This results in migration of these cells to the regional lymph nodes where they can participate in immune responses (126). In addition to IL-1α and TNF-α release, UVB can stimulate the release of IL-10 (127 ,128 ,129 ,130), and IL-6 directly (131). IL-10 has been shown to mediate local (132) as well as systemic UV-induced immunosuppression (133). Chemokines are chemo-attractive proteins that are associated with inflammatory cell recruitment. UVB irradiation of primary human keratinocytes, in the presence of pro-inflammatory cytokines such as IL-1 and TNF-α or IFN-γ significantly enhances the expression of the inflammatory chemokines CCL5, CCL20, CCL22, and CXCL8 (134). This is of relevance in cutaneous lupus as CCL5 and CXCL8 are highly upregulated in lesional skin (134). Following photo-testing, elevated levels of CCL27, a novel skin-specific chemokine known to recruit memory T cells into the skin was also found in the dermis of LE patients (134).

Table 29-2: Mediator Release by Ultraviolet Radiation

Source of Mediator	UVB	UVA
Keratinocyte	IL-1α, TNF-α	
	GM-CSF, IL-6, IL-8	IL-8
	IL-10	IL-10, IL-12
	TGF-ß	PGE2, PGF2α
	PGE2, PGF2α	
Mast cell	TNF-α LTC4, LTD4, PGD Histamine	
Endothelial cell	TNF-α, PCI2	PCI2
Langerhans cell		IL-12

Ultraviolet radiation results in the release of interleukins (IL), prostaglandins (PG), prostacyclin (PC), leukotrienes (LT), and other mediators.
UVA, ultraviolet A; UVB, ultraviolet B.

Transgenic overexpression of IL-6 in murine keratinocytes has been associated with an increase in the thickness of the stratum corneum but not with significant cutaneous inflammation when expressed alone (135). Both IL-10 and IL-6 have further been shown to induce local heat-shock protein synthesis (136 ,137). UVB induced primary cytokine production and release is likely the result of UVB-induced DNA damage: UV-damaged DNA, specifically UV thymidine dimer formation, induces DNA repair enzymes that also regulate cytokine transcriptional activity (138).

In contrast to UVB, UVA upregulates ICAM-1 in keratinocytes directly by producing oxygen-free radicals that affect gene transcription (139). UVA also upregulates IL-8 and IL-10 production in keratinocytes and FasL expression in dermal mononuclear cells (140 ,141). The longer wavelength of UVA allows it to penetrate into the dermis and to upregulate vascular endothelial ICAM-1 and E selectin thereby increasing leukocyte-vascular adhesion (142). Acute low-dose UVA administration, but not UVB, also results in IL-12 production by keratinocytes in vivo (143 ,144). UVA results in a rapid increase in interferon-γ (IFN-γ) levels in the skin, the source of which may be resident epidermal T cells (144). This IFN-γ has been postulated to potentiate IL-12 release (144). IL-12 is a potent immunostimulant that can abrogate tolerance induced by low-dose UVB (145) by enhancing DNA repair (146). IL-12 also decreases UV-induced serum TNF-α in mice and UV-induced keratinocyte TNF-α (147 ,148), possibly through the effects on DNA repair. Interestingly, UVA inhibits chemokine CCL17 production by keratinocytes (149). The chemokine CCL17 or thymus and activation-regulated chemokine (TARC) is one of the major chemokines that attract T cells into inflamed skin.

Ultraviolet Light and Th-1/Th-2 Cytokine Balance

Overall, exposure to UVB radiation correlates with a predominance in cytokines that promote T helper 2 (Th2) immune responses at the expense of T helper 1 (Th1) immune responses and that may result in photo-immunosuppression (150). While potentially suppressing cellular immune responses, Th2 responses generally promote antibody production (151). Exposure to physiologic levels of UVA radiation alone, or together with UVB (like natural sunlight), results in the local predominance of cytokines that promote Th1 immune responses. These Th1 responses are “immunopotentiating” and result in strong cellular immune responses including $CD8^+$ cytotoxic T cell responses (151). Whether patients with cutaneous LE have a

unique primary or secondary sensitivity to UV light-induced cytokine changes is not yet clear. Significant interindividual variability in UV light-induced ICAM-1 expression and TNF-α release has been noted in keratinocyte cell lines (120) and this suggests that there may be genetic variability in the human skin response to UV. Indeed, the presence of the TNF-α promoter -308A allele, increased in patients with SCLE, has been associated with enhanced TNF-α production following UV exposure of keratinocytes (152). An increased frequency of this TNF-α promoter allele has also recently been noted in neonates with cutaneous manifestations of neonatal LE. Lesional skin, but not skin from normal neonates, demonstrated TNF-α on immunostaining (153).

Possible Benefit of Ultraviolet in Lupus Erythematosus Patients

Although the above discussion has focused on the potential inflammatory effects of UV light, recent work has suggested that selective UV radiation may have salutatory effects in LE. UVA irradiation of NZB/NZW mice has resulted in increased survival and decreased levels of circulating anti-DNA antibodies (154). Subsequently, a randomized, double-blind, cross-over study of low-dose UVA1 light (light limited to the longer wavelength spectrum of UVA, 340 to 400 nm) compared to visible light showed significant clinical and serologic improvements in SLE patients treated with UVA1 light (155). Recently, treatment of patients with moderately active SLE was shown to significantly decrease disease activity scores (156) and to improve skin disease and this correlated with a decrease in the proportion of circulating IFN-γ–producing T cells, particularly of the $CD8^+$ T cell subset (157).

Humoral Factors in Cutaneous Lupus Erythematosus

Autoantibody production is a sine qua non of SLE and the autoantibodies can be pathogenic. Extracutaneous lupus-like disease can be induced in animals by the introduction of anti-DNA antibodies either transgenically (158) or by intravenous injection (159). Autoantibodies can initiate cellular cytotoxicity and activate the complement cascade and also can promote the recognition of epitopes related to the original autoantigen through a process termed epitope spreading.

Immunopathology of Cutaneous LE

Immunofluorescence studies of cutaneous LE lesions show lesional deposition of immunoglobulins (Fig. 29-4). In 80% to 90% of CCLE or ACLE, and in 50% to 60% of SCLE, a thick band of immunoglobulins and complement components is deposited along the dermo-epidermal junction (160). These complexes have been localized on the upper dermal collagen fibers and along the lamina densa of the epidermal basement membrane zone (161). As these deposits also are found in clinically normal skin of patients with SLE, their role in the local induction of cutaneous tissue injury is still unclear (162). Further, the specificities of these skin basement membrane-deposited antibodies has not been defined although colocalization of the lupus band deposits with collagen VII has been determined using confocal laser scanning microscopy (163).

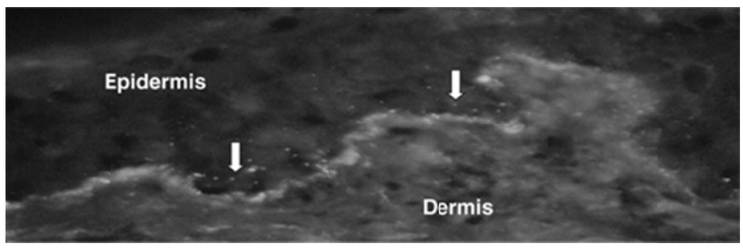

Figure 29-4. (See color plate.) Immunopathology of subacute cutaneous lupus. Direct immunofluorescence analysis for the presence of IgG reveals a “dustlike” distribution of IgG deposits in the suprabasilar keratinocytes (arrowheads mark specific IgG “dust” deposits). There is also IgG deposition in the basement membrane zone.

Ro/SS-A and La/SS-B Autoantibodies and LE Photosensitivity

Several specific autoantibodies have a special relationship to cutaneous LE. SCLE was recognized as a distinct and uniquely photosensitive subset of cutaneous LE by Sontheimer et al. (164). This form of cutaneous disease is strongly associated with a particular autoantibody specificity, anti-Ro/SSA (165); Ro/SSA antibodies have been observed in frequencies ranging from 40% to 100% of SCLE patients by immunodiffusion techniques (166). Neonatal LE also is strongly associated with an anti-Ro response (167). In this disorder, infants have maternally acquired IgG Ro/SSA antibodies and develop SCLE-like skin lesions. As the maternally derived Ro/SSA antibodies are cleared from the infant's circulation several months after birth, the skin lesions resolve (168). The anti-Ro antibody specificity has been implicated in animals as a direct cause of heart block, another manifestation of neonatal LE (169 ,170 ,171 ,172). It has been proposed that Ro/SSA and La/SSB antibodies bind human cardiocytes only after appropriate developmental apoptosis and that in vivo opsonized apoptotic cardiocytes then promote an inflammatory response damaging surrounding tissue (173). Anti-Ro/SSA antibody infusion can lead to the deposition of anti-Ro/SSA antibodies onto human skin grafts in severe combined immunodeficient (SCID) mice (174). The deposition pattern is identical to “dustlike particles” of immunoglobulin deposition over the cytoplasm and nuclei of cells in the lower epidermis and upper dermis seen in adults with SCLE and babies

with NLE and first described by Nieboer (175) (see also Fig. 29-3). The cellular pathology of SCLE however, is not reproduced in the SCID mouse model arguing that autoantibodies may be participatory, but other factors must be present as well. In favor of this, no difference has been noted in the frequency of Ro/SSA autoantibodies among lupus patients with positive or negative phototest reactions (9 ,15). Additionally, titers of antibodies to Ro/SSA do not always correlate with skin activity (176). The presence of levels of Ro/SSA antibodies similar to SCLE in disorders such as Sjogren syndrome (not typically associated with cutaneous lesions) also argues that factors other than the serologic presence and local Ro/SSA antibody deposition must participate in the genesis of LE skin disease.

The Ro/SS-A Autoantigen System

The originally described Ro antigen is a protein of 60 kd that may be bound in vivo to four small RNA molecules called “Y RNA” or “hY RNA” (177). Subsequent studies have indicated that the hY RNA molecules also can be targets of autoantibody production in SLE patients (178). The term “Ro” derives from the first two letters of the last name of the index patient from whom the antibody was characterized. It is identical to an antigen characterized from patients with Sjögren syndrome and given the name “SSA” (179). It is now known that Ro/SSA autoantibodies can variably bind to at least four antigenically distinct polypeptide components of the Ro/SSA ribonucleoprotein (180) in addition to the hY RNA molecules themselves. The function of the 60-kd antigen still is unknown but it has been proposed that it may be involved in ribosome synthesis, assembly, or transport (181) or that it may be part of a salvage pathway for mutant rRNA precursors (182). In this way, it may be a quality-control mechanism for ribosome biogenesis. A recent study has linked a bacterial homologue of Ro/SSA to a UV-resistant phenotype in bacteria (183). Based on these observations, Ro/SSA may bind to UV-damaged RNA and protect the cell from UV damage. Mice lacking Ro protein develop an autoimmune syndrome characterized by antiribosome antibodies, antichromosome antibodies, and glomerulonephritis (184). Further, mice lacking Ro demonstrate increased sensitivity to irradiation with UV light re-enforcing the role of this protein as a UV survival factor. It was proposed that Ro functions to sequester defective ribonucleoproteins and thereby protects against autoantibody development (185).

Reactivity against a 52-kd polypeptide is another antibody specificity commonly found in anti-Ro/SSA positive sera (186). The expression of 52 kd Ro/SSA is upregulated in keratinocytes by TNF-α (187). The function of the 52 kd Ro/SSA antigen is likewise still unknown but the protein recently has been shown to interact with the de-ubiquinating enzyme UNP, suggesting an involvement in the ubiquitin pathway (188). A physical association between the 52-kd and 60-kd proteins has been demonstrated by immunoprecipitation assays (189). A protein-protein interaction between these two polypeptides recently has been confirmed (190). Calreticulin, a 46-kd calcium-binding protein also has been reported to bind both hY RNA and 52-kd Ro (191) and may play a role in facilitating the binding of 60 kd Ro/SSA to hY RNA. Calreticulin binds calcium in the endoplasmic reticulum and has been found to have multiple functions including the inhibition of C1q-mediated immune functions (192 ,193). Calreticulin also has been shown to have in vivo peptide-binding activity and to facilitate the priming of CTL against such bound peptides (194).

The La/SS-B Autoantigen System

The La/SSB antigen is a 48-kd protein that participates in the control of RNA polymerase III transcription termination (195). Recently, La/SSB has been shown to control the synthesis of the x-linked inhibitor of apoptosis protein (XIAP), a key inhibitor of apoptosis upregulated in cells under physical stress (196). La/SSB also is associated with 60 kd Ro, likely through mutual binding to hY RNA (197). The functions and cellular redistribution of calreticulin (198), the 52-kd and 60-kd Ro/SSA polypeptides (199 ,200 ,201), and the La antigen (200) all have been associated with the heat-shock response. The heat-shock response is characterized by the production and activation of ubiquitous cellular proteins that detect and bind proteins damaged by heat or other physiologically stressful stimuli (202). The potential involvement of the variable cutaneous LE-associated autoantibody antigens with the heat-shock response may relate to the general importance of this response to cellular stresses or may be a function of a potentially unique relationship of this response to the abrogation of self-tolerance. Heat shock proteins (HSPs), which are induced in heat-shock responses, have been shown to promote cellular immune responses by both chaperoning peptides into cellular antigen-processing compartments and by directly activating antigen-presenting cells (203 ,204). HSP induction by UV light has been correlated to the increased binding of Ro/SSA and La/SSB antibodies in keratinocytes in vitro (200). Finally, increased HSP70 expression is increased in both sun-exposed and sun-protected skin of SLE patients (205).

Epitope Spreading

Epitope spreading is the process by which specific immune responses that arise to particular determinants on a macromolecule diversify over time (206). B cells, by virtue of their immunoglobulin receptors, can process and concentrate antigen prior to T cell presentation and are central to this process. This spread of immune responses can occur to epitopes within the primary macromolecule (intramolecular epitope spreading) or to physically associated molecules (intermolecular epitope spreading). Both inter- and

intramolecular epitope spreading have been reported in cases of murine immunization with peptides of 60 kd Ro/SSA, 52 kd Ro/SSA, and La (207 ,208 ,209). This is consistent with a physical linking of these antigens (210). Spreading of the immune response for 52 kd Ro/SSA and 60 kd Ro/SSA (but not La/SSB) to calreticulin is consistent with the notion that calreticulin may associate with a subpopulation of Ro/SSA particles from which La/SSB already has dissociated (209). The observation that human anti-Ro/SSA immune responses segregate with either anticalreticulin responses or anti-La/SSB responses (211) also is consistent with a differential compartmentalization of Ro/SSA and La/SSB antigens at the time of initiation of the immune response. The secondary recruitment of antibodies to the inducible heat shock proteins Grp78 and HSP70, following immunization of mice with either 52-kd Ro/SSA, 60-kd Ro/SSA, but not La/SSB, suggests physical association and colocalization of these proteins with the Ro/SSA polypeptides under conditions such as apoptosis, that may promote autoimmunization (199). In addition to providing evidence for the physical association of autoantigens, the phenomenon of epitope spreading to specific antigens associated with cutaneous LE in these animal models suggests that such epitope spreading may occur in human disease. These autoantibodies thereby may enhance and perpetuate cell-mediated autoimmune inflammation.

Interaction of Ro/SS-A and La/SS-B Autoantibodies and Human Skin

There is compelling evidence that antibodies to Ro/SSA and La /SSB bind to human epidermis in vivo (174). The binding of these antibodies to keratinocytes in vitro can be enhanced by UV radiation in the presence (52) or absence (212) of apoptosis. Estrogens (213), heat shock (200), and viral-induced apoptosis (214) also can enhance binding. What are the potential consequences of this binding? The predominant IgG subclass that is deposited in lesions is IgG1, a form that is known to activate complement and initiate antibody-dependent cellular cytotoxicity (ADCC) (215). The presence of complement membrane attack complex at the dermal-epidermal junction (DEJ) of cutaneous LE lesions further suggests an antibody-mediated pathogenesis for the cell damage seen in cutaneous LE (216). The presence of the complement membrane attack complex (C5b-9) in only the lesional skin of patients with SLE, SCLE, or CCLE (217 ,218) suggests that this complex may then play a role in the pathogenesis of the lesions. MRL/lpr mice deficient in decay accelerating factor, an inhibitor of both the classical and alternative complement pathways, develop more severe skin findings associated with C3 deposition (219). Furukawa et al. have shown that keratinocytes from patients with lupus are more susceptible to binding of anti-Ro/SSA antibodies following UV exposure than controls and that these keratinocytes can be lysed by ADCC when sera and peripheral blood leukocytes from patients are added to the keratinocytes (220 ,221). This increased binding may be from an increased susceptibility to UV-induced apoptosis or from other causes of increased Ro/SSA antigen availability (50). Considerable interindividual variation in levels of keratinocyte Ro/SSA and La/SSB epitope expression has been noted (222) and expression is higher in lupus patients with documented photosensitivity (223). Despite this evidence, anti-Ro/SSA, La/SSB, and other autoantibodies may not have an initiating role in the clinical lesions of cutaneous lupus because the deposition of immunoglobulin and complement components as detected by fluorescence microscopy generally follows the appearance of perivascular inflammation in photo-provoked lesions (8 ,13). Keratinocytes generally are susceptible to ADCC but are able to resist cytotoxic damage by the complement membrane attack complex (224). Although anti-Ro/SSA antibodies can potentiate ADCC in vitro (225), NK cells are the most common mediators of ADCC and these are seen rarely in cutaneous lupus infiltrates (226 ,227). It remains possible that other cell types, including monocytes or lymphocytes, are participating in ADCC, but this has not been confirmed in vivo.

While the anti Ro/SSA response is clearly associated with SCLE, another clinical type of cutaneous lupus, CCLE is not exquisitely photosensitive. The majority of CCLE patients do not have anti-Ro/SSA responses as detected by standard immunodiffusion techniques. Additionally, epidermal IgG deposits are found rarely, and immunoglobulin deposition is limited to the DEJ (166). When sensitive ELISA techniques were used, 11 out of 15 CCLE patients were found to have low-level IgG anti-Ro/SSA antibodies. Other, as yet undefined, antibody sensitivities or cell-mediated responses may be paramount in CCLE.

Cellular Factors

Immunogenetics

Anti-Ro/SSA antibody responses have been linked to susceptibility loci associated with Class II MHC alleles. There is a strong association between SCLE, anti-Ro/SSA antibodies, and the HLA-B8, DR-3, DRw52 phenotypes (228). Associations between Ro antibody responses and DQA1 alleles, DQ2 alleles, and HLA-DR3 in different populations suggest that specific MHC class II molecules participate in the anti-Ro response (229 ,230 ,231). Diversification of the Ro/SSA and LA/SSB antibody response also has been linked to specific HLA class II phenotypes (232). This would imply the participation of Ro/SSA antigen-specific T cells in the generation of the Ro/SSA antibody response. Although 52 kd Ro/SSA-specific T cells have been described in the salivary glands of Sjögren patients (233), no antigen-specific T cells have been described in cutaneous LE. Murine T cell epitopes have been defined in a number of lupus autoantigen systems including the La/SSB autoantigen (234) and the Ro/SSA antigen (235). The specificity

and role of similar autoantigen–specific T cells in humans is an obvious area of ongoing investigation.

T Cells and Murine Models of Cutaneous LE

As in the examples above, there is growing evidence that the highly specific humoral immune response to autoantigens in SLE is T cell dependent (236). Murine models of cutaneous LE include the spontaneously occurring and UV accelerated forms of disease in MRL/lpr mice, graft-versus-host disease, and NZB/NZW mice (reviewed in (237)). None of these accurately recapitulate the cutaneous pathology seen in human disease. They nevertheless have been useful in a dissection of the potential cellular mechanisms of autoimmune inflammation.

The NZB/NZW mouse offers a model of immune globulin deposition at the DEJ, but these animals do not develop clinical cutaneous inflammation (238). The acute phase of graft-versus-host disease generated by minor histocompatibility disparity simulates the histopathology of cutaneous lupus (239) but immunoglobulin deposition at DEJ is uncommon.

MRL/lpr mice develop alopecia and scab formation associated with histopathologic changes similar to cutaneous lupus including DEJ immunoglobulin deposition (240 ,241). These lesions are characterized by a T cell inflammatory infiltrate. The important accelerator role of the lpr mutation on the MRL background has been documented in backcross experiments. The lpr mutation results in deficient Fas expression and this interferes with the apoptotic death of potentially self-reactive B and T cells (242). Both conventional (αß) and nonclassical (γδ) T cells have been shown to participate in the MRL/lpr disease phenotype including the skin disease (243 ,244) and autoantigen–specific αß T cells are absolutely required for full penetrance of disease (245). The spontaneous activation of T cells in MRL/lpr mice is highly B cell dependent (246) but is dissociated from antibody production (247) suggesting that antigen processing and presentation to T cells by B cells is important (248). A protective role for nonclassical MHC restricted NKT cells is suggested by the fact that CD1d-deficient MRL/lpr mice have exacerbated skin disease (249) and treatment with α-galactosylceramide, which results in the expansion and activation of CD1d-reactive NKT cells, improves dermatitis in MRL/lpr mice (250). Humans with active systemic LE also have diminished numbers of NKT cells (251).

Recently, the critical role of the costimulatory molecules B7–1 (CD80) and B7–2 (CD86) in this model has been shown. MRL/lpr mice deficient in both of these molecules have diminished skin lesions and do not develop renal pathology (223). These molecules provide essential signals for T cell activation and immunoglobulin class switching, again establishing a crucial role for B and T cells in this disease. Another costimulatory molecule that may have a primordial role is CD40, expressed on DC. Transgenic expression of CD40 ligand in the skin using a keratin promoter resulted in constitutive activation of cutaneous DC and a $CD8^+$ T cell–mediated autoimmune disease characterized by dermatitis, myositis, pneumonitis, nephritis, and autoantibody formation (252). This model recapitulates a number of features of SLE and places the skin dendritic cell at the center of disease initiation.

Role of Activated T Cells in Human Cutaneous Lupus Erythematosus

The pathology of cutaneous lupus is one of a lichenoid tissue reaction in which the basal keratinocytes are the primary focus of injury (253) (Fig. 29-5). This injury is associated with keratinocyte hyperproliferation, with normal early differentiation and premature terminal differentiation (254). The inflammatory-cell infiltrate is characterized by mononuclear cells at the DEJ as well as around blood vessels and dermal appendages. Inflammatory cells in the infiltrate of established cutaneous LE lesions are predominantly CD3 positive cells with CD4 positive cells present in higher numbers than CD8 cells (reviewed in (160)). The study of photo-induced lesions has allowed an analysis of early histologic changes and their evolution. In early lesions, this analysis has demonstrated $CD4^+$ T cells predominantly at the DEJ associated with rare HLA class II expression by keratinocytes. In spontaneous lesions and late photo-induced lesions, an increased number of $CD8^+$ T cells was observed, epidermal class II MHC expression was increased and the number of Langerhans cells was reduced (255 ,256 ,257 ,258). The decrease in Langerhans cell number may reflect DC activation and migration into the regional lymph nodes. The predominant type of T cell in established inflammatory infiltrates remains controversial. Volc-Platzer et al. have suggested that T cells of a specific γδ T cell receptor phenotype are preferentially expanded within the infiltrates (259). They proposed that these cells may recognize heat shock proteins induced or translocated in keratinocytes by UV or stress. Fivenson et al., however, reported that γδ T cells are virtually absent in the infiltrates (260). The Vß usage of infiltrating T cells in lesions of cutaneous LE recently was compared to that in

peripheral blood and in other inflammatory skin conditions (261). The percentage of Vß 8.1 $CD3^+$ cells was elevated in skin lesion from both CCLE and ACLE when compared to patients with other inflammatory skin disease. There was a significant skew to this Vß type when compared to peripheral blood. This selective expansion is consistent with an antigen-driven response. Sequencing of TCR clonotypes derived from the inflammatory infiltrates further suggests antigen-induced clonal accumulation (262). The expression of class II MHC molecules and CD28 by infiltrating T cells and expression of B7–1 (CD80) and B7–2 (CD86) costimulatory molecules by antigen-presenting cells in the lesional but not nonlesional skin suggests ongoing active and productive antigen presentation to T cells in cutaneous LE (263). Recently, a primary role for $CD8^+$ T cells in SLE has been suggested by the observation that activated $CD8^+$ T lymphocytes expressing perforin and granzyme B correlate with disease activity (264). Scarring CCLE has now been associated with the presence of lesional and circulating $CD8^+$ T cells that express CCR4, the receptor for CCL17/TARC, implicating cytotoxic $CD8^+$ T cells in the scarring subtype of CCLE (265).

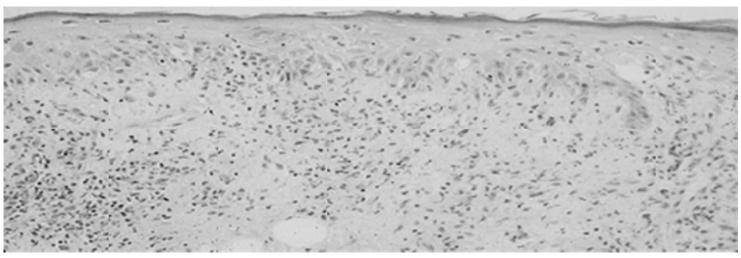

Figure 29-5. (See color plate.) Photomicrograph of a biopsy of subacute cutaneous lupus. There is disarray in the maturation pattern of the keratinocytes. There is evidence of hyperkeratosis (increase the thickness of the horny-cell layer). The basement membrane zone is disorganized with a mononuclear cell infiltrate and thickening of the basement membrane zone. There is a dermal mononuclear cell infiltrate that is predominantly perivascular. The mononuclear cells are predominantly CD4 T cells, many showing an activation phenotype, and macrophages.

Cofactors in Cutaneous Lupus Erythematosus

Ultraviolet Effects on Cutaneous Vasculature

Observational studies in humans and animal models have furthered our understanding of the roles of autoantibodies and cellular mechanisms in cutaneous lupus. Dermal blood vessels are involved in all forms of cutaneous lupus as targets for the cytokines and other mediators released from keratinocytes. These vessels also are affected directly by UV light. The potential importance of UV light in contributing to dermal and perivascular inflammation is underscored by the exquisite photosensitivity of lupus tumidus, a dermal variant of cutaneous LE without epidermal or interface changes (255 ,266). In a model of UV-induced erythema in the guinea pig, infusion of sera from patients with SCLE greatly enhanced UV-induced blood flow and this was greatest with sera containing high titers of anti-Ro/SSA (267). This observation highlights the potential interactions of the various soluble factors present in circulation of patients with cutaneous LE with the vasculature. Passive transfer of serum from patients with vesiculobullous LE into guinea pigs followed by UV irradiation also results in lesion induction (268) providing further evidence that circulating factors contribute to LE photosensitivity.

Vascular Activation

Enhanced expression of adhesion molecules on the surface of endothelial cells is an essential point of control for leukocyte attachment and migration through the endothelial barrier into cutaneous tissues (reviewed in (269 ,270)). A subpopulation of human memory T cells preferentially recirculates to the skin. These cells interact via cutaneous lymphocyte antigen (CLA) molecules on their surface with E-selectin molecules on dermal microvascular endothelial cells. In addition to CCR4, the $CD8^+$ T cells in the peripheral blood of patients with CCLE express elevated levels of CLA (265). E-selectin can be upregulated by UVB (271) and its expression is increased in CCLE and SLE photo-induced lesions (272). Elevated levels of soluble E-selectin in LE patients with widespread and active cutaneous disease further suggests an important role for endothelial-cell activation in the pathogenesis of disease (273). Intracellular adhesion molecule 1 (ICAM-1) expression by endothelial cells is a crucial step in the initiation of endothelial T cell binding, which occurs via LFA-1. ICAM-1 expression also facilitates T cell adhesion to keratinocytes (274). Endothelial ICAM-1 also is upregulated following UV irradiation, and this is stimulated by TNF-α and IFN-γ (275). In the MRL/lpr mouse model of SLE, TNF-α and IL-1 sequentially induce endothelial ICAM-1 in vivo with disease evolution (276). Vascular cell adhesion molecule (VCAM)-1 is necessary for leukocyte emigration from the microvasculature and is the ligand for VLA-4 on leukocytes. Immunohistochemical studies have confirmed that the endothelium underlying cutaneous lupus lesions is activated: VCAM-1 is expressed both in lesional and nonlesional cutaneous endothelium in active systemic lupus (277) and levels of VCAM-1 are increased in lesional skin (272 ,278). ICAM-1 is expressed by the endothelial cells in lesional skin of most patients with CCLE or SCLE (279). Figure 29-6 summarizes the role of these molecular interactions in facilitating leukocyte migration into the skin. Chemokines are induced by UV light and are upregulated in lesional lupus skin. The TH1 associated CXCR3 ligands CXCL10 and, to a lesser amount CXCR9 and CXCL11, are expressed at the dermo-epidermal junction in CCLE (280) and are the most abundantly expressed chemokine family members in cutaneous LE (134). A functional role for these ligands is suggested by the expression of CXCR3 by infiltrating dermal T cells. The CXCR3 ligands cooperate with the homeostatic chemokine CXCL12 to recruit CLA^+ memory T cells into the skin (134). The functional relevance of lymphocyte CCR4 expression and tissue expression of CCL17 in patients with scarring CCLE was likewise confirmed by in vitro migration assays (265).

Nitric Oxide

The clinically normal appearing skin of patients with active SLE demonstrates elevated levels of inducible nitric oxide synthase (iNOS) in both the epidermis and adjacent vascular endothelium (281). Aberrant regulation of iNOS expression also has been noted in photo-induced lesion of cutaneous lupus (282). Healthy controls were shown to have short-term expression (days 1 to 2) of iNOS after either UVA or UVB irradiation. Patients with cutaneous LE were noted to have significantly delayed, but prolonged expression (days 3 to 24) of iNOS. Both IL-1 and TNF-α promote the expression of iNOS (283) and this abnormal expression

in cutaneous LE may be secondary to a genetic dysregulation of these cytokines (see below). Synthesis of iNOS leads to nitric oxide (NO) production, which is known to promote apoptosis and have multiple pro-inflammatory effects. When applied to normal human skin, NO induced accumulation of $CD4^+$ and $CD8^+$ T cells, expression of ICAM-1 and VCAM-1, and accumulation of p53, followed by apoptosis (284). Altered expression of this molecule, if confirmed, may link dysregulated apoptosis and inflammation.

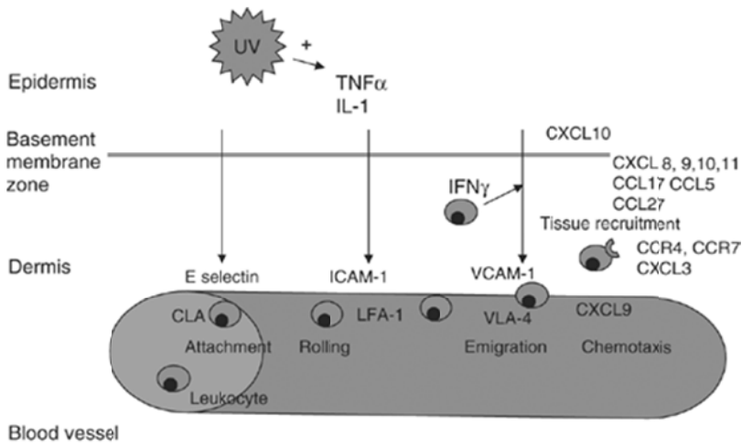

Figure 29-6. Ultraviolet (UV)-induced leukocyte migration into the skin. UV radiation induces cytokine release in cutaneous tissues. These cytokines then induce adhesion molecule expression on endothelial cells and leukocytes promoting inflammatory cell recruitment to the skin. Likewise, cytokines released by the inflammatory cells can enhance and perpetuate this recruitment. Specific chemokines promoting local inflammation are then expressed in lesional skin and promote chemotaxis of inflammatory cells. The selectins, chemokines, chemokine receptors and adhesion molecules depicted all have been shown to be upregulated in cutaneous lupus erythematosus.

Cytokines

An appropriate cytokine milieu can facilitate and modulate immune responses. Abnormalities in the production and function of cytokines could underlie the abnormal photoreactivity noted in cutaneous LE. Analysis of interleukin-2, -4, -5, and -10 and IFN-γ mRNA levels in lesions of cutaneous LE has revealed increased local levels of IL-5 and significant levels of IL-10 and IFN-γ (285). These results indicate a mixed cytokine pattern favoring cell adhesion and cellular inflammation via IFN-γ–induced ICAM-1 expression and a T-helper 2 response, favoring antibody production, with IL-5 and IL-10.

TNF-α

TNF-α is a primary cytokine that can be induced in keratinocytes (118) and in dermal fibroblasts by UVB (286 ,287 ,288). TNF-α has numerous effector functions and has been termed a master regulator of leukocyte movement (289). Abnormal TNF-α expression can promote autoimmunity. Prolonged overexpression of TNF-α in the pancreas of mice has been shown to initiate organ-specific autoimmune disease (290). In the skin, TNF-α can induce rapid Ro/SSA and La/SSB antigen translocation and surface expression in keratinocytes (291). In mice, transgenic overproduction of TNF-α by the epidermis, results in: (a) epidermal basal-cell degeneration; (b) a pleomorphic dermal leukocyte infiltrate with macrophage engulfment of degenerating cells; (c) hyperkeratosis, and ultimately (d), a graft-versus-hostlike histology (292). Some of these features are reminiscent of cutaneous lupus. Unfortunately, these mice also have high levels of TNF-α in the serum and soon die of cachexia, which has presumably prevented further analysis for features of autoimmunity. In the MRL/lpr mouse model of lupus, systemic TNF-α (and IL-1) levels are elevated and induce endothelial adhesion molecule expression (276). Raised circulating levels of TNF-α may correlate with disease activity in human systemic lupus (293). A polymorphic variant in the TNF-α promoter in humans (TNF-α 308A) is associated with increased production of TNF-α (261). The presence of this promoter is associated with an increased risk of SLE in African Americans (294). It is an independent susceptibility factor for systemic lupus in Dutch Caucasians (295). TNF-α production in keratinocytes shows interindividual variability and it has been proposed that this variability may underlie a predisposition to cutaneous lupus (29 ,120). Recently, this concept has found support in that the TNF-α –308A promoter

polymorphism associated with increased TNF-α production has been shown to be highly associated with photosensitive subacute cutaneous LE (152), and confirmed in a separate population (296), as well as the related cutaneous neonatal LE (153). Direct involvement of TNF-α in the pathogenesis of cutaneous LE inflammation could explain the clinical benefit of thalidomide in this setting (297 ,298 ,299). Thalidomide decreases TNF-α production in monocytes, DC, and keratinocytes (300 ,301 ,302 ,303), possibly via an increase in the rate of TNF-α mRNA degradation (300 ,304) although it may also inhibit TNF-α–independent keratinocyte apoptosis (302).

IL-1

Another early response to UV light is the production of and release of IL-1 by keratinocytes. Like TNF-α, IL-1 has broad pro-inflammatory activities (305). IL-1α predominates in keratinocytes while IL-1ß is the predominant form in monocytes, macrophages, and DC. Transgenic overexpression of IL-1α by basal keratinocytes in mice results in hair loss, scaling, and focal inflammatory lesions (306). Keratinocytes also contain an excess of IL-1 receptor antagonist, which binds to the IL-1 receptor and inhibits IL-1–induced activation (307). This molecule is particularly polymorphic and a significant increase of a null allele has been reported in SLE patients (308), especially those with photosensitivity (309). A correlation with CCLE also has been published as an abstract (310).

Interleukin-10

IL-10 is a key mediator of UV-induced immune suppression (133). Skin disease in MRL/lpr mice is exacerbated by IL-10 deficiency in association with increased IFN-γ production by T cells (311). IL-10 also can promote B cell activation (312). Polymorphisms within the IL-10 gene promoter associated with high levels of expression of this cytokine have been associated with anti-Ro/SSA sero-positivity (313). Abnormalities in either constitutive or UV-induced cytokine expression or the genes governing these factors thus may be central to the cutaneous-lupus phenotype. The associations of gene polymorphisms with disease described so far remain preliminary in nature and will need to be confirmed in multiple populations.

Interferon-γ

Interferon-γ is detected in normal skin after low-dose UVA exposure (144) and elevated IFN-γ mRNA levels are noted in lesions of cutaneous LE (285). This cytokine recently has been shown to be pivotal in the induction of keratinocyte apoptosis in two skin inflammatory conditions: allergic contact dermatitis and atopic dermatitis (314). In these conditions, T cell derived IFN-γ promotes keratinocyte apoptosis by enhancing the expression of the Fas death-inducing molecule on the keratinocytes (314). Overexpression of IFN-γ alone in the suprabasal skin of transgenic mice by the use of the involucrin promoter (a protein restricted to suprabasal keratinizing epithelium) results in a model of systemic lupus with the production of antinuclear antibodies and immune-complex glomerulonephritis (315 ,316). The mice also develop scaly skin, alopecia, and cutaneous inflammation—suggestive but not characteristic of cutaneous LE. This murine model demonstrates increased keratinocyte apoptosis and requires the presence of αß T cells (317). This is currently the most provocative experimental evidence that the upregulation of cutaneous immune responses through cytokine overexpression can promote T cell–mediated local and systemic lupus-like disease.

Interferon-α

Recent gene array studies have demonstrated that type I interferons (IFN-α/ß) play an important role in the pathophysiology of SLE. Interferon-inducible protein transcripts were found to be upregulated in pediatric and adult SLE patients and the levels of these transcripts correlated with disease activity (318 ,319). Curiously, natural IFN-α–producing cells, also termed plasmacytoid DC, are found in the skin (but not the peripheral blood) of LE patients and in the cutaneous lesions of LE (320 ,321 ,322) and are found in association with the presence of type I interferon inducible proteins such as Mx (323). This suggests that local IFN-α production by these cells promotes Th1-biased inflammation. In favor of this, the number of infiltrating $CXCR3^+$ lymphocytes correlated closely with the expression of Mx and the type 1 interferon–inducible chemokine CXCL10 (323) and IFN-α was shown to potently induce CXCR3 ligands expression by keratinocytes, endothelial cells, and dermal fibroblasts in vitro (134). IFN-α has also been shown to confer a pro-inflammatory function to IL-10, resulting in the enhanced production of CXCL10 and CXCL9 (324). The potential central role of plasmacytoid DC-derived IFN-α production in cutaneous LE is underscored by the further observations that immune complexes containing nucleic acid released by necrotic or late apoptotic cells and opsonized by lupus IgG potently induce IFN-α production by plasmacytoid DC, thereby, potentially promoting ongoing disease activity (325).

Complement

Homozygous deficiencies of the complement components C2 and C4 have been associated with both SCLE and systemic LE (326 ,327), and most patients with homozygous C2 or C4 complement deficiency possess anti-Ro/SSA antibodies. Likewise, most individuals with a complete deficiency of C1q or C1r/C1s develop systemic LE (reviewed by (328)). These patients also have prominent photosensitive cutaneous involvement. Such deficiencies may enhance

the lupus phenotype by decreasing the clearance of apoptotic cells, thereby allowing immune-cell activation. Such abnormalities thus may both initiate and potentiate cutaneous lupus. In this regard, a silent C1q A chain mutation (C1QA single nucleotide polymorphism Gly70 [GGG.GGA]) in exon 2 has been associated with SCLE and with decreased expression levels of C1q (329). This mutation has also been found in virtually all C1q-deficient patients (330). Such a “silent” mutation may affect gene product function through effects on pre-mRNA splicing.

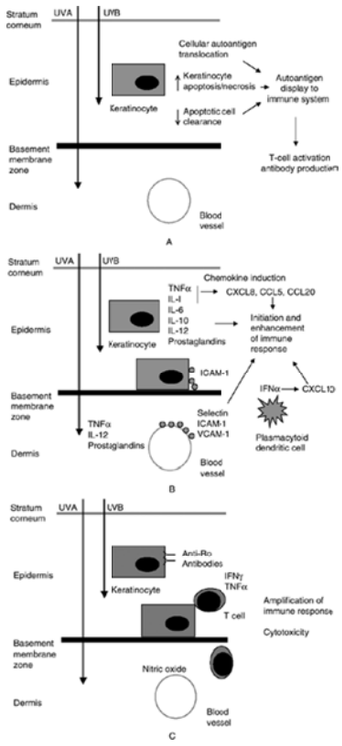

Figure 29-7. A, A model of the pathogenesis of photosensitive cutaneous lupus erythematosus. Recent studies of photosensitive lupus patients have demonstrated and increased number of apoptotic keratinocytes in both established lesions and following photoprovocation. Either increased apoptosis/necrosis or a delay in the clearance of apoptotic cells could result in an increase in autoantigen packaging and processing in a form accessible to the immune system. **B,** Ultraviolet radiation can induce keratinocyte apoptosis and/or necrosis and can also stimulate local cytokine release. This cytokine release can then lead to the observed increase in local mediateor of inflammation including selectins, adhesion molecules, chemokines and prostanoids. These molecules serve to recruit and activate dendritic cells and T cells. Plasmacytoid dendritic cells release IFN-α locally resulting in further chemokine release. **C,** The end result is a stimulation of the immune system to produce antibodies and to activate dendritic cells to prime T cells directed against stress-induced or stress-altered molecules (Ro antigen, La antigen, calreticulin). These agents of the immune system then act to promote further inflammation and tissue damage by processes such as epitope spreading mediated by antibodies and B cells, and cellular cytotoxic mechanisms mediated by T cells, natural-killer cells, and monocyte-macrophages.

A Model of Pathogenesis of Cutaneous Lupus Erythematosus

David Norris has proposed a four-step model for the pathogenesis of cutaneous lupus (49): (a) exposure to UV light induces the release of proinflammatory epidermal and dermal mediators such as IL-1 and TNF-α; (b) these mediators induce changes in the dermal and epidermal cells including the induction of adhesion molecules and the promotion of translocation of normally intracellular autoantigens such as Ro/SSA to the surface of epidermal cells; (c) autoantibodies then bind to the translocated autoantigens; and (d) keratinocyte cytotoxicity ensues as the result of lymphoid cells that are recruited from the circulation via an antibody-dependent cellular cytotoxicity mechanism. According to this model, several factors are required concurrently for the development of cutaneous LE, including: (a) abnormal susceptibility to UV light, resulting in altered cytokine expression and possibly increased keratinocyte apoptosis induction; (b) the presence of antibodies with appropriate specificities, targeting keratinocyte components upregulated by stress; and (c) the presence of activated lymphocytes, specific for autodeterminants.

Since this model was first proposed, a great deal of new data has accumulated (also reviewed in (331 ,332 ,333 ,334 ,335)). Clinical and experimental data now suggest that apoptosis may be an important mechanism leading to autoantigen display in cutaneous LE and that UV light may be an important initiator of apoptosis and possibly necrosis. Abnormalities may exist in either apoptosis induction or in apoptotic cell clearance that result in an increased load of apoptotic and necrotic cells. In addition to promoting cell death and neo-antigen generation (such as UV-DNA), UV light induces and modulates inflammatory mediator release. Genetic abnormalities in TNF-α, IL1 receptor antagonist, and IL-10 have been linked tentatively to cutaneous lupus. The dysregulation of such cytokines may allow the upregulation of adhesion molecules, chemokines, and costimulatory molecules to allow self-antigen recognition and the initiation of an immune response in genetically predisposed individuals. The autoantibodies linked with cutaneous LE are directed at antigens involved in cellular-stress responses and in the heat-shock response. Autoantibody production and directed T cell responses may perpetuate and amplify autoantigen recognition as well as keratinocyte toxicity leading to the clinical hallmarks of cutaneous LE disease. A central role for skin resident DC such as plasmacytoid DC and for dysregulated IFN-α production in the initiation and perpetuation of skin disease is emerging. Figure 29-7 shows the salient points of this revised model. Ongoing research will no doubt shed light on the in vivo role of cellular apoptosis in disease induction and perpetuation, the primary or secondary pathophysiologic role of specific autoantibodies, and the nature of the underlying genetic makeup that predisposes to disease.

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Chapter 30

Lupus-Specific Skin Disease (Cutaneous LE)

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Realizing that the classification of cutaneous lupus erythematosus (LE) remains somewhat controversial, the authors have chosen to use the Gilliam classification system for LE skin disease (1) to organize the material in this chapter (Table 30-1). See Table 30-2 for an overview of the organization of this chapter. LE-nonspecific skin disease and pathomechanisms of cutaneous LE and photosensitivity are discussed in Chapters 31 and 29 , respectively

Historical Perspective

In his review of the history of LE, Thomas Benedek (see Chapter 1) points out that this disease initially was recognized by its visible cutaneous manifestations long before its systemic manifestations were known to exist (2 ,3 ,4). In this section, we will attempt only to highlight the people and events that relate primarily to the evolution of thought concerning the cutaneous manifestations of this protean disease process.

In 1851, Cazenave first used the term “lupus érythÜmateaux” in referring to Biett's earlier description of what appears to have been discoid LE (DLE) skin lesions (5). The term “lupus érythÜmateaux” helped to further distinguish this entity as a cutaneous malady that was distinct from cutaneous tuberculosis (lupus vulgaris) with which it had been earlier confused. Cazenave stated that LE preferentially occurred in outdoor workers and that exacerbations were related to cold, heat, fire, and the action of air.

In 1875, Kaposi further expanded Hebra's earlier description of what is now recognized as the systemic manifestations of LE and employed the “butterfly” simile first used by Hebra to describe the facial skin lesions of LE (6). He also commented upon the relationship between DLE skin lesions and the systemic manifestations of this disease, noting that DLE can, on occasion, progress to SLE. Payne reported the treatment of DLE with the natural antimalarial, quinine, in 1893 (11). Hutchinson (7), and later Osler, at the turn of the century (8), emphasized the multisystem nature of LE and the patient-to-patient variation with which its cutaneous and systemic manifestations are expressed. Lehmann and Ruzicka (9) have pointed out that Osler went on to say that LE patients did not tolerate sun well (10). Lehmann and Ruzicka also note that in 1913, Pussey commented on the exacerbating effects of sunlight on cutaneous LE in a young woman (12). Brocq's initial description of “symmetrical erythema centrifugum” (13), probably representing the earliest delineation of what Gilliam later referred to as “subacute cutaneous LE” (SCLE), the bridging form of LE-specific skin disease in his revised classification of cutaneous LE (14).

Lehmann and Ruzicka point out that in 1929, Fuhs reported the exacerbating effect of an artificial light source on a patient with “lupus erythematosus subacutus,” a term that appears to be referring to what we recognize today as SCLE (15).

In 1934, O'Leary's introduction of the term “disseminated DLE” as part of his new classification scheme included both its cutaneous and systemic manifestations (16). The term “disseminated” in his classification scheme was used somewhat ambiguously in referring both to a widespread distribution of LE skin lesions and the transition from disease limited to skin to the systemic illness that we now recognize as SLE. Thus, several forms of widespread LE-specific skin disease (i.e., generalized DLE and SCLE) were thereafter often lumped together under the designation “disseminated DLE” or “subacute-disseminated LE,” thereby obscuring the clinical and laboratory correlates of each of these clinically distinctive types of cutaneous LE. As a result, when the large LE population studies were later presented in which the clinical features of LE patients were correlated with the newly identified autoimmune serologic manifestations of the disease, the distinction between patients having generalized DLE and SCLE was often blurred.

In 1936, Freidberg presented the heretical idea that the systemic manifestations of LE could occur in the absence of any type of skin lesion (17). In 1951, the synthetic antimalarial, quinacrine (18), and corticosteroids (19) were introduced for the treatment of LE. In 1948, Hargrave's description of the LE-cell factor (20) and Friou's subsequent description of the antinuclear antibody assay in 1957 (21) ushered in the era of serologic-clinical correlation in LE. In 1963, Neville Rowell described EM-like lesions that

occurred in LE patients in the context of autoantibodies to the Sjögren syndrome antigen, Sj-T, which are now thought to represent the La/SS-B specificity (22). Dr. Rowell's other work has contributed significantly to our current understanding of various aspects of cutaneous LE including chilblains lupus (perniotic LE) and classical DLE.

Table 30-1: The Gilliam Classification of Lupus Erythematosus (LE)-Associated Skin Lesions

I. Histopathologically Specific (LE-Specific)
A. Acute cutaneous LE
1. Localized
2. Generalized
B. Subacute cutaneous LE
1. Annular
2. Papulosquamous
C. Chronic cutaneous LE
1. “Classical” DLE
a. Localized
b. Generalized
2. Hypertrophic (verrucous) DLE
3. Lupus profundus (LE panniculitis)
4. Mucosal LE
5. LE tumidus
6. Chilblains LE (perniotic LE)

DLE, discoid lupus erythematosus; LE, lupus erythematosus.

In 1963, Burnham et al. identified the “lupus band” (23) and Edmund Dubois and Denny Tuffanelli presented their landmark description of 520 consecutive SLE patients, 29% of whom had DLE skin lesions (24). Dubois was among the first to use the “spectrum” analogy for LE, emphasizing that this illness represented a disease continuum extending from localized DLE at the more benign pole to fully expressed SLE at the more severely affected pole. He also emphasized the fact that various transitional forms commonly occurred within this spectrum, including the appearance of SLE in patients who had initially presented with DLE lesions alone and the development of DLE lesions in patients with pre-existing SLE. These concepts were emphasized in the first edition of his classic book (25 ,26). Dubois' spectrum concept influenced Jim Gilliam's thinking concerning the classification of cutaneous LE.

Table 30-2: Chapter Organization

I.	Historical perspective
II.	Definition and classification
III.	LE-Specific skin disease
A.	Epidemiology
B.	Cutaneous manifestations
C.	Relationship with systemic disease features
1.	Risk of developing SLE
2.	Lupus band test
D.	Pathology and immunopathology
E.	Laboratory findings
F.	Differential diagnosis
G.	Management
H.	Prognosis

LE, lupus erythematosus; SLE, systemic lupus erythematosus.

In 1969, Reichlin et al. described anti-Ro/SS-A antibodies (27), which were later identified as the serologic markers for SCLE and neonatal LE, and in 1975, James N. Gilliam extended Dubois' spectrum analogy, which focused on the relationships between the various cutaneous and systemic manifestations of LE (28). He also stressed the value of the various forms of cutaneous LE as markers for subsetting LE (the exercise of identifying more homogeneous subgroups of LE patients based upon the sharing of common clinical, pathologic, and laboratory features) that had become popular by the 1970s (29).

In 1976, Marian Ropes published a comprehensive monograph (30) that detailed the experience gained from a large number of SLE patients followed over several decades in the inpatient and outpatient settings at Massachusetts General Hospital. Careful observations concerning the mucocutaneous manifestations of this disease were reported.

In 1977, Barba and Gonzalez presented one of the earliest reports on the beneficial effects of thalidomide on cutaneous LE (31). The tremendous impact of thalidomide on cutaneous LE inflammation subsequently was documented by a number of other workers (reviewed in (32)). Thalidomide subsequently has been shown to inhibit tumor necrosis factor-α (TNF-α) gene expression. These observations taken together with the modern work indicating abnormal TNF-α expression in photosensitive cutaneous LE such as SCLE could lead to new therapeutic approaches.

Gilliam et al. characterized the immunogenetically homogeneous LE subset in 1979, which was marked by the presence of SCLE skin lesions (33 ,34 ,35). These were virtually the same group of patients being described concurrently by Provost et al. under the designation of “ANA-negative SLE” (36). The concept of SCLE originally was introduced by Gilliam as a component of a new classification of cutaneous LE that emphasized the clinical differences existing between different types of histopathologically specific LE skin lesions (29). Gilliam's earlier work at Stanford with the dermatopathologist, Alvin Cox, and later at University of Texas Southwestern with Robert Freeman greatly influenced his thinking in this area.

In 1981, Weston and Provost described the anti-Ro/SS-A autoantibody as the serologic marker for neonatal LE (37) and in 1990, Lehmann et al. redefined LE photosensitivity in modern photobiological terms by documenting that SCLE is among the most photosensitive subsets of cutaneous LE and that ultraviolet A (UVA) and ultraviolet B (UVB)-play significant roles in SCLE photosensitivity (38 ,39).

In 1984, Norris et al. showed that ultraviolet B (UVB) radiation modulates Ro/SS-A autoantigens to the surface of

human epidermal keratinocytes and proposed a model for the pathogenesis of Ro/SS-A autoantibody–associated photosensitive cutaneous LE involving antibody-dependent, cell-mediated cytotoxicity (40 ,41). In 1992 and 1993, Beutner et al. attempted to systematically classify the clinical forms of cutaneous LE (42 ,43).

In 1994, Casciola-Rosen et al. first demonstrated that autoantigens (e.g., Ro/SS-A, calreticulin) that are targeted in SLE are clustered in two populations of surface structures on UVB-induced apoptotic keratinocytes (44). In 1995, a systematic characterization of cutaneous LE subsets in a relatively homogenous ethnic population (45) was described by Watanabe and Tsuchida. In 1996 these two authors further characterized LE profundus/panniculitis (46).

In 2000, the most modern characterizations of the subsets of LE tumidus (47) was reported. Werth et al. initially reported a specific genetic marker for SCLE, the presence of the -308A TNF-α promoter polymorphism (48).

Definition and Classification

LE is a systemic autoimmune disorder associated with polyclonal B cell activation that is thought to result from an interplay of genetic, environmental, and hormonal elements. It is convenient to consider the heterogenous clinical expression of this disorder as constituting a disease continuum or spectrum extending from a limited cutaneous disorder to a life-threatening systemic disease process.

The term “discoid LE” (DLE) often was used in the past to generically designate the subgroup of LE patients whose disease was expressed only in the skin (49). The use of the term “DLE” in this sense can create confusion because this same term also is commonly used to identify one of several clinically distinctive forms of LE-specific skin disease. In the following discussion, the term “DLE” will be used in the latter, more restricted sense to refer only to a certain clinical form of chronic cutaneous LE. The term “cutaneous LE” will be used in an umbrella fashion in this chapter to refer to all skin lesions that have some form of association with LE.

The term “SLE” has been used in the past synonymously with the term “LE” to generically designate all patients suffering from this autoimmune disease process (unfortunately, many in the present continue to use the term in this manner). However, in this sense, the term “SLE” unfairly stigmatizes those LE patients whose disease is expressed clinically only in their skin throughout their entire disease course. The term “SLE” will be used in this discussion to refer only to the systemic manifestations of the fundamental underlying disease process, LE. It must be remembered that the large majority of patients with SLE will express some form of cutaneous LE during the course of their disease.

The American College of Rheumatology (ACR) has formulated a set of revised clinical and laboratory criteria for the classification of SLE primarily for the purpose of providing some degree of uniformity to the patient populations of clinical studies (50). This classification system is used commonly by clinicians to establish a diagnosis of SLE in a given patient. However, this system is not perfect. For example, based on the devised criteria, a patient can be classified as SLE based on mucocutaneous findings only (i.e., the four criteria being photosensitivity, oral ulcers, discoid rash, and malar rash). It also is unfortunate that this classification did not include histopathologic evidence of cutaneous LE as a diagnostic criterion (e.g., evidence of vacuolar degeneration in the basal layer of the epidermis). Dr. Victoria Werth led an effort, which included the two authors of this chapter, to develop recommendations on revising the clinical and laboratory criteria for the classification of LE (51).

The LE disease spectrum can be subdivided in a number of ways—clinically, serologically, or pathologically. Using this approach, various patterns (or subsets) of illness can be identified, i.e., some patients will share common patterns of clinical, immunologic, and pathologic abnormalities. One example would be SCLE. Patients who present with SCLE skin lesions have a clinically and pathologically distinctive form of cutaneous LE, frequently produce anti-Ro/SS-A autoantibodies, and share a common HLA phenotype (HLA-B8, DR3, DRw52, DQ 1/2) (52). The clinical expression of skin involvement in LE is very common and extremely heterogenous (Table 30-3). Because the type of skin involvement in LE can be reflective of the underlying pattern of SLE activity, it is important to have a common language when referring to LE skin lesions. Much attention has been paid to the issue of classifying LE from the dermatologic perspective in the past and there continues to be considerable debate in this area (53). For the purpose of this discussion, we will use the classification system developed by Jim Gilliam, which divides the skin lesions that can be encountered in LE patients into those that are histologically specific for LE (i.e., LE-specific skin disease) and those that are not histologically specific for this disease (i.e., LE-nonspecific skin disease) (29). The histologically specific skin lesions share the following elements of a lichenoid tissue reaction (54): hyperkeratosis; epidermal atrophy; liquefactive (vacuolar, hydropic) degeneration of the epidermal basal-cell layer; a mononuclear cell infiltrate focused at the dermal-epidermal junction (DEJ), perivascular areas, and perifollicular areas; thickening of the epidermal-basement membrane; and melanin-pigment incontinence. There are three broad categories of LE-specific skin disease: acute cutaneous LE (ACLE), subacute cutaneous LE (SCLE), and chronic cutaneous LE (CCLE). LE-nonspecific skin lesions such as cutaneous vasculitis have histopathologic changes that are seen in conditions other than LE and thus are not specific for this disorder (see Chapter 31).

It is important to note, however, that other cutaneous disorders can have LE-like histopathology. These disorders include dermatomyositis, graft-versus-host disease, erythema

multiforme, drug eruptions, and polymorphous light eruption, which sometimes can clinically mimic the gross morphologic features of cutaneous LE. An experienced dermatologist usually can differentiate between these various disorders by analyzing additional information from the patient's history, physical examination, and laboratory evaluation.

Table 30-3: Clinical Feature Comparisons between the LE-Specific Skin Diseases

	ACLE	SCLE	Classical	DLE
Clinical Features:				
Induration		0	0	+++
Scarring		0	0	+++
Pigment changes		+	++	+++
Follicular plugging		0	0	+++
Hyperkeratosis		+	++	+++
Histopathology:				
Thickened basement membrane		0	+	+++
Lichenoid infiltrate		+	++	+++
Periappendaggeal inflammation		0	+	+++
Lupus band:				
Lesional		++	++	+++
Nonlesional		++	+	0
Laboratory				
Antinuclear antibodies		+++	++	+
Ro/SS-A antibodies:				
By immunodiffusion		+	+++	0
By ELISA		++	+++	+
Antinative DNA antibodies		+++	+	0
Hypocomplementemia		+++	+	+
Prognosis				
Risk for developing SLE		+++	++	+

+++Strongly associated.
++Moderately associated.
+Weakly associated.
0 Negative, or no association;
ACLE, acute cutaneous lupus erythematosus; DLE, discoid lupus erythematosus; ELISA, enzyme-linked immunosorbent assay; SCLE, subacute cutaneous lupus erythematosus; SLE, systemic lupus erythematosus.

With any arbitrary subdivision of a disease continuum such as LE, overlapping features can occur. For example, patients who have predominately SCLE lesions also can develop scarring DLE or ACLE lesions at some point in their course. However, in most patients, one form of LE-specific skin involvement will predominate.

An occasional patient will be encountered who has skin lesions that demonstrate LE-specific histopathology but whose cutaneous disease does not conform fully to one of the categories of LE-specific skin disease under the Gilliam classification scheme. The few such patients that have been encountered have been considered to have a clinically generic variety of LE-specific skin disease. It has been the impression of one of the authors (RDS) that this situation arises more commonly in African American patients.

It also should be remembered that LE patients not infrequently develop unrelated common dermatologic disorders that are not the direct result of LE activity in the skin. Harm to patients can result when skin disorders are misdiagnosed as cutaneous LE causing patients to be subjected to unnecessary tests and/or potentially toxic therapies. In their study of 84 consecutive SLE patients, Weinstein et al. found that (42%) had dermatoses attributable to SLE while 58 (69%) had dermatoses that were not directly attributable to SLE (55). Some conditions can simulate the clinical appearance of different forms of cutaneous LE. One example would be acne rosacea simulating the clinical appearance of ACLE on the face (56). Additionally, cutaneous complications of treatment of SLE, such as corticosteroid-induced acne vulgaris, might be attributed to the underlying systemic autoimmune process by observers less familiar with the cutaneous manifestations of this disorder.

Lupus Erythematosus-Specific Skin Disease

Epidemiology

There is very little population-based data concerning the epidemiology of LE—cutaneous or systemic. Most studies are reported by rheumatologists or dermatologists working separately, and as a result, selection bias often is present in the data that are commonly reported. In most large clinical studies of selected LE patients, the skin is second only to joints as the most frequently affected organ system, and skin disease is the second most common way that LE initially presents clinically (57). This also was true for a population-based cohort of 80 patients (58). In some study populations, cutaneous disease has been the most common disease manifestation (59).

Together, all forms of cutaneous LE have a significant socioeconomic impact within the United States. It has been suggested that cutaneous LE is the third most common cause of industrial disability from dermatologic disease, with 45% of cutaneous LE patients experiencing some degree of vocational handicap (60). It is most informative to discuss the epidemiology of the three major types of LE-specific skin disease in more detail.

Acute Cutaneous Lupus Erythematosus

In most large clinical studies carried out by rheumatologists or internists, ACLE is reported under the designations “malar rash” or “butterfly rash” (i.e., presumably localized ACLE) and “rash,” “maculopapular rash,” or “photosensitive lupus dermatitis” (presumably generalized ACLE). In such studies, the skin lesions usually are not biopsied and thus it is difficult to know whether they in fact represent LE-specific skin disease. Because dermatologists usually do not primarily mange such patients because of the strong association between ACLE and systemic LE activity, little data concerning this form of LE-specific skin disease is available in the dermatologic literature. Because of its strong association with SLE, the epidemiology of ACLE might be expected to closely parallel that of unselected SLE patients, which have been comprehensively reviewed (61).

Demographics

Malar rash or butterfly rash has been reported in 20% to 60% of large LE patient cohorts (59). Limited data suggest that the “maculopapular rash” of SLE is present in about 35% of SLE patients (57). Malar rash is more common in women than men (57). Because photosensitivity is seen more frequently in whites than African Americans (62), one might infer that the same could be true for ACLE. Malar rash has been suggested to be associated with a younger age of disease onset (57). A clinically nonspecific maculopapular rash resembling a drug eruption but felt to be related to SLE was present in 59% of the 81 patients studied by Wysenbeek et al. (63); however, because biopsy data were not presented, it cannot be certain whether this “rash” was related to generalized ACLE.

Genetic Associations

Curiously, little effort has been made to determine whether ACLE has any specific HLA associations. ACLE lesions usually occur in the context of SLE and SLE has been associated with HLA-DR2 and -DR3 (64). Familial associations and concordance in twins suggest that SLE has an important genetic component (64).

Environmental Factors

Exposure to natural or UV irradiation is a frequent precipitating factor for SLE patients (65). Although photosensitivity was more common in patients with anti-Ro/SS-A autoantibody in one study (66), this association has not been confirmed in a more recent phototest study (67). Chemicals such as L-canavanine present in alfalfa sprouts can induce a SLE-like illness (61). Numerous drugs have been implicated in inducing various features of SLE (68), although the skin often is spared in classical drug-induced SLE. Infections, especially with subtle types of viruses, have long been suspected to be capable of precipitating and/or exacerbating SLE (69).

Subacute Cutaneous Lupus Erythematosus

Demographics

SCLE patients comprised 9% of the total LE patient population examined by Sontheimer et al. (33). Others have found SCLE lesions in 7% to 27% of their LE patient populations (70). SCLE is primarily a disease of white females of all ages. Seventy percent of the original cohort of SCLE patients were female and 85% were white (33). The mean age of this group of patients was 43.3 years with a range from 17 to 67 years. Others have reported similar racial and sexual demographic data (70). There have been at least five case reports of children affected by SCLE, ranging in age from 18 months to 9 years (71 ,72 ,73 ,74 ,75).

Genetic Associations

The majority of studies have found that at least one half of their SCLE patients have the HLA-DR3 phenotype (76), although some groups have found a lower frequency of HLA-DR3 (77). HLA-DR3 normally is found in only 25% of the U.S. white population (78). One group has suggested that the HLA-DR3 phenotype is most strongly associated with the annular form of SCLE (79) and that annular SCLE lesions denote the most homogeneous subgroup of SCLE patients immunogenetically (79). HLA-DR2 also has been associated

with SCLE (80). SCLE patients with overlapping features of SCLE and Sjögren syndrome are more likely have the HLA-B8, DR3, DRW6, DQ2, and DRW52 extended haplotype (81). It has been suggested that the HLA-DR antigen associations of SCLE relate more to the anti-Ro/SS-A antibody response in these patients than to the SCLE skin lesions (80). Patients with the extended haplotype HLA-B8, DR3, DRW6, DQ2, and DRW52 produce very high levels of Ro/SS-A autoantibodies (82). High Ro/SS-A antibody titers also have been associated in individuals with HLA-DQw1/DQw2 (82).

There is a growing body of evidence that genetic factors may influence the putative role TNF-α plays in the pathogenesis of the Ro/SS-A associated LE-specific skin diseases—SCLE and neonatal LE skin disease (83). Werth et al. have demonstrated that SCLE patients often have a TNF-α gene promoter polymorphism (–308A) that appears to be associated with higher levels of TNF-α expression in cells exposed to UVB radiation (84). There is linkage disequilibrium between the –308A allele and HLA-DRB1*03, the latter being found more frequently in patients producing Ro/SS-A antibodies. Of interest, Werth et al. found that the proportion of Caucasians with a –308A allele and the HLA-DRB*3 allele was 100% in SCLE patients (85). Sixty-four percent of 22 patients with neonatal LE skin disease have the TNF-α –308A allele (86). In this same study, TNF-α staining was observed in lesional skin from three neonatal LE patients but not in skin from a normal infant. Another study has found that TNF-α expression is increased in epidermal cells within SCLE skin lesions compared to cells from uninvolved skin from SCLE patients and cells from other inflammatory or neoplastic skin diseases (87).

Genetically based deficiencies in various complement components including C2, C3, C4, and C5 have been associated with SCLE and/or DLE (88 ,89). Additionally, genetic deficiency of C1q is a very strong risk factor for photosensitive SLE (90 ,91). There is preliminary evidence that a synonymous single nucleotide polymorphism in the Gly70 codon in the second exon of the C1QA gene (C1QA-Gly70GGG/A) is associated with the SCLE phenotype (92). The C1QA-Gly70GGG/A single nucleotide polymorphism also curiously appears to be linked to other DNA sequence variations that result in complete congenital deficiency of C1q (93).

Table 30-4: Drugs That May Precipitate or Exacerbate LE-Specific Skin Disease

SCLE	acebutolol, angiotensin-converting enzyme inhibitors (captopril, cilazapril), antihistamines (cinnarizine, ranitidine, thiethylperazine), calcium channel blockers* (diltiazem, nifedipine, nitrendipine, verapamil), carbamazepine, etanercept, griseofulvin, hydrochlorothiazide*, interferon-alpha and beta, leflunomide, naproxen, oxprenolol, d-penicillamine, phenytoin, piroxicam, procainamide, proton pump inhibitors (lansoprazole, omeprazole), spironolactone, statins (pravastatin, simvastatin), sulfonylureas (glyburide), tamoxifen, terbinafine*, taxotere, tiotropium
DLE	etanercept, infliximab, uracil-tegafur, voriconazole
Chilblain LE	infliximab, terbinafine
LE Tumidus	angiotensin-converting enzyme inhibitors, bupropion, antiretroviral therapy, hydrochlorothiazide

*bold text = many reports

Environmental Factors

Earlier studies (data reviewed in (94 ,95)) have indicated that a large majority of SCLE patients are highly sensitive to ultraviolet irradiation. One recent phototesting study demonstrated photosensitivity in 100% of SCLE patients and that the majority of the photosensitivity reactions occurred more than a week after exposure (67). SCLE has also has developed following radiation therapy (96), UVA therapy in combination with oral psoralen (97), and from fluorescent light exposure (98) and photocopier light (99). A single case of SCLE has been associated with pesticide exposure (100). A number of drugs, many of which are photosensitizers, have been implicated in inducing SCLE (Table 30-4). Ro/SS-A autoantibodies have been found in most reported cases of drug-induced SCLE (101). The skin lesions began between 4 and 20 months after the medication was started, and the lesions improved 6 to 12 weeks after the offending agent was withdrawn (101). These drugs include spironolactone (102), angiotensin-converting enzyme inhibitors (captopril, cilazapril) (103 ,104), calcium-channel blockers (verapamil, diltiazem, nifedipine) (105), hydrochlorothiazide (106), interferon-α and -ß (101 ,107), procainamide (108), beta blockers (acebutolol (109) and oxprenolol) (110), d-penicillamine (94), sulfonylureas (94), terbinafine (111 ,112), griseofulvin (113), piroxicam (114), naproxen (115), phenytoin (116), carbamazepine, (117), tamoxifen (118), docetaxel (119), pravastatin and simvastatin (101), leflunomide (120 ,121 ,122), etanercept (123) and PUVA (97). One patient developed annular SCLE after taking the combination of cinnarizine and thiethylperazine (124). The implication that the antihistamine cinnarizine might induce SCLE is of additional interest in light of the recent report of antihistamine-induced SCLE-like dermatitis (125). One of the authors (DPM) has witnessed a patient who developed SCLE shortly after

starting the gastric acid/proton-pump inhibitor omeprazole that resolved after discontinuing this medicine. The patient noted a recurrence of her lesions 1 to 2 years later after starting the proton-pump inhibitor lansoprazole (unpublished observation). The other author (RDS) has observed ranitidine associated SCLE and is also aware of a case of omeprazole (Prilosec, Astra Merck)-induced annular SCLE in a male (A personal communication, Dr. Mark DeMay, Sioux City, Iowa; April, 2000). Inhalation of the bronchodilator tiotropium has also been demonstrated to induce SCLE in a patient with Ro/SS-A autoantibodies (126).

It is difficult to envision a mechanism by which so many different classes of drugs might trigger the appearance of SCLE lesions. However, it is interesting that the majority of drugs that have been observed to be capable of triggering SCLE skin lesions are also known to be capable of triggering photosensitive skin reactions in individuals who have no evidence of LE autoimmunity. It is tempting to speculate that drug-induced SCLE lesions might result when an individual who is immunogenetically predisposed to SCLE develops what might otherwise be viewed as an innocent drug-induced photoallergic or phototoxic reaction that results in the induction of a secondary SCLE skin reaction via the Köebner or isomorphic response (i.e., the well-documented cutaneous phenomena of LE specific skin inflammation being triggered by different types of nonspecific skin injury).

Smoking may be a risk factor for contributing to the development of SCLE and DLE (89 ,127).

Chronic Cutaneous Lupus Erythematosus

No reliable population-based data exist pertaining to the true prevalence or incidence of classical DLE (the most common form of CCLE) presenting in the absence of systemic LE activity. Such patients tend to be underrepresented in studies reported by rheumatologists and internists and over represented by those reported by dermatologists. Additionally, the epidemiologic data that is available pertaining to DLE is hard to interpret. Studies prior to 1970 did not stratify patients according to the American Rheumatism Association's (now known as the American College of Rheumatology ACR) classification criteria for SLE. Additionally, these workers did not have access to the various laboratory parameters that gauge the presence and degree of activity of systemic autoimmunity. Also, prior to 1979, investigators did not distinguish between DLE and SCLE, usually lumping these two varieties of cutaneous LE together under the generic designations of “DLE” or “disseminated DLE.” Thus, it can be difficult to gain an accurate view of the prevalence of or incidence of DLE from the data presented in studies prior to that time.

Demographics

Based upon data from the available studies, the following points can be made concerning the epidemiology of DLE, as distinguished from SCLE. DLE skin lesions are present in 15% to 30% of variously selected study populations of SLE (59). Approximately 5% to 10% of SLE populations have DLE skin lesions as their presenting disease manifestation (24). It has been suggested that for every patient affected primarily with DLE, seven will be affected primarily by SLE (128). The most common age of onset of DLE is between 20 and 40 years in both males and females (129). DLE lesions, however, can appear in infancy as well as the elderly. The female:male ratio for DLE has been reported to be between 3:2 to 3:1 (130 ,131), which is much lower than that of SLE. In a review of 27 children with DLE, the ratio of females to males was 5:1 (132). Another study of 16 cases of childhood DLE from Tunisia found and equal sex ratio (133). DLE patients in any race can be affected and most of the classical studies have found DLE to be more frequent in whites (134). Some earlier studies suggested that African Americans were relatively protected from DLE; however, Hochberg et al. have presented data that argue that DLE might actually be more prevalent in African Americans (62). There are few reports in the medical literature of children with chilblain LE, LE tumidus, and LE profundus.

Genetic Associations

Knop et al. found significant increases of HLA-B7, B8, Cw7, DR2, DR3, DQW1, and a significant decrease in HLA-A2 in a large group of German DLE patients (135). The combinations of HLA-Cw7, DR3, DQW1, and HLA-B7, Cw7, DR3 conferred the maximum relative risk (7.4) for DLE. Partial C2 and C4 complement deficiencies have been reported in chronic cutaneous LE including DLE and LE panniculitis (89 ,136 ,137 ,138).

Environmental Factors

Skin lesions are precipitated or aggravated by ultraviolet irradiation, especially UVB, in approximately half of patients with DLE. A phototesting study found 41 of 46 (89%) of DLE patients to develop an abnormal reaction to UV light and visible light exposure (67). The skin lesions often took 1 to 4 weeks after irradiation to develop. The validity of the irradiation protocol and the significance of these finding might have been better appreciated if a control group had been included. However, histopathology of the photo-provoked skin lesions did show characteristic findings of LE (including vacuolar degeneration and necrotic basal cell keratinocytes).

Several reports have suggested that smoking may predispose patients to develop DLE (89 ,139 ,140 ,141 ,142). We have observed several patients whose previously refractory DLE lesions improved spontaneously in a dramatic fashion following the cessation of cigarette smoking. It is possible that smoking may predispose patients to develop other forms of chronic cutaneous LE, and/or make them more resistant to antimalarial treatment. This might be ascertained if authors of future reports include the smoking history of their patients.

Nonspecific injury to the skin can precipitate the development of DLE activity within the areas of trauma (i.e., the Köebner/isomorphic phenomenon) (143 ,144 ,145).

In contrast to many drug-induced SCLE reports, there are only a few drug-induced chronic cutaneous LE reports in the literature (Table 30-4). There has been one case report of DLE-like lesions developing after a rheumatoid arthritis (RA) patient was treated with infliximab (146) and three cases apparently induced by etanercept (123 ,147). A patient developed DLE-like lesions on her neck after being treated with the azole antifungal drug voriconazole (148) and another on her cheek after receiving uracil and tegafur for lung cancer (149). Infliximab appeared to induce chilblain LE in a RA patient (150). Terbinafine induced both SCLE and chilblain LE in one patient (151). There has been one case report of possible hydrochlorothiazide-induced (152), and angiotensin-converting enzyme inhibitor–induced tumid LE (153). There has also been one case report of lupus erythematous tumidus being worsened by bupropion therapy for smoking cessation. (154). This later report is disconcerting as bupropion is sometimes prescribed to help cutaneous LE smokers quit so that they might become more antimalarial responsive. LE tumidus has also occurred following antiretroviral therapy in an HIV patient (155).

Cutaneous Manifestations

Acute Cutaneous Lupus Erythematosus

ACLE can present in either a localized or generalized distribution. Localized ACLE (the classic “butterfly rash” or “malar rash” of SLE) is the most common pattern (Fig. 30-1). Localized ACLE typically is characterized by confluent, symmetrical erythema and edema centered over the malar eminences (unilateral involvement with ACLE has been described (30)). Inflammation extending over the bridge of the nose completes the body of the classic butterfly. However, the nasolabial folds typically are spared. The forehead and V area of the neck can be similarly involved. Facial swelling may be severe in some patients with ACLE (156). Occasionally, ACLE begins on the face as small, discrete macular and/or papular lesions that later become confluent and hyperkeratotic (157) (Fig. 30-2).

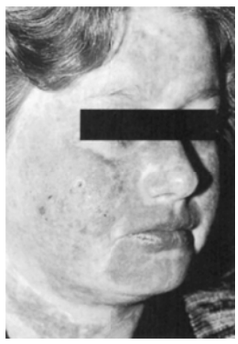

Figure 30-1. (See color plate.) Localized acute cutaneous lupus erythematosus (ACLE). ACLE confined to the face and neck is classified under the Gilliam scheme as localized ACLE. The most typical presentation, as seen in this case, is that of a confluent erythema that can be associated with some degree of induration because of edema formation. As the inflammation subsides, postinflammatory hyper- or hypopigmentation can remain; however, atrophic dermal scarring is not seen.

Generalized ACLE is less common than DLE. It presents as a more widespread morbilliform or exanthematous eruption (Fig. 30-3). This presentation has been referred to in the past as the “maculopapular rash” or “photosensitive lupus dermatitis.” Some patients experience an extremely acute form of ACLE that can simulate toxic epidermal necrolysis. This form of bullous LE results from dissolution of the epidermal basal-cell layer as a result of intense lichenoid inflammation (158) (Fig. 30-4). Su and Alegre present a framework for considering how this form of bullous LE skin change relates to the other clinical patterns of bullous cutaneous injury that can be seen in LE patients (159).
ACLE is very photosensitive and can be quite transient, lasting only several days or weeks. Occasionally, lesions will wax and wane over a period of several hours and some patients will experience more prolonged disease activity. Postinflammatory pigmentary change is most prominent in LE patients with darkly pigmented skin (Fig. 30-5). ACLE lesions do not result in scarring. Patients with localized ACLE occasionally will have SCLE lesions elsewhere on their body, however, the simultaneous occurrence of ACLE and active DLE is very unusual.

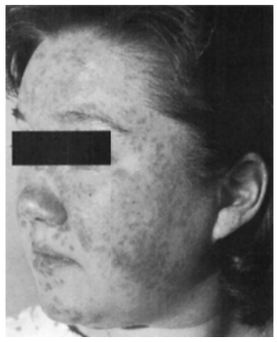

Figure 30-2. (See color plate.) Macular/papular acute cutaneous lupus erythematosus (ACLE). ACLE less commonly presents as a macular and/or papular erythema, as demonstrated in this young woman who ultimately died from systemic lupus erythematosus.

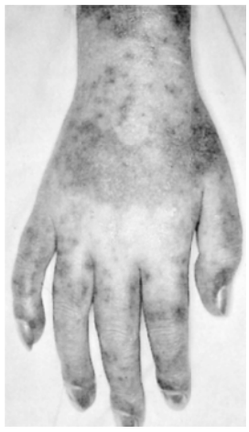

Figure 30-3. Generalized acute cutaneous lupus erythematosus (ACLE). ACLE that presents both above and below the neck is classified as generalized ACLE. Note the macular erythema over the extensor aspect of the wrists that becomes confluent over the dorsal aspect of the hand and interphalangeal areas.

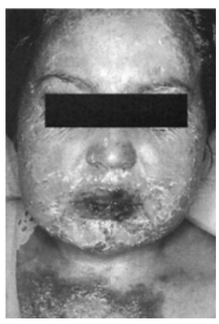

Figure 30-4. Acute cutaneous lupus erythematosus (ACLE) simulating the appearance of toxic epidermal necrolysis. Note the hemorrhagic crusting of the lips and the erosions over the V-area of the neck and upper chest. These skin changes developed during an acute flare of this patient's systemic lupus erythematosus that followed her decision to abruptly discontinue all medications. Therefore, there was no possibility that her toxic epidermal necrolysis-like skin changes resulted from a drug hypersensitivity reaction.

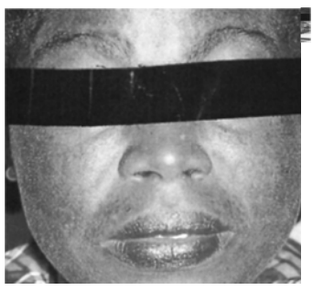

Figure 30-5. Hyperpigmented acute cutaneous lupus erythematosus (ACLE). Hyperpigmentation often can soon follow the onset of ACLE inflammation in darkly pigmented individuals. This rather stoic patient's only presenting complaint was darkening of the skin of her face. However, her evaluation revealed the presence of active systemic lupus erythematosus, including renal involvement. Soon after starting prednisone and hydroxychloroquine, she developed an acute abdominal crisis, resulting in an unrevealing exploratory laparotomy. The source of her abdominal pain subsequently was realized to have been antimalarial-induced hepatotoxicity in the setting of clinically silent porphyria cutanea tarda.

Subacute Cutaneous Lupus Erythematosus

SCLE was first discussed as a distinct disease entity by Gilliam in 1977 (14) and expanded further in 1981 (29). SCLE skin lesions have been referred to in the past under a number of other designations including symmetric erythema centrifugum, disseminated DLE, autoimmune annular erythema, subacute-disseminated LE, superficial-disseminated LE, psoriasiform LE, pityriasiform LE, and maculopapular-photosensitive LE (original data reviewed in (33)). The entity “LE gyratum repens” now is also considered to be synonymous with annular SCLE (159). The original cohort of SCLE patients presented in 1979 (33) and the others that have followed indicate that SCLE is a distinct subset of cutaneous LE having characteristic clinical, serologic, and genetic features (76).

SCLE consists of nonscarring papulosquamous or annular skin lesions having an LE-specific histopathology (161) that occurs in a characteristic photodistribution (33). Approximately 85% of all SCLE patients report photosensitivity (162), although some ethnic groups report this less frequently. While circulating autoantibodies to the Ro/SS-A ribonucleoprotein particle supports this diagnosis (35), their presence is not required to make a diagnosis of SCLE according to its original definition (33).

SCLE lesions present initially as erythematous macules or papules that evolve into scaly, papulosquamous or annular/polycyclic plaques (Figs. 30-6 and 30-7). Approximately 50% of patients have predominately papulosquamous- or psoriasis-form lesions (Fig. 30-6), whereas the other half

have the annular/polycyclic form (Fig. 30-7). A few patients may develop both types of lesions. Some workers have observed a predominance of papulosquamous lesions (77), whereas others have noted an abundance of the annular/polycyclic form (163). SCLE lesions characteristically occur in sun-exposed areas (i.e., upper back, shoulders, extensor aspects of the arms, V area of the neck, and less commonly, on the face) (Figs. 30-8 and 30-9).

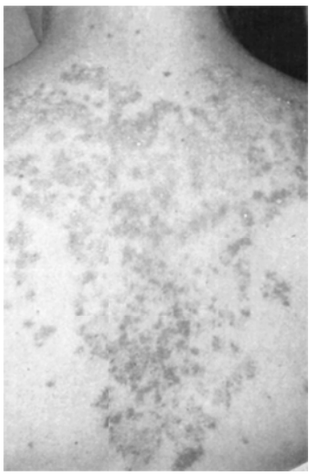

Figure 30-6. Papulosquamous subacute cutaneous lupus erythematosus on the mid- and upper back of a patient with no extracutaneous manifestations of systemic lupus erythematosus. Note the superficial scale and tendency for the individual lesions to merge into a retiform pattern.

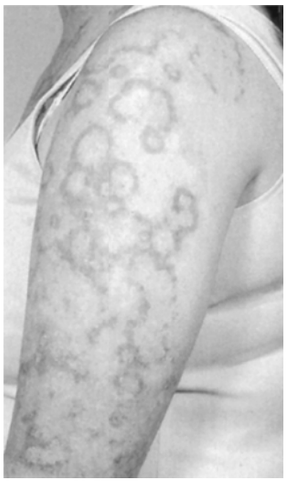

Figure 30-7. (See color plate.) Annular subacute cutaneous lupus erythematosus. Note the polycyclic array resulting from the confluence of the individual annular lesions. This young woman ultimately died from complications of chronic active hepatitis.

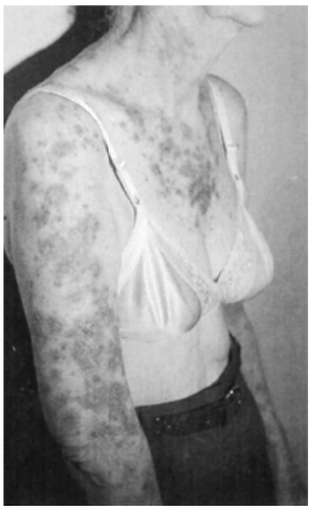

Figure 30-8. (See color plate.) Characteristic photodistribution of subacute cutaneous lupus erythematosus (SCLE). Curiously, the face of this patient with hydrochlorothiazide-induced SCLE was not affected. In the experience of one of the authors (RDS), sparing of the central face in patients with SCLE has been the rule rather than the exception.

Infrequently, SCLE lesions present initially looking like erythema multiforme (Fig. 30-10) (164). Occasionally, arcuate lesions are seen resembling erythema annulare centrifugum (Fig. 30-11). Such cases can simulate the appearance of

Rowell syndrome (erythema multiforme–like lesions occurring in SLE patients in the presence of La/SS-B autoantibodies (22). As a result of hyperacute basal-cell-layer injury, the active edge of annular SCLE lesions on rare occasion undergoes a vesicular change that breaks down to produce a striking crusted appearance (Fig. 30-12) (165). On at least one occasion, such lesions have progressed to mimic toxic epidermal necrolysis (166). One SCLE patient was reported to initially present with exfoliative erythroderma (167), whereas another presented with a curious acral distribution of annular lesions (168). Pityriasiform (94) (Fig. 30-13), poikilodermatous (169), and exanthematous (164) variants of SCLE have been mentioned anecdotally on rare occasion.

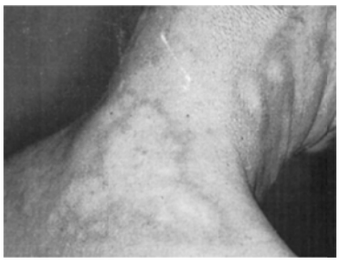

Figure 30-9. Annular subacute cutaneous lupus erythematosus (SCLE) with central hypopigmentation. As opposed to other types of annular skin lesions, the central portions of annular SCLE lesions characteristically display hypopigmentation in areas where the inflammation has subsided, reflecting the pigment-compartment injury pattern commonly seen on biopsy specimens. Although not visible in this photograph, telangiectasia also frequently can be observed in the inactive central areas of annular SCLE lesions.

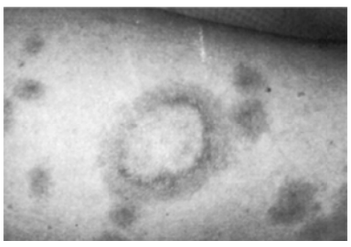

Figure 30-10. Annular subacute cutaneous lupus erythematosus (SCLE) presenting with an appearance highly similar to that of erythema multiforme. Note the targetoid appearance of the small primary lesions surrounding the more established annular SCLE lesion in the center.

Lesions typically heal without scarring but can sometimes leave long-lasting or permanent vitiligo like leukoderma and telangiectasias (Figs. 30-9 and 30-14). In one case, annular SCLE lesions were observed over time to progress to plaques of morphea (170).

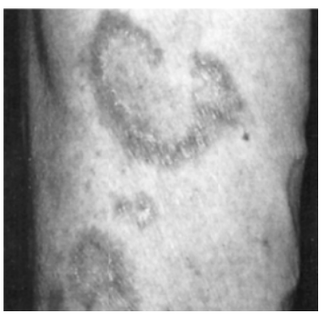

Figure 30-11. An arcuate lesion of biopsy-confirmed subacute cutaneous lupus erythematosus (SCLE) simulating the appearance of erythema annulare centrifugum. Although the fine scale at the trailing edge of the actively inflamed border is characteristic of erythema annulare centrifugum, it also can be seen in arcuate and annular lesions of SCLE.

Patients with SCLE lesions also may develop ACLE (Fig. 30-15) or classical DLE (Fig. 30-16). Localized facial ACLE lesions have been seen in 20% of the SCLE patients examined by one of the authors (RDS). Others have reported ACLE lesions in 7% to 100% of SCLE patients (163). Figure 30-17 illustrates the patterns of overlap that can occur between the three forms of LE-specific skin disease. ACLE skin lesions tend to be more transient than SCLE and heal with less pigmentary change. They tend to be more edematous and less scaly than SCLE lesions. ACLE more commonly affects the malar areas of the face. The experience of one of the authors (RDS) and others (95) suggests that SCLE affects the central face much less often.

Various reports have noted that 0% to 29% of SCLE patients manifest DLE lesions sometime during their clinical course (171). Nineteen percent of the original cohort of SCLE patients had classical DLE lesions (33). DLE lesions can predate the onset of SCLE lesions. DLE lesions in patients with SCLE usually are confined to the head and neck but may be more widely distributed (Fig. 30-16). DLE lesions in this setting generally are associated with a greater degree of hyper- and hypopigmentation, may display atrophic dermal scarring, and are more characteristically associated with follicular plugging and adherent scale. DLE lesions are characteristically indurated whereas SCLE lesions are not (95). Calcifying lupus panniculitis has occurred in a patient with SCLE (172).

A number of other skin lesions that are not histopathologically specific for LE can be found in SCLE patients (data reviewed in (94)). The most frequently encountered of these include alopecia, painless mucous-membrane lesions, livedo reticularis, periungual telangiectasias, vasculitis, and Raynaud phenomenon (173). Cutaneous sclerosis (174) and calcinosis (175) may be seen rarely in SCLE patients. Multiple HPV-11-associated squamous-cell carcinomas of the skin were noted in one SCLE patient (176). Polymorphic light eruption, DLE, and SCLE may sometimes occur together and may share a common genetic predisposition (177).

Chronic Cutaneous Lupus Erythematosus

Classical DLE

The most common form of chronic cutaneous LE is classical DLE (hereafter, the unqualified term “DLE” will be used to refer to this form of DLE lesion). DLE lesions of this type begin as flat or slightly elevated, well-demarcated, red-purple macules or papules with a scaly surface. Early DLE lesions most commonly evolve into larger, coin-shaped (i.e., “discoid”) erythematous plaques covered by a prominent, adherent scale that extends into dilated hair follicles (Fig. 30-18). These discoid plaques can enlarge and merge to form even larger, confluent, disfiguring plaques (Fig. 30-19).

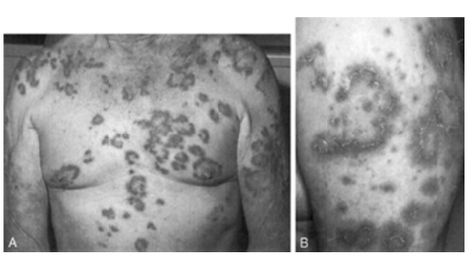

Figure 30-12. (See color plate.) Vesicobullous annular subacute cutaneous lupus erythematosus **(**SCLE). On rare occasions, the active advancing border of annular SCLE lesions undergoes a vesiculobullous change because of extensive liquefactive degeneration of the epidermal basal cell layer **(A)**. As these vesicular elements break down, crusts can form (more evident on the closer view shown in **(B)**. At times, such eroded areas can become secondarily infected with gram-positive bacteria. Intact pustules occasionally can be seen intermixed with vesicles at the border of annular SCLE lesions such as this.

Involvement of hair follicles is a prominent clinical feature of DLE lesions. Scales accumulate in dilated follicular openings, which soon become devoid of hair (Fig. 30-20). When the adherent scale is peeled back from more advanced lesions, follicle-sized keratotic spikes similar in appearance to carpet tacks can be seen to project from the undersurface of the scale (i.e., the carpet-tack sign) (Fig. 30-20).

Erythema and hyperpigmentation are present during the initial phase of DLE lesions. The lesions slowly expand with active inflammation and hyperpigmentation at the periphery leaving depressed central scarring, telangiectasia, and depigmentation. The central atrophic scarring is highly characteristic (Figs. 30-18 and 30-19). Pigment changes alone do not constitute scarring when referring to cutaneous LE lesions. In some ethnic backgrounds such as Asian Indians, the DLE histopathology can present clinically as isolated areas of macular hyperpigmentation (178).

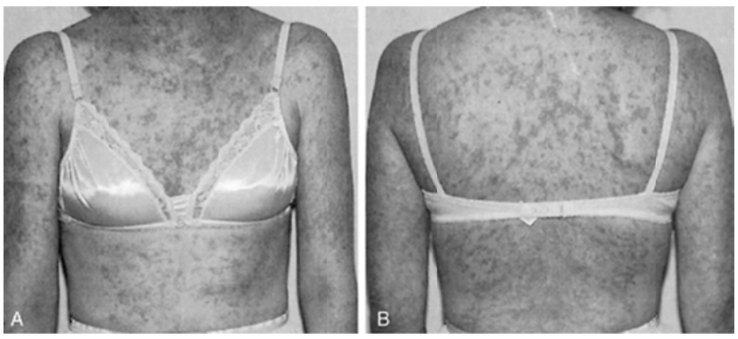

Figure 30-13. (See color plate.) Pityriasiform subacute lupus erythematosus. This anti-Ro(SS-A) antibody-positive patient had a generalized eruption of local, minimally scaling, nonscarring lesions demonstrating lupus erythematosus-specific skin disease on biopsy.

Typical DLE lesions occur most often on the face, scalp, ears, V-area of the neck, and extensor aspects of the arms. Any area of the face can be affected, including the nose (Figs. 30-18 an 30-19), lips (Figs. 30-19 and 30-22), eyebrows (Fig. 30-19), and eyelids (Fig. 30-21). There have been multiple cases of DLE occurring in and around the eyes, which are often initially misdiagnosed (179 ,180 ,181).

DLE can masquerade as blepharitis and chronic blepharoconjunctivitis (182) and has presented as periorbital edema and erythema (183), madarosis (loss of eyelashes) (184), and cicatrizing conjunctivitis (185).

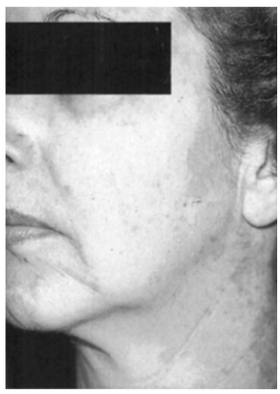

Figure 30-14. Subacute lupus erythematosus of the neck and face resolving with permanent vitiligo-like depigmentation.

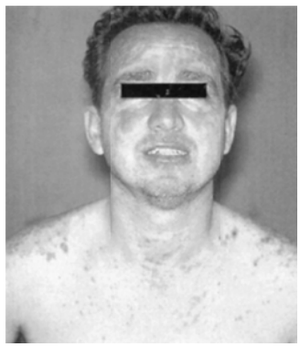

Figure 30-15. (See color plate.) Facial acute cutaneous lupus erythematosus (ACLE) occurring in a patient with subacute cutaneous lupus erythematosus (SCLE). This man had experienced uncomplicated papulosquamous SCLE for several years before developing confluent malar erythema at a point in his illness where he experienced clear evidence of extracutaneous SLE activity, including nephritis. It has been the experience of one of the authors (RDS) that the superimposition of ACLE on SCLE is a worrisome prognostic sign regarding the risk for developing extracutaneous manifestations of SLE.

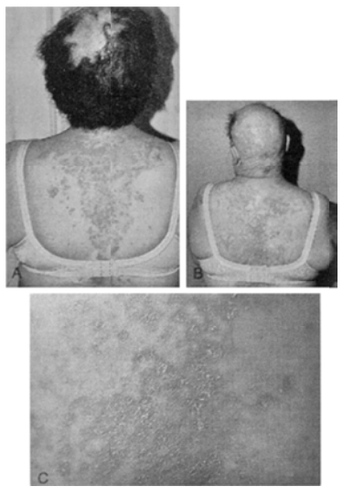

Figure 30-16. (See color plate.) Coexistence of subacute cutaneous lupus erythematosus (SCLE) and discoid lupus erythematosus (DLE). Approximately 20% of patients with SCLE will develop classical scarring DLE skin lesions at some point. This patient presented initially with DLE of her scalp and, several years later, developed typical nonscarring papulosquamous SCLE lesions on her back **(A)**. Her cutaneous disease was extremely resistant to therapy, and over the ensuing 5 years, she experienced progression of her DLE scalp involvement, resulting in extensive scarring alopecia **(B)**. The SLCE activity on her back smoldered throughout this period, resulting in the development of superficial atrophy **(C)**. This is one of two such cases observed by one of the authors (RDS) in which dermal atrophy developed within long-smoldering papulosquamous SCLE lesions. Such a progression has not been observed in annular SCLE lesions.

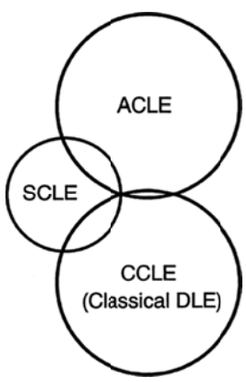

Figure 30-17. Potential for overlap between the three major forms of lupus erythematosus-specific skin disease.

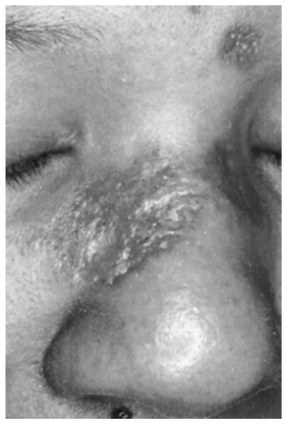

Figure 30-18. Classical discoid lupus erythematosus lesion. Note the erythema (indicating disease activity), keratin-plugged follicles, and dermal atrophy. This is a relatively early lesion that lacks the typical adherent scale usually accompanying more established lesions.

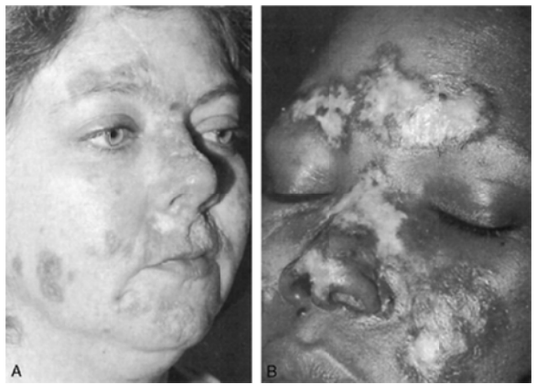

Figure 30-19. More extensive examples of therapeutically refractory facial discoid lupus erythematosus lesions that produce large areas of disfigurement on confluence. The characteristic pattern of hyperpigmentation at the active border and hypopigmentation at the inactive center is especially evident in African American patients **(B)**. Facial involvement of this sort can produce extreme psychosocial disability. The proper application of corrective camouflage cosmetics can be of great psychologic benefit to such patients.

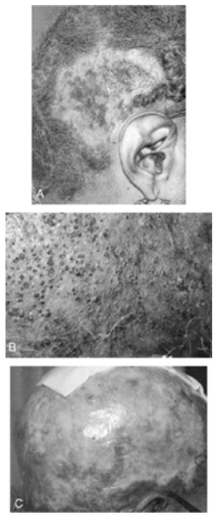

Figure 30-20. Discoid lupus erythematosus (DLE) of the scalp. **A,** A relatively early lesion. At this stage, the alopecia might be reversible to some degree with effective treatment. **B,** Close-up of the pattern of keratin-plugged follicles that can be observed in such lesions. **C,** A patient in whom the DLE disease process has progressed to the point of total, irreversible scarring alopecia. The areas of crusting represent a superimposed secondary bacterial infection.

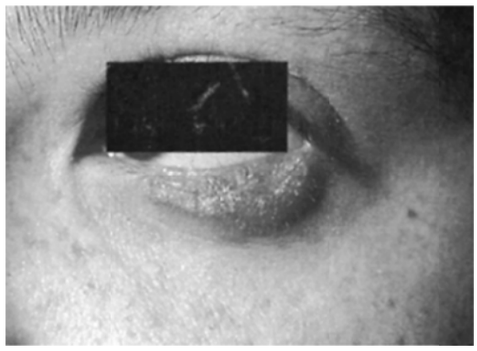

Figure 30-21. Discoid lupus erythematosus of the lower eyelid resulting in loss of the eyelashes.

A symmetrical, butterfly shaped DLE plaque occasionally will be found over the malar areas and bridge of the nose. Such DLE lesions are not to be confused with the more transient, edematous-erythema reactions that occur over the same distribution in ACLE lesions. As with ACLE, DLE usually spares the nasolabial folds. Perioral DLE lesions can occur and often resolve with a striking acneiform pattern of pitted scarring (Fig. 30-23). DLE often involves the external ear, including the outer portion of the external auditory canal. The earliest lesions in this area present as dilated, hyperpigmented follicles (Fig. 30-24).
Scalp involvement occurs in 60% of DLE patients, and is the only area involved in approximately 10% (186) (Fig. 30-20). Irreversible scarring alopecia from permanent follicular destruction occurred in 34% of patients in one recent series (187). The irreversible, scarring alopecia that occurs as the result of persistent DLE activity in a localized area differs from the more widespread, reversible, nonscarring alopecia that SLE patients often develop during periods of disease activity.

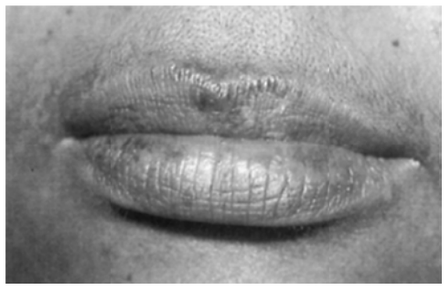

Figure 30-22. Discoid lupus erythematosus of the vermillion border of the upper lip. Although the lower lip is more exposed to sunlight, the upper lip for some reason more often is affected with lupus erythematosus-specific skin disease.

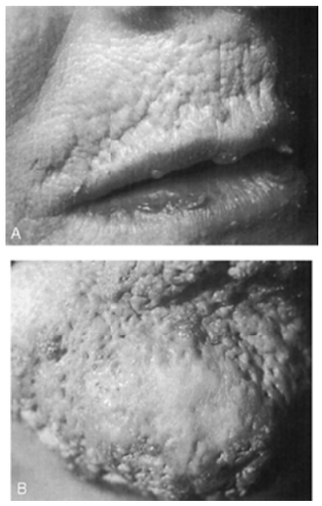

Figure 30-23. (See color plate.) **A,** Perioral pitted scarring resulting from prior discoid lupus erythematosus involvement. **B,** This pattern of scarring over the chin of a different patient was improved by dermabrasion. Note that smoother surface and appearance of the central part of the photograph that was treated as a test area. This patient was on antimalarial treatment at the time of the dermabrasion to minimize the chance for Köebnerization.

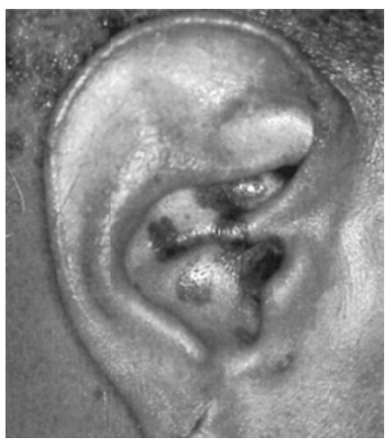

Figure 30-24. Discoid lupus erythematosus (DLE) involvement of the ear. Note the patulous, keratin-plugged follicles within areas of hyperpigmentation in the conchae. This pattern of clinical involvement is highly specific for DLE.

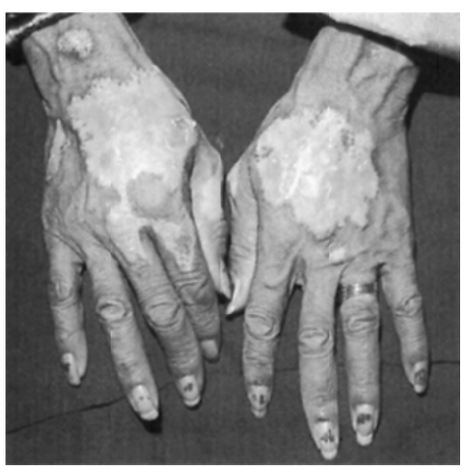

Figure 30-25. Discoid lupus erythematosus (DLE) lesions of the dorsal hands indicating generalized DLE. There is a somewhat higher risk of developing systemic lupus erythematosus in patients with generalized DLE compared to those patients with localized DLE (i.e., head and neck involvement only).

DLE lesions that occur only on the head or neck are referred to as “localized DLE.” DLE lesions occurring both above and below the neck are referred to as “generalized DLE.” The presence of DLE lesions below the neck only is extremely uncommon. DLE lesions occurring below the neck are most commonly found on the extensor aspects of the arms, forearms, and hands (Fig. 30-25), although DLE lesions can occur at virtually any site on the body, including completely sun-protected sites such as the perineal area (188) (Fig. 30-26). Unusual locations can reflect the fact that DLE, as well as other forms of LE skin disease activity, can follow in the wake of any form of trauma to the skin (i.e., the Köebner or isomorphic response) (Fig. 30-27). Painful, erosive palmar-plantar DLE involvement can predominate in some cases producing significant disability and presenting an especially difficult management problem (189) (Fig. 30-28). On occasion, DLE lesions can remain as small, discrete, follicule-based papules having a diameter of less than 1 centimeter or less. Such “follicular DLE” lesions often are seen around the elbow (Fig. 30-29), but can occur elsewhere as well.

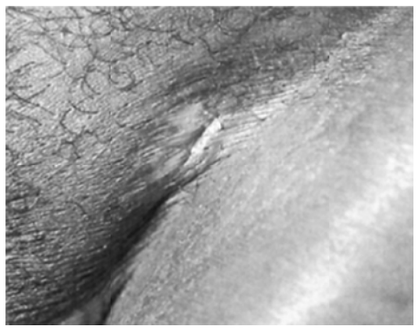

Figure 30-26. Biopsy-confirmed discoid lupus erythematosus lesion of the inguinal fold.

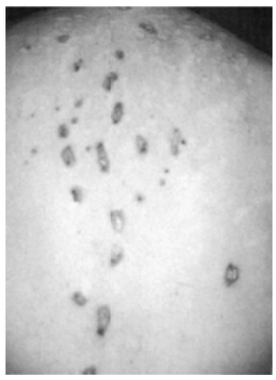

Figure 30-27. Köebnerized discoid lupus erythematosus (DLE). DLE lesions occurring in areas of neurotic excoriation. Note the linear shape of some of these lesions.

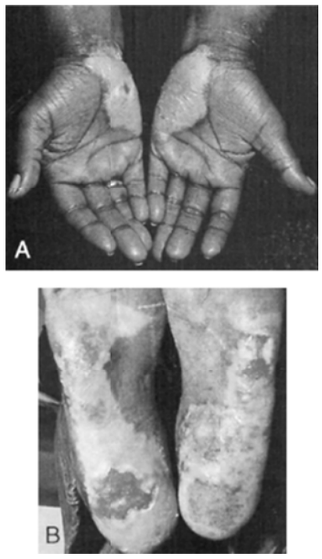

Figure 30-28. (See color plate.) Palmar-plantar discoid lupus erythematosus (DLE). DLE involvement of the palms of the hands **(A)** and soles of the feet **(B)** can present a very difficult management problem. Erosions that can develop in such areas can cause extreme pain and result in occupational disability **(B)**.

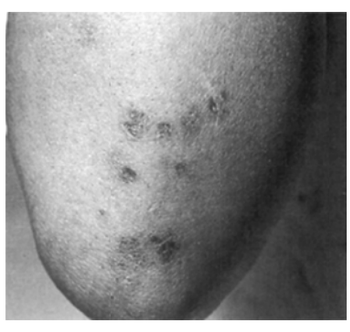

Figure 30-29. Discoid lupus erythematosus presenting as discrete follicular lesions around the elbow, the most common site for this pattern of involvement.

Linear DLE lesions rarely have been reported and may be more common in children (190 ,191), usually following the lines of Blaschko (192).

DLE lesions can be potentiated by sunlight exposure however, this occurs less frequently than with ACLE and SCLE. Patients often are unaware that UV irradiation exacerbates or precipitates their skin disease, perhaps from the fact that there may be a 1- to 4-week lag time from the time of light exposure until the skin lesions develop (38) (67). It is predominately UVB that aggravates DLE lesions, although increasing evidence suggests that the longer UVA wavelengths also can be deleterious in some LE patients (193). However, in as many as 50% of patients, sun exposure does not appear to be related to the cause of their DLE lesions. DLE lesions in the hair-bearing scalp, external auditory canal, or perineal areas are examples where this form of cutaneous LE is not related to light exposure.

DLE also can localize to the nail unit (194). Focal lesions of DLE occurring over the nailfold can produce nail-plate dystrophy (Fig. 30-30). The nail unit can be impacted by other forms of cutaneous LE as well as SLE-producing nail-fold erythema and telangiectasia, red lunulae, clubbing, paronychia, pitting, leukonychia striata, and onycholysis (primary data reviewed in (3)).

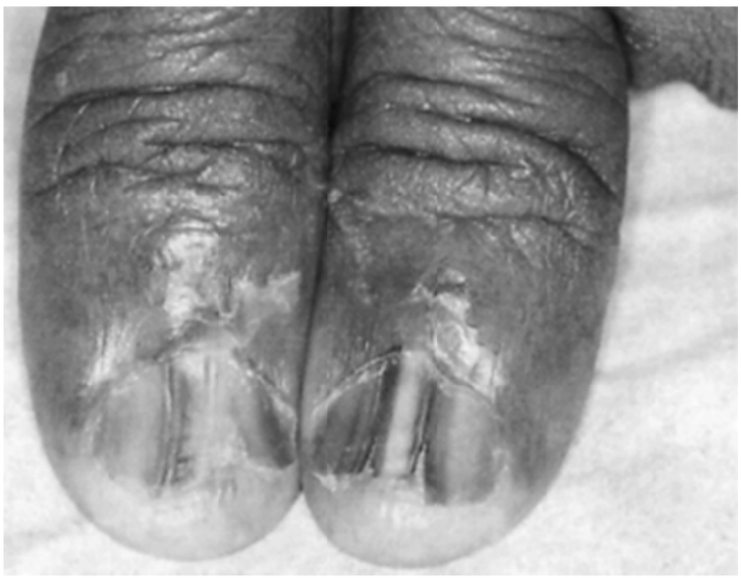

Figure 30-30. Symmetric discoid lupus erythematosus lesions of the nailfold producing medial nailplate dystrophy. None of the other fingernails were affected.

Hypertrophic Discoid Lupus Erythematosus

Hypertrophic DLE (i.e., hyperkeratotic DLE, verrucous DLE) is a rare variant of chronic cutaneous LE in which the hyperkeratosis that normally is present in classical DLE lesions is greatly exaggerated (Fig. 30-31). These lesions can occur at any site that classical DLE lesions develop, although the extensor aspects of the extremities, the upper back, and the face are the areas most frequently affected. The histopathology sometimes reveals features of squamous-cell carcinoma or keratoacanthoma, which can lead clinicians to make the wrong diagnosis (195 ,196). However, it is important to realize that squamous-cell carcinoma sometimes can develop in DLE lesions and can metastasize (197 ,198). When multiple widespread lesions with histopathologic features of squamous-cell carcinoma or keratoacanthoma are present, one should consider a diagnosis of hypertrophic LE. Overlapping features of hypertrophic LE and lichen planus also have been described (199). Fortunately, patients with hypertrophic DLE lesions often have classical DLE lesions elsewhere on their body that helps clinicians make the correct diagnosis.

The entity “LE hypertrophicus et profundus,” that was described originally by Bechet in 1942 (200) appears to represent a very rare form of hypertrophic DLE affecting the face associated with the additional features of violaceous (or dull red), indurated, rolled borders, and striking central, crateriform atrophy. The name of this entity is somewhat ambiguous because its pathology does not include a significant degree of LE panniculitis.

LE Profundus/LE Panniculitis

Historically referred to as Kaposi-Irgang disease (201), this rare form of chronic cutaneous LE is characterized by inflammatory lesions in the lower dermis and subcutaneous tissue. Approximately 70% of the patients with this type of chronic cutaneous LE also have typical DLE lesions often overlying the panniculitis lesions (202 ,203). The term “LE profundus” has been used by some (including one of the authors [RDS]) to arbitrarily designate those patients who have both LE panniculitis and DLE lesions. However, traditionalists might considered this to be an artificial distinction.

The lesions present as deep, firm, 1- to 3-cm diameter nodules often with normal-appearing overlying skin (204). The skin ultimately becomes attached to the firm, subcutaneous nodular lesions and is drawn inward to produce deep, saucerized depressions as the lesions mature (Fig. 30-32). The head, proximal upper arms, chest, buttocks, and thighs are the sites of predominant involvement. Confluent involvement

of the face can simulate the appearance of lipoatrophy (Fig. 30-33). LE profundus also can present as periorbital edema (205 ,206).

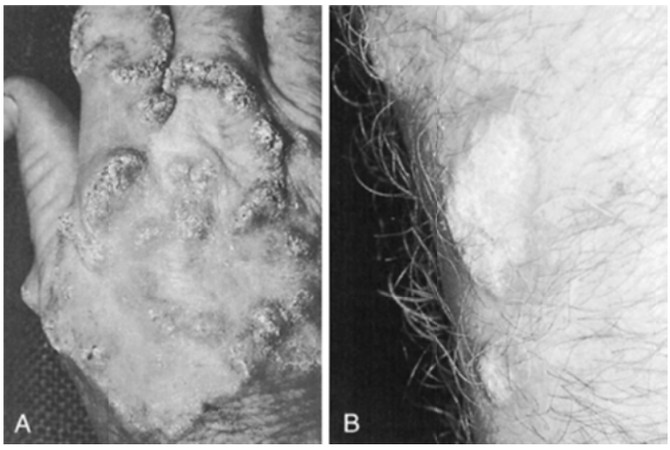

Figure 30-31. Two clinical patterns of hypertrophic (verrucous) discoid lupus erythematosus (DLE). **A,** A pattern where warty hyperkeratosis occurs at the active edge of an active DLE lesion. **B,** A DLE lesion that is entirely covered by a thickened shield of hyperkeratosis, presenting a clinical appearance that could be mistaken for other common dermatoses such as keratoacanthoma, squamous-cell carcinoma, or prurigo nodularis.

Linear involvement of the extremities also has been observed (207). Dystrophic calcification within older lesions of LE profundus is common and at times can be a prominent clinical feature of the disease requiring surgical excision. Additionally, LE panniculitis may produce breast nodules that can mimic carcinoma clinically and radiologically (208). Rarely anetoderma, a peculiar form of skin atrophy that is often associated with antiphospholipid antibodies, and is a LE-nonspecific skin disorder, has been found in patients with LE panniculitis (209). Cases of subcutaneous panniculitis-like T cell lymphoma may mimic LE panniculitis (210 ,211).

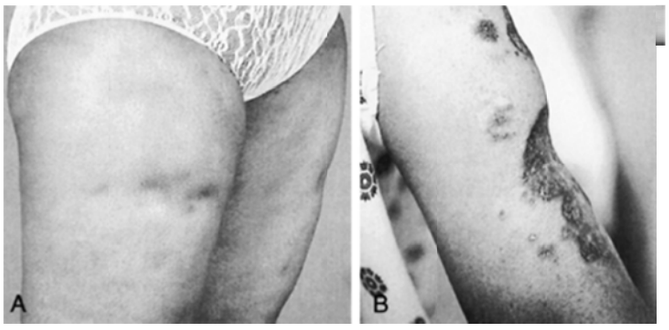

Figure 30-32. Lupus erythematosus (LE) panniculitis/profundus. **A,** An example of LE panniculitis without overlying discoid lupus erythematosus (DLE). The primary lesion is a firm subcutaneous nodule that is associated with saucerized areas of atrophy at the surface of the skin. **B,** The combination of LE panniculitis with overlying DLE involvement, referred to as LE profundus.

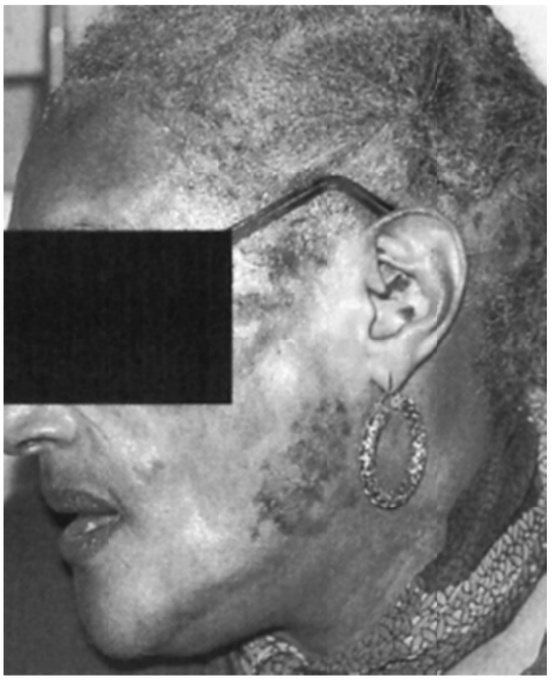

Figure 30-33. Lipoatrophy of the face resulting from lupus erythematosus profundus.

Mucosal DLE

It long has been recognized that mucosal-membrane involvement can occur in chronic cutaneous LE patients (212). Studies by Burge et al. have confirmed that the prevalence of mucous-membrane involvement in chronic, cutaneous LE is about 25% (213). While the oral mucosa is most frequently involved, nasal, conjunctival, and genital mucosal surfaces also can be affected.

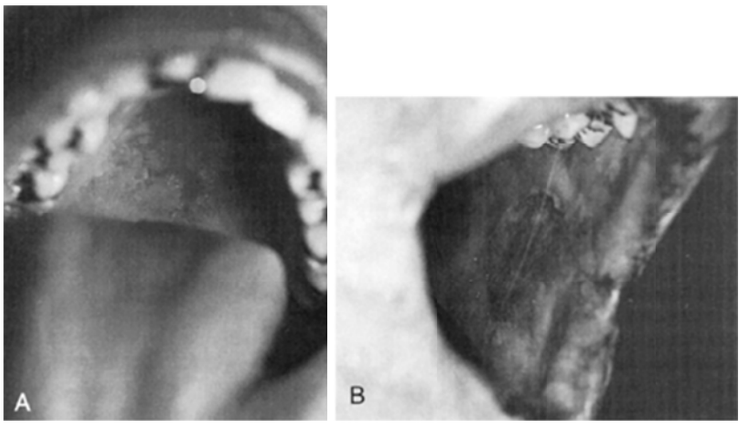

Figure 30-34. (See color plate.) Discoid lupus erythematosus (DLE) of the oral mucosa. **A,** Typical appearance of a lesion on the hard palate. Note the honeycomb appearance. These lesions show a typical lupus erythematosus-specific histopathology and usually are painless. **B,** Margins of a DLE lesion on the buccal mucosa of another patient are demarcated by staining with a toluidine blue mouthwash. This patient ultimately developed a squamous-cell carcinoma within a different area of chronic DLE involvement on his buccal mucosa.

Within the mouth, the buccal mucosal surfaces are most commonly involved, with the palate, alveolar processes, and tongue being sites of less-frequent involvement. Individual lesions begin as painless, erythematous patches later maturing to a chronic plaque that can present an appearance quite similar to that of lichen planus (Fig. 30-34). The chronic buccal-mucosal plaques have a sharply marginated, irregularly scalloped white border with radiating white striae and telangiectasia (213). The surface of these plaques overlying the palatal mucosa often has a well-defined meshwork of raised hyperkeratotic white strands that encircle zones of punctate erythema, which gives a “honeycomb” appearance (213). The center of older lesions can become depressed and occasionally undergoes painful ulceration. Well-defined chronic DLE plaques also can appear on the vermillion border of the lips (Fig. 30-22). At times, DLE involvement of the lips can present as a diffuse cheilitis, especially on the more sun-exposed lower lip. Although lesions can appear on the tongue, this location is quite rare (213).

Chronic oral-mucosal DLE lesions occasionally can degenerate into squamous-cell carcinoma (214), similar to cutaneous DLE lesions. Any area of asymmetrical nodular induration within a mucosal DLE lesion should be carefully evaluated for the possibility of malignant degeneration.

Discrete DLE plaques also can develop on the nasal, conjunctival, and genital mucosa (213). Nasal septum perforation is relatively rare and is more often associated with SLE

than DLE (215). However, nasal-mucosal involvement has been suggested to be relatively common in LE patients (216). Conjunctival DLE lesions begin as small areas of nondescript inflammation most commonly affecting the palpebral conjunctivae or the margin of the eyelid. The lower lid is affected more often than the upper lid. As the early lesions progress, scarring becomes more evident and can produce permanent loss of eyelashes and ectropion (Fig. 30-21). DLE involvement of the eyelid can produce considerable disability (217). It has been suggested that corneal, stromal keratitis also can occur as a result of DLE ocular involvement (218). Although quite rare, anogenital mucosal DLE lesions also have been observed (213).

Chilblains Lupus/Lupus Pernio

Some LE patients develop red-purple patches and plaques on their toes, fingers, and face that are precipitated by cold, damp climates. Such lesions are highly reminiscent of simple chilblains or pernio lesions (219). As these lesions evolve, however, they take on the typical appearance of DLE lesions clinically and histopathologically. The terms “chilblains lupus” and “perniotic LE” have been used to describe such lesions (Fig. 30-35). Unfortunately, the term “lupus pernio” also has been used for such lesions; however, this term is more properly used to designate a form of cutaneous sarcoidosis (220). Chilblains lupus patients often have typical DLE lesions on the face and head (219).

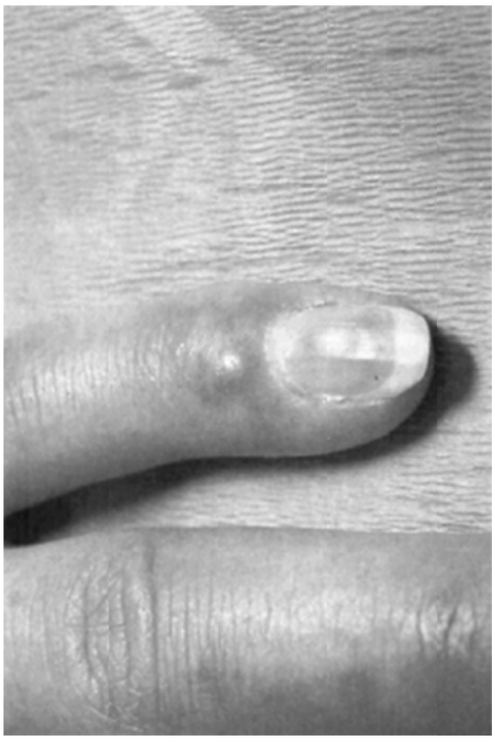

Figure 30-35. Chilblains lupus erythematosus (LE) (perniotic LE). Note the rather nondescript appearance of this early lesion. At this stage, the pathology might be nonspecific; however, with time, such lesions can present a typical discoid lupus erythematosus histopathology.

Lupus Erythematosus Tumidus

The accumulation of excessive dermal mucin early in the course of a cutaneous LE lesion can result in the succulent, edematous, urticaria-appearing plaques of LE tumidus (Fig. 30-36). The characteristic liquefactive degeneration and basement membrane thickening seen microscopically in lesions of SCLE and DLE are usually absent in lupus tumidus lesions (221). The lack of these histologic features, and absence of an associated serologic marker, make it more difficult to diagnose LE tumidus, compared to diagnosing other forms of LE-specific skin disease.

Kuhn et al. have reported a series of 40 LE tumidus patients and have traced the line of thought relating to this clinical entity from its apparent original description by Gougerot and Bournier in France in 1930 (222). Three other smaller patient cohorts of LE tumidus also have appeared recently (222 ,223 ,224). It now is clear that terms such as the “urticarial plaque” form of LE (225), “lupus tumidus” (223 ,226), and “tumid LE” (222) in the modern

English literature have been functionally equivalent to the classical term “LE tumidus” described by Gougerot and Bournier in 1930 and others since. LE tumidus patients accounted for 16% of their total population of cutaneous LE patients examined by Kuhn et al. LE tumidus has accounted for a much lower percentage of the total number of cutaneous LE lesions seen by the workers outside Germany including the United States (204 ,223 ,227 ,228). Additionally, in epidemiologic studies of large series of SLE patients in which cutaneous lesions have been recorded, lesions consistent with LE tumidus are mentioned rarely (4 ,24 ,55 ,229). This plus the dearth of specific published experience on LE tumidus from the United States suggests the possibility that the epidemiology of LE tumidus might differ somewhat between Western Europe and the United States. Alternatively, some of these latter workers might not have recognized LE tumidus as a distinct form of cutaneous LE or have included patients with LE tumidus under other designations such as “urticaria” in the context of SLE.

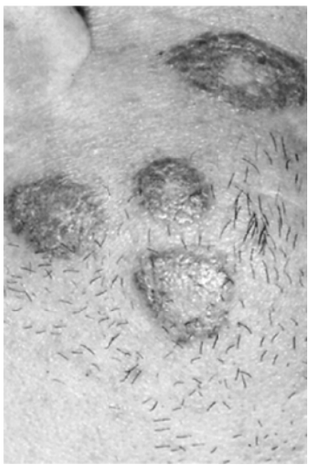

Figure 30-36. Lupus tumidus. This form of chronic cutaneous lupus erythematosus (CCLE), which is very rare in the authors' experience, presents succulent, elevated plaques occurring in a sun-exposed distribution. In this case, which is not typical of all cases of lupus tumidus, there was a pattern of surface-color change reminiscent of Wickham striae. However, the biopsy specimen did not show changes of lichen planus, nor were LE-specific skin disease changes clearly present at the DEJ. The predominant pathologic change was mononuclear cell infiltration around the superficial and deep dermal blood vessels. It is quite likely that lupus tumidus lesions and lesions described by other investigators under different designations such as urticarial plaque lupus erythematosus represent the same disease process (personal communication, Irwin M. Braverman, MD).

Other intriguing aspects of the large LE tumidus case series presented by Kuhn et al. include male predominance, negative lesional direct immunofluorescence, and virtual absence of associated SLE disease activity. However, the other three recently published cases series of LE tumidus, although much smaller in patient number, did not find a male predominance and some of their patients had LE tumidus occurring in the context of discoid LE and systemic LE (224 ,230).

The systematic phototesting studies performed by Kuhn et al. revealed a rather strong presence of UVA in the action spectrum of LE tumidus. This is in agreement with earlier such work (231). The extreme photosensitivity noted in their patients occurred in the virtual absence of epidermal changes and Ro/SS-A antibody production. These findings suggest the possibility that there is a fundamentally different mechanism of photosensitivity in LE tumidus compared to SCLE. However, it is interesting to note that when Lehmann et al. (38) challenged the nonlesional skin of SCLE patients with UVA alone, only dermal changes of LE-specific skin disease were noted.

Table 30-3 presents a comparison of the selected clinical and laboratory features of patients presenting with ACLE, SCLE, and classical DLE skin lesions.

Relationship between Cutaneous and Systemic Disease

Figure 30-37 illustrates the different relationships that exist between the three major forms of LE-specific skin disease and the systemic autoimmune manifestations of LE.

Acute Cutaneous Lupus Erythematosus

Because the “malar rash” or “butterfly rash” is so common in patients with SLE and because such lesions are considered to be a fundamental component of the clinical expression of SLE disease activity, few workers have directly examined the relationship that exists between ACLE and SLE disease activity.

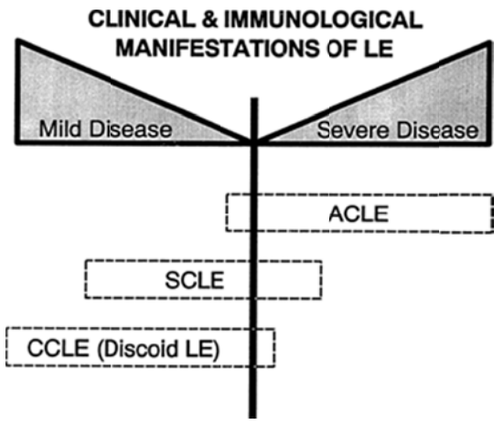

Figure 30-37. Relative risks for systemic lupus erythematosus disease activity that are associated with the three major forms of lupus erythematosus-specific skin disease.

Ropes noted that the “butterfly rash” of SLE often waxed and waned in parallel with underlying SLE disease activity (30). However, Dr. Ropes was unable to demonstrate a positive correlation between skin disease activity and evidence of LE nephritis. Curiously, a significant negative correlation was noted with psychosis and serum protein electrophoresis abnormalities (30). Vlachoyiannopoulos et al. found that flaring of the “rash” of SLE frequently accompanies flares of SLE activity (59). Wysenbeek et al. also have demonstrated that the generalized “nonspecific rash” of SLE (presumably generalized ACLE) correlated positively with lymphadenopathy, antinative DNA antibody, low-complement levels, and higher prednisone doses (63). However, these workers found no such correlation with renal disease, central nervous system (CNS) disease, or SLE disease activity index.

It has always been our impression that either localized or generalized ACLE is associated with a high risk for aggressive SLE activity, including the risk of LE nephritis.

Subacute Cutaneous Lupus Erythematosus

Approximately one half of SCLE patients meet the ACR's revised criteria for the classification of SLE (50) (data reviewed in (94)). However, it has been the experience of most observers that severe SLE (i.e., LE nephritis, CNS disease) develops in only ~10% of SCLE patients (data reviewed in (94 ,232)). While most SCLE patients who develop four or more SLE classification criteria tend to have a relatively mild disease course overall, there are reports of such patients who have had severe and sometimes fatal outcomes, usually from renal and CNS involvement (233).

There is some indication that patients with the papulosquamous variety of SCLE might be at more risk of developing renal involvement (70). Sontheimer et al. identified

five patients in a cohort of 47 individuals with SCLE that had evidence of lupus nephritis (234). All five of these patients had the papulosquamous type of SCLE, leukopenia, high-titer antinuclear antibody (ANA) (>1:640) and anti-double–stranded (ds) DNA antibodies. All five also developed ACLE lesions at some point during their disease course and all had been refractory to antimalarial treatment. Another report noted that SCLE patients with renal disease were more likely to have the papulosquamous type of lesions (235). Men with papulosquamous SCLE may have higher risk of severe SLE (70).

SCLE can overlap with other autoimmune disorders. Some patients with SCLE will later manifest clear evidence of Sjögren syndrome, whereas some patients whose illness begins as Sjögren syndrome will later develop SCLE lesions. Three to 12% of patients who present with SCLE skin lesions will later developed Sjögren syndrome (34). It is interesting to note in this regard that both SCLE and Sjögren syndrome have been associated with circulating Ro/SS-A and La/SS-B autoantibodies and the presence of the HLA-B8, DR3, DRW6, DQ2, and DRW52 extended haplotype.

A number of Japanese patients having Sjögren syndrome and Ro/SS-A and La/SS-B autoantibodies have been reported to have an annular erythema reaction that appears somewhat similar clinically to annular SCLE, although biopsies of the lesions have revealed histopathologic findings somewhat distinct from SCLE (236). While some consider this to be a distinct entity (i.e., annular erythema of Sjögren syndrome), it is possible that genetic differences between occidentals and whites could account for this difference in disease expression. For example, the HLA-DR3 allele is distinctly unusual in the citizens of Japan.

Roughly one third of SCLE patients produce rheumatoid factor (94). It is therefore not surprising that SCLE patients have been noted to subsequently develop RA on occasion (237). Additionally, patients presenting with RA can subsequently developed SCLE lesions. One group reported that of 12 RA patients who produced Ro/SS-A autoantibodies, two had SCLE skin lesions (238). An association between SCLE and antithyroid autoantibodies and Hashimoto thyroiditis also has been reported (239).

There have been case reports of malignancies occurring in patients with SCLE (breast, lung, gastric, uterine, lymphoma, hepatic, and melanoma) (240 ,241 ,242 ,243 ,244). In some of these reports, the SCLE lesions have been suggested to represent a paraneoplastic manifestation of the underlying malignancy. Other disorders that have been anecdotally related to SCLE have been Sweet syndrome (245), porphyria cutanea tarda (246), gluten-sensitive enteropathy (247), X-linked chronic granulomatous disease (248), hereditary angioedema (249), and autoimmune polyglandular syndrome type II (Schmidt syndrome) (250) and Crohn disease (251). The infrequency of these associated conditions mitigates against anything more than a casual association. However, it should be kept in mind that the usual dose of aminoquinoline antimalarial agents used in the management of SCLE (or DLE) can cause significant hepatotoxicity in patients who might have underlying subclinical porphyria cutanea tarda.

Chronic Cutaneous Lupus Erythematosus

The relationship that exists between classical DLE and SLE has been the subject of debate throughout the 20th century. Daniel Wallace has presented a comprehensive overview of this subject, which systematically and thoughtfully addresses the following issues (134).

What is the Risk for Patients Presenting with Classical DLE Subsequently Developing SLE?

Five percent to 10% of patients presenting with classical DLE lesions subsequently will develop unequivocal evidence of the extracutaneous manifestations SLE (134). Considerable effort has been made to identify clinical or laboratory features that might correlate with such a pattern of disease progression. One clinical feature that can be useful in this regard is the extent and distribution of the DLE lesions. Generalized DLE patients (i.e., lesions both above and below the neck) have a higher rate of immunologic abnormalities and risk for progressing to SLE compared to localized DLE patients (228). Additionally, generalized DLE patients are at higher risk for developing the more severe manifestations of SLE (228). In some instances, SLE patients may develop DLE skin lesions late in the course of their disease when their SLE disease activity has fully remitted (252). A 36-month follow-up of 27 children who initially presented with DLE (15 localized and 12 with disseminated disease) revealed that 7 (26%) had developed SLE (132).

Other physical findings that should alert one to the possibility of underlying systemic disease are diffuse, nonscarring alopecia, generalized lymphadenopathy, periungual nailfold telangiectasia, Raynaud phenomenon, SCLE, and LE-nonspecific skin lesions such as vasculitis (186).

The following laboratory findings have been reported to be risk factors for the development of SLE in DLE patients: unexplained anemia, marked leukopenia, false-positive tests for syphilis, persistently positive high-titer antinuclear antibody assay, anti-single–stranded DNA antibody, hypergammaglobulinemia, an elevated erythrocyte sedimentation rate (especially of greater than 50 mm/hour), and immune deposits at the dermal-epidermal junction of nonlesional skin (i.e., a positive lupus band test) (228).

A recent multivariate study of 51 SLE patients and 245 patients with SCLE and/or DLE revealed that those patients with evidence of nephropathy (e.g., proteinuria, hematuria), arthralgias, or an ANA titer 1:320 were at significantly greater risk of having systemic disease (253). This analysis found that the erythrocyte sedimentation rate, anti-dsDNA antibodies, photosensitivity, and recurrent headaches

were not useful in distinguishing between patients with or without systemic disease. Recent work also has suggested that elevated soluble interleukin 2 (IL-2) receptor levels might also be a risk factor for SLE in patients with DLE (254).

What Are the Clinical Implications of Classical DLE Lesions Occurring in SLE Patients?

Roughly one fourth of SLE patients will develop DLE lesions at some point in the course of their disease. Some work has suggested that such patients tend to have less severe forms of SLE with life-threatening complications such as diffuse proliferative glomerulonephritis are being uncommon in these patients and survival is increased when compared to SLE patients without DLE lesions (255).

What Are the Risks for Developing Systemic Disease Associated with Other Forms of Chronic Cutaneous LE?

Hypertrophic DLE patients do not appear to have a greater risk for developing SLE than do patients with classical DLE lesions (256). Several reports have indicated that approximately 50% of the patients with LE profundus/panniculitis have a relatively mild form of SLE (200). In a recent retrospective review of 40 LE panniculitis patients, only 10% met the ACR criteria for SLE (257). In an earlier study, only 25% of 16 LE panniculitis patients that were followed over a decade on average ever developed SLE (258). A more recent review of 12 LE panniculitis cases from Singapore reported 3 of the 12 cases with evidence of SLE. (203). The systemic features of patients with LE panniculitis/profundus tend to be less severe, similar to those in SLE patients who have DLE skin lesions (200). Severe nephritis is an uncommon complication in SLE patients with LE panniculitis. In one study, 3 of 15 (20%) patients presenting with chilblain LE later developed SLE (219).

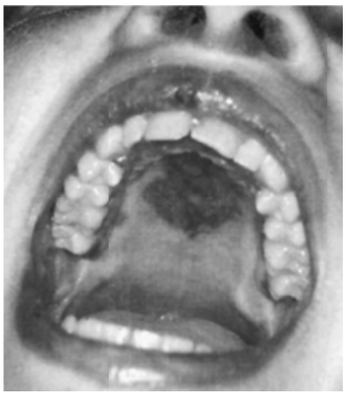

Figure 30-38. Oral mucosal ulceration in systemic lupus erythematosus (SLE). As seen in this case, superficial ulcers often occur in the junctional area of the hard and soft palates; however, ulceration of the gingival, buccal, and lingual mucosa also occur. In the early stages of such lesions, the histopathology often is nonspecific. While difficult to see in this photograph, this young woman with active SLE, including nephritis, also had ulceration of the nasal mucosa. Oral and nasal mucosa ulceration in this setting can be associated with severe pain, or it can be painless.

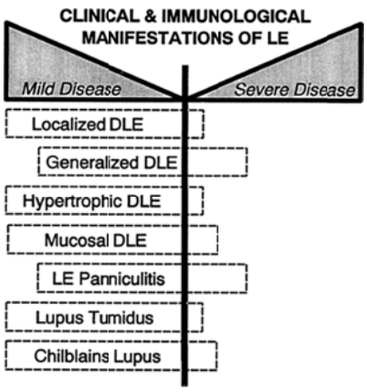

Figure 30-39. Relative risks for systemic lupus erythematosus disease activity that are associated with the various clinical varieties of chronic cutaneous lupus erythematosus (CCLE).

The risk for systemic disease activity in LE patients having mucosal involvement is a function of the type of mucosal lesion that is present. Superficial, transient oral or nasal mucosal ulcerations having a relatively nonspecific histopathology are commonly seen in patients with active SLE (Fig. 30-38) (such mucosal lesions represent one of the 11 ACR's revised classification criteria for SLE). Chronic mucosal LE plaques, the mucosal equivalent of DLE lesions are seen most commonly in LE patients who do not have life-threatening SLE. The more severe manifestations of SLE such as renal disease appear to be quite uncommon in SLE patients who develop chronic mucosal plaques (213).

Figure 30-39 illustrates the relative risks for systemic disease activity that are associated with the various clinical varieties of chronic cutaneous LE.

The Lupus Band Test

No discussion of the relationship between the cutaneous and systemic manifestations of LE would be complete without addressing the lupus band test. Burnham et al. first identified the presence of immunoglobulins and complement components in a continuous bandlike array at the DEJ of lesional skin biopsies from LE patients using direct immunofluorescence microscopy (23). This phenomenon, subsequently referred to as the “lupus band” (259), initially was felt to be quite specific for LE; however, subsequent work has documented

that the lupus band can be found in a number of skin disorders other than those caused by LE (260).

Cormane (261) later described similar findings in biopsies from clinically normal skin of SLE patients in the complete absence of any signs of cutaneous inflammation. Because DLE patients lacked this finding in their nonlesional skin, it was suggested that such results might have diagnostic specificity for SLE. The search for immunoreactant deposition in nonlesional skin of LE patients subsequently has been referred to by many as the lupus band test (LBT).

Controversies concerning terminology have clouded this field from the beginning. Although some workers use the term “LBT” to refer to LE lesional as well as nonlesional immunopathologic findings (262), others reserve this designation for reference to the results of immunopathologic examination of nonlesional skin (263). Less confusion might exist in this area if the terms “lesional LBT” and “nonlesional LBT” were uniformly adopted by those working in this area.

The immunopathologic findings in nonlesional skin of LE patients must be distinguished from those present in LE lesional skin biopsies. Issues related to immunopathologic findings in LE-specific skin lesions (i.e., the lesional LBT) will be discussed later in this chapter (see Pathology/Immunopathology). The remainder of this discussion will be limited to the presence or absence of immunoreactants at the DEJ of nonlesional skin (i.e., the nonlesional LBT).

All three major immunoglobulin classes (IgG, IgM, and IgA) and a variety of complement components including constituents of the membrane attack complex have been identified in these DEJ deposits (260). Studies have suggested that the presence of membrane attack complex at the dermal-epidermal junction of lesional skin is relatively sensitive and specific for LE (264). The immunoglobulin-staining pattern of immunoreactants in nonlesional LE skin under low power generally is described as being granular. Upon high-power magnification, the pattern of fluorescence has been variously described as appearing homogeneous, stippled, fibrillar, shaggy, lumpy-bumpy, linear, or thready (details related to the appearance and significance of these various patterns have been reviewed (265)). Ultrastructurally, these immunoreactants are seen to be deposited on and below the lamina densa of the DEJ (266). The intensity of the staining and the number of immunoreactants present in these deposits can vary considerably (262). Most authorities require a continuous pattern of immunoreactant deposition along the DEJ for a

positive nonlesional LBT. Numerous studies have suggested that a discontinuous or interrupted nonlesional LBT can be seen in a number of other disorders and thus is much less specific for SLE (260). Additionally, the presence of IgM alone appears to be very nonspecific (260). The diagnostic and prognostic significance of the nonlesional LBT has been the subject of much controversy over the past two decades. In-depth analyses of these issues are available elsewhere (262). The following summary points can serve to acquaint the reader with the clinically relevant issues pertaining to the nonlesional LBT:

- Standardization. It is quite curious that a test such as this that has been used clinically for well over four decades has been so poorly standardized. Clinically normal individuals have been studied to determine the incidence of a false-positive nonlesional LBT in both sun-exposed and nonexposed skin regions. Such studies have suggested that as many as 20% of healthy young adults have a positive LBT in sun-exposed nonlesional skin regions such as the lateral aspect of the neck whereas virtually none are positive in sun-protected nonlesional sites such as the buttocks (267). Thus, considerable caution must be taken when interpreting the results of immunopathologic findings in both lesional and nonlesional biopsies taken from sun-exposed skin sites (e.g., face, neck, extensor aspect of forearm and hand) or partially sun-exposed skin sites (flexor aspect of the forearm, deltoid region) with respect to the diagnosis of cutaneous or systemic LE.

  Autofluorescence of dermal collagen and elastin fibers at low power can give the appearance of a positive nonlesional LBT (i.e., the “fibrillar pseudoband” (259)). At higher power, the artifactual nature of this false-positive finding becomes apparent. A false-negative, nonlesional LBT can occur when high levels of extravascular dermal IgG is present. This finding, which correlates with hypergammaglobulinemia, can obscure the distinctness of the lupus band at the DEJ (268).

  It also is important to understand that considerable anatomic regional variation exists with respect to the nonlesional LBT. It has been suggested that there is a cephalocaudal gradient in the frequency of a positive lesional LBT in DLE lesions, with lesions on the head more often being positive than those on the trunk (262). There are indications that a similar phenomenon might exist with the LBT in nonlesional skin of NZB/W mice, a murine model of SLE (33). Firm data addressing this issue in human LE patients have not yet been presented.

- Diagnostic specificity. Because the strongest clinical association of the nonlesional LBT has been with SLE, it is not surprising that classical DLE patients without clinical or laboratory evidence of extracutaneous disease have been uniformly nonlesional LBT negative (186). Only about 25% of SLE patients who have DLE lesions have a positive, nonlesional LBT (186). Approximately 25% of SCLE patients (an LE subset that frequently has mild symptoms of SLE) are nonlesional LBT positive in relatively sun-protected flexor forearm skin (33). As noted earlier, the diagnostic specificity of the nonlesional LBT for SLE has become a point of controversy. Factoring in the profile of individual immunoreactants present in a positive nonlesional LBT has been suggested to be one approach to enhancing the specificity of this test. Several studies have suggested that when three or more immunoreactants are present in the nonlesional LBT, the diagnostic specificity for SLE is very high (269). These observations taken together with the fact that actinically damaged skin can yield false-positive results (267) would suggest that a positive nonlesional LBT (confirmed under high-power observation to exclude the artifacts such as the fibrillar pseudoband) in fully sun-protected skin from the buttock or inner aspect of the upper arm that consists of three or more immunoglobulin or complement components might have the greatest specificity for SLE. Under these conditions, a positive nonlesional LBT can serve as a very useful piece of additional diagnostic information in those difficult cases of SLE where the clinical and laboratory manifestations of this disorder are being expressed atypically.
- Prognostic significance. Although the nonlesional LBT was initially adopted because of its perceived diagnostic specificity for SLE, subsequent work suggested that it also correlated positively with a more aggressive course of systemic disease including the development of lupus nephritis (270). The presence of IgG in the nonlesional LBT was suggested to be more indicative of severe SLE than the presence of IgM alone (271). The idea that the nonlesional LBT had prognostic value also became a point of contention. However, a prospective follow-up study has confirmed the predictive value of a positive nonlesional LBT (272). Whether the nonlesional LBT provides incremental value over more generally available and less invasive tests such as circulating dsDNA antibody levels in prognosis assessment is not clear; however, there are those who continue to feel that this is the case (269). It is the authors' opinion that a properly interpreted nonlesional LBT has its greatest utility as an additional diagnostic maneuver in patients with atypical clinical and laboratory presentations of SLE.

Pathology and Immunopathology

Acute Cutaneous Lupus Erythematosus

Histopathology

The histologic picture in ACLE is generally less impressive than that seen in SCLE and DLE lesions. The dermal cellular infiltrate is often relatively sparse. The most prominent changes are edema of the upper dermis and focal liquefactive degeneration of the epidermal basal-cell layer. In the most severe forms of ACLE, epidermal necrosis may occur, producing a histopathologic pattern strongly resembling toxic-epidermal necrolysis.

Immunopathology

Curiously, there is very little published data concerning direct immunofluorescence findings in ACLE. In one study, five of five (100%) skin biopsies from ACLE (i.e., “diffuse erythema”) lesions were reported to be lesional LBT positive (273). More recent work has indicated that the lesional lupus band test is positive in 60% of patient having the “malar rash” of SLE (55).

Subacute Cutaneous Lupus Erythematosus

Histopathology

While the histopathology of SCLE is clearly that of LE-specific skin disease, it may be impossible to clearly differentiate SCLE from ACLE and DLE. Characteristically, ACLE, SCLE, and DLE have variable degrees of hyperkeratosis, basal-cell degeneration, dermal edema, and mononuclear cell infiltration around the DEJ extending into the dermis. In SCLE, there is focal basal-cell injury and disorientation with liquefaction degeneration, sparse upper-dermal mononuclear-cell infiltrate that may partially obscure the DEJ, dermal edema, and rarely epidermal necrosis (161). The mononuclear infiltrate usually is limited to perivascular and adnexal structures in the upper third of the dermis and the epidermis may be mildly atrophic. Vesicular changes can occur in SCLE lesions, particularly at the active border of annular SCLE lesions (274). It has been suggested that such patients are more likely be HLA-DR3 and have Ro/SS-A autoantibodies (274).

SCLE lesions generally have less hyperkeratosis, follicular plugging, mononuclear cell infiltration of adnexal structures, and dermal melanophages compared to DLE lesions. Qualitative differences in the histopathology of SCLE versus DLE have been noted (275); however, not all agree on this point (276). Bangert et al. were unable to differentiate papulosquamous from annular SCLE by blinded histopathologic examination (161).

Immunopathology

As in other LE-specific lesions, immune deposits can be detected frequently by immunofluorescence staining in SCLE skin lesions. The initial studies indicated that these deposits consist of immunoglobulin (IgM, IgG, and/or IgA) and complement components arranged in a granular bandlike pattern along the DEJ (33). Approximately 60% of SCLE lesions from the original cohort of SCLE patients had such deposits, compared to somewhat higher percentages for ACLE and DLE lesions. Others have found similar results in SCLE skin lesions (274), whereas some workers have found even higher frequencies (178). A more recent study revealed IgM +/- C3 was the predominant immunoreactant in perilesional biopsies from patients with LE, being found in 75% of 199 patients. Of 13 SCLE patient biopsies in this study, 62% had IgM, 46% had IgG, 23% had IgA, 62% had C3, and 31% had fibrin, alone or in various combinations (277). Thus, the presence of immune deposits at the DEJ can help confirm a diagnosis of SCLE, but its absence does not necessarily rule it out. These immune deposits are not specific for LE as similar deposits can be found in normal or sun-damaged skin (267) and in other non-LE dermatologic conditions (260).

Nonlesional deltoid and flexor forearm skin biopsies from the original group of SCLE patients contained junctional immune deposits in 46% and 26%, respectively (33). The prognostic significance of such deposits has not yet been determined.

Nieboer et al. reported finding a “dust-like particle” pattern of IgG deposition deposited in and around the epidermal basal keratinocytes and subepidermal regions in 30% of SCLE lesional skin biopsies (278). This group suggested that this pattern of immunoglobulin deposition is specific for SCLE although its presence did not correlate with the presence of circulating Ro/SS-A autoantibodies. Others have noted this same immunofluorescence pattern in SCLE patients (279). A recent review of 4,374 skin-biopsy specimens submitted for direct immunofluorescence exam revealed 66 samples from 60 patients with “dust-like particles.” Fifty-three percent of these patients had SCLE, but only 36% of these 60 patients had Ro/SS-A autoantibodies (280).

It is curious, however, that one of the authors (RDS), as well as a number of other observers (269), have not been impressed by this pattern of immunofluorescence in SCLE lesional biopsies, suggesting that it might be somewhat technique dependent. This dust-like pattern of IgG is similar to that found in human skin explants grafted onto nude mice that resulted from intravenous infusion of human Ro/SS-A autoimmune sera (281). One of the authors (RDS) has noted a similar pattern of IgG and IgM deposition in guinea-pig skin following intradermal injections of human Ro/SS-A autoimmune sera.

Chronic Cutaneous Lupus Erythematosus

Classical Discoid Lupus Erythematosus

The epidermal basal cell layer is the principal site of injury in all three forms of LE-specific skin disease (161). In classical DLE, there is also prominent hyperkeratosis and follicular plugging. The nucleated layer of the epidermis generally is not thickened and may be somewhat atrophic. Epidermal basal-layer changes include: loss of the normal organization and orientation of basal cells, edema with vacuole formation between and sometimes within basal cells (i.e., liquefaction or vacuolar degeneration), partial obliteration of the DEJ by a mononuclear-cell infiltrate, thickening of the epidermal-basement membrane, increased melanin-pigment formation, and interruption of pigment transfer between melanocytes and keratinocytes leading to the accumulation of melanin by phagocytosis in dermal macrophages.

The dermal histopathologic changes are less specific. A mononuclear-cell infiltrate composed predominantly of T lymphocytes and macrophages is present most predominately in the periappendageal and perivascular areas. Plasma cells occasionally are seen in the more chronic lesions and dermal mucin deposition can at times be quite prominent. The chronic scarring DLE lesions more often have a denser inflammatory cell infiltrate that extends well into the deeper reticular dermis and/or subcutis. In contrast, ACLE and SCLE lesions contain a less dense inflammatory infiltrate that is confined to the upper dermis but still shows the distinctive pattern of injury along the DEJ (161). The periappendageal inflammation that is characteristic of DLE is less prominent in SCLE and ACLE.

Direct immunofluorescence examination of biopsy specimens taken from DLE lesions often reveals a thick, continuous band of immunoreactants along the DEJ (262). This band also extends along the basement membrane of the hair follicle, a finding that is not often seen in those other disorders that have been reported to have similar DEJ immunoreactants deposited in a bandlike pattern. Multiple immunoglobulin classes (IgG, IgA, IgM) usually are present within this band and various complement components (C3, C4, Clq, properdin, factor B, and the membrane attack complex, C5b–C9) also can present in many of these lesions (264).

Early reports suggested that over 90% of DLE lesions had lesional immunoreactants at the DEJ (273), however, subsequent studies have reported somewhat lower frequencies (262). A more recent study of 50 DLE patient biopsies found 78% had IgM, 58% had IgG, 38% had IgA, 72% had C3, and 66% had fibrin, alone or in various combinations (277).

The frequency with which immunoreactants are found in DLE lesions also appears to vary with the anatomic region from which the biopsy is taken. In one study, lesions on the head, neck, and arms were more often positive (80%) than those on the trunk (20%) (282). The frequency of bandlike immunoreactant deposition at the DEJ appears also to be a function of the age of the lesion being examined with older lesions (i.e., greater than 3 months) being more often positive than younger ones (i.e., less than 1 month) (283). Ultrastructural localization of immunoglobulin at the DEJ has confirmed that these proteins are deposited on the upper dermal collagen fibers and along the lamina densa of the epidermal basement membrane zone (266).

Hypertrophic Discoid Lupus Erythematosus

The histopathology and immunopathology is similar to that of classical DLE lesions except for a much greater degree of epidermal acanthosis and hyperkeratosis. The histopathology sometimes reveals features of squamous-cell carcinoma or keratoacanthoma, which can lead clinicians to make the wrong diagnosis (23 ,24). Overlap between the histologic features of hypertrophic LE and lichen planus have been described (199). A more recent clinicopathologic study of 14 hypertrophic LE patients found they all had concomitant classical DLE lesions (284). Histopathology of the hypertrophic LE lesions often showed hydropic degeneration of the basal cell layer, thickening of the basement membrane, a lichenoid reaction at the dermoepidermal junction and a perivascular and periadnexial lymphocytic infiltrate. Pseudoepitheliomatous hyperplasia engulfing elastotic material was also frequently encountered.

Lupus Erythematosus Panniculitis/Profundus

LE panniculitis is the only LE-specific skin lesion that spares the epidermis; however, Dr. James Gilliam felt that the pathologic changes within the subcutaneous tissue were characteristic enough to classify this entity as a form

of LE-specific skin disease. Absence of the characteristic epidermal and dermal changes of LE can make the histologic diagnosis difficult and controversy has existed in the past as to the specificity of the histopathologic changes of LE panniculitis when overlying changes of DLE are not present at the DEJ. The histologic features are that of a lobular lymphocytic panniculitis: perivascular infiltration with lymphocytes, plasma cells, and histiocytes in the deep dermis and subcutaneous fat (including lymphoid nodule formation); vessel-wall thickening and invasion by mononuclear cells (“lymphocytic vasculitis”); absence of polymorphonuclear leukocytes; hyaline-fat necrosis, prominent fibrinoid degeneration of collagen; as well as mucinous degeneration and calcification in old, established lesions (285). Immunoglobulin and complement deposits usually are found in blood-vessel walls of the deep dermis and subcutis by direct immunofluorescence staining of biopsy specimens (202). Immunoglobulin deposits at the DEJ may or may not be present depending on the site biopsied, the presence or absence of accompanying SLE, and the presence or absence of overlying changes of DLE at the DEJ.

A retrospective clinicopathologic study on 12 patients from Singapore found fat necrosis in all cases, and hyaline necrosis in 8 (203). The majority of cases showed lobular and paraseptal lymphocytic inflammation. One third of the cases had evidence of lymphocytic vasculitis and 75% had mucin deposition. Sixty-seven percent had histologic features diagnostic of DLE in the overlying skin, although only a third had overt evidence of DLE. Direct immunofluorescence was positive in only a third of cases compared to 70% in a previous study (284). A more recent retrospective study of 11 biopsy specimens from 9 patients with LE panniculitis found lobular panniculitis in all specimens and co-existent septal involvement in 82% of cases. Epidermal involvement occurred in 73% of cases. Superficial and deep dermal infiltrates were found in 82% of cases, mucin deposition in 73%, and reactive germinal centers in 45%. The subcutaneous infiltrate was composed of lymphocytes in all cases, and contained plasma cells in 91% of the specimens. Immunohistochemistry studies revealed a predominance of α/ß T-helper cells and cytotoxic lymphocytes admixed with B cells in 80% of the cases (211).

Mucosal Discoid Lupus Erythematosus

Except for the differences related to the absence of hair follicles and stratum corneum in mucous membranes, the microscopic changes are highly reminiscent of those seen in cutaneous DLE lesions (286).

Chilblain Lupus Erythematosus

Several studies have addressed the histopathologic findings in chilblain LE lesions in an attempt to differentiate these lesions from idiopathic chilblain lesions (287 ,288). One study found spongiosis, dermal edema and deep perieccrine inflammation may be more common to idiopathic chilblains, although vacuolar change in the basal keratinocytes is more common in chilblain LE (287). However, another study only found deep perieccrine inflammation as a distinguishing characteristic more common to idiopathic chilblains (288). A verrucous form of chilblain LE has recently been reported with prominent hyperkeratosis (289).

Lupus Erythematosus Tumidus

A recent histopathologic study of 80 LE tumidus patients including 53 primary lesions and 38 UVA- and/or UVB-induced lesions showed a characteristic and diagnostic pattern of periadnexal and perivascular lymphocytic infiltrate that in some cases included a few scattered neutrophils. (221). All specimens demonstrated interstitial mucin deposition. Epidermal atrophy or alteration of the dermoepidermal junction was absent. Direct immunofluorescence of LE tumidus lesions have been negative in most cases.

Laboratory Findings

Acute Cutaneous Lupus Erythematosus

Little data are available concerning specific laboratory associations of ACLE. Wysenbeek et al. reported that anti-dsDNA antibodies and low complement levels were more common in patients who had the nonspecific “rash” of SLE (presumably generalized ACLE) (63).

Subacute Cutaneous Lupus Erythematosus

Autoantibodies

ANAs have been detected in 60% to 81% of SCLE patients when human-tissue substrate was used (171), but only in 49% to 55% of patients when mouse or rat substrates were used (274). Ro/SS-A antibodies have been observed in frequencies ranging from 40% to 100% of patients by immunodiffusion techniques (290) (earlier data reviewed in (94)). Higher percentages of patients are anti-Ro/SS-A positive by the more sensitive enzyme-linked immunosorbent assay (ELISA) (290). Some have suggested that anti-Ro/SS-A precipitins more often are associated with annular SCLE patients (79), although this has not been the experience of the authors. Most SCLE patient series report finding La/SS-B antibodies by immunodiffusion in 12% to 42% of their SCLE patients (291), however, two series outside of the United States found that considerably higher percentages of their patients had these antibodies (163). Table 30-3 presents a comparison of the frequency of lab abnormalities seen in SCLE to those seen in ACLE and DLE.

False-positive Venereal Disease Research Laboratory (VDRL) reactions, indicative of antiphospholipid antibodies, have been detected in anywhere from 7% to 33% of SCLE patients (94). Anticardiolipin antibodies have been detected by ELISA in approximately 10% to 16% of patients (292). Rheumatoid factor has been present in approximately one

third of SCLE patients (94); however, relatively few SCLE patients have developed RA (237). Sm, dsDNA, and U1RNP antibodies have been reported to occur in approximately 10% of SCLE patients (data reviewed in (94)). One report has found anti-U1RNP antibodies in eight of 15 SCLE patients (53%) (293), though others have noted a much lower frequency (35). Antilymphocyte antibodies were found in 33% of patients in one study (35). Antithyroid antibodies have been reported in 18% (235) and 44% (294) of SCLE patients.

Other Laboratory Findings

Patients with SCLE, particularly those with systemic involvement, may have a number of laboratory abnormalities. Various studies have found the following: anemia, leukopenia, thrombocytopenia, an elevated erythrocyte sedimentation rate, hypergammaglobulinemia, proteinuria, hematuria, urine casts, elevated serum creatine and blood urea nitrogen, and depressed complement levels (data reviewed in (94)). Levels of complement components such as C2 or C4 can be depressed as a result of genetic deficiencies that have been associated with SCLE (295). One small study suggests that SCLE patients with normal lymphocyte counts are unlikely to have SLE (296).

Chronic Cutaneous Lupus Erythematosus

Autoantibodies

Only a small percentage of patients with classical DLE who have no evidence of systemic disease by history or physical examination will have detectable immunologic abnormalities (186). Antinuclear antibodies may be detected in low titer in as many as 30% to 40% of DLE patients; however, less than 5% will have the higher levels that are characteristically seen in severe SLE. While antibodies to single-stranded DNA are not uncommon in DLE, antibodies to dsDNA are distinctly uncommon (297). Precipitating antibodies to U1RNP sometimes are found in patients whose disease course is dominated by DLE lesions, however, such patients usually have evidence of mild SLE or overlapping connective-tissue disease (228). Ro/SS-A precipitins also can be seen occasionally in DLE patients (290). The presence of precipitating Sm and La/SS-B antibodies are, however, distinctly unusual in patients with isolated DLE lesions (298). Fewer than 10% of DLE patients have IgG anticardiolipin antibodies (299).

Antinuclear antibodies are present in 70% to 75% of patients with LE profundus/panniculitis, but anti-dsDNA antibodies are uncommon (200).

The frequency that autoantibodies have been detected in chilblain LE patients has varied. A recent study has reported ANA in 9 of 14 patients, anti-DNA antibodies in 4 of 14 and Ro/SS-A and /or La/SS-B antibodies in 2 of 14. It is likely that the true frequency of autoantibodies in chilblain LE may be lower, as some patients without antibodies may have been deemed idiopathic chilblains, from selection bias (288). Another study found Ro/SS-A antibodies in the sera of eight out of nine chilblain lupus patients, suggesting that these antibodies might be a useful clinical marker of this disorder (300). The majority of these patients also complained of photosensitivity and Raynaud phenomenon.

Other Laboratory Abnormalities

A small percentage of DLE patients will have a biologic false-positive serologic test for syphilis (VDRL), positive rheumatoid factor tests, slight depressions in serum-complement levels, modest elevations in gamma globulin, and modest leukopenia. The significance of such findings will be discussed below. One study found that 10 of 14 patients with severe chilblains had leukocytopenia and/or lymphopenia, 8/14 had hypergammaglobulinemia, and 5/14 had hypocomplementemia (288).

Differential Diagnosis

Acute Cutaneous Lupus Erythematosus

There are a number of dermatoses unrelated to LE that can produce a red face (56 ,301 ,302). Among those more commonly confused with ACLE are acne rosacea, dermatomyositis, and seborrheic dermatitis. Facial swelling may be severe in patients with ACLE and SLE, sometimes simulating the facial skin changes that are characteristic of dermatomyositis (156). Generalized ACLE can be confused with other causes of widespread exanthematous reactions such as drug hypersensitivity reactions as well as erythema multiforme.

Subacute Cutaneous Lupus Erythematosus

The cutaneous lesions of papulosquamous SCLE can be most closely mimicked by psoriasis (particularly photosensitive psoriasis). Psoriasis has been reported in SLE patients (303). Psoriasis and SCLE have occurred concurrently in the same patient (304 ,305). Histopathology can usually easily distinguish between the two disorders.

Occasionally, SCLE lesions can be confused with pityriasis rubra pilaris (306) and crusted scabies (307). Seborrheic dermatitis, polymorphous-light eruption, dermatophyte infections, nummular eczema, contact dermatitis, dermatomyositis, and cutaneous T cell lymphoma/mycosis fungoides also can be confused with SCLE on occasion. Annular SCLE lesions are more apt to be misdiagnosed as granuloma annulare (308), erythema multiforme, or types of figurate erythemas such as erythema annulare centrifugum and erythema gyratum repens. The photodistribution of SCLE lesions and the LE-specific histopathology often are crucial in helping the clinician differentiate SCLE from these other skin diseases. The presence of circulating Ro/SS-A autoantibodies can serve to further support a diagnosis of SCLE.

Chronic Cutaneous Lupus Erythematosus

Classical Discoid Lupus Erythematosus

With respect to diagnosis, discoid-shaped skin lesions that have erythema and hyperpigmentation at their active borders and depigmentation, telangiectasia, and atrophy at the centers are very unlikely to result from dermatological disorders other than cutaneous LE. However, there are other dermatoses that can produce persistent red plaques on the face that at times can be confused with DLE.

Polymorphous light eruption (PLE), as the name implies, is an exclusively photo-triggered dermatosis that can be expressed in several clinical forms, including succulent red plaques that occasionally can mimic the earlier phases of evolving DLE lesions. PLE lesions, however, clinically lack the keratinaceous follicular plugging, telangiectasia, and atrophy that are characteristic of DLE lesions. Histopathologically, PLE usually lacks the prominent liquefaction degeneration of the epidermal basal-cell layer and basement membrane thickening that is characteristic of DLE lesions. In the dermis, the lymphoid-cell infiltrate is predominately perivascular in PLE and does not involve the cutaneous appendages as in DLE.

Immunoglobulins and complement components are not deposited at the DEJ in PLE as in DLE. Ro/SS-A autoantibodies have been detected in 3.5% to 14% of PLE sera (309 ,310 ,311). ANA titers 1:80 were found in 14% (28 of 198) of PLE patient sera (310), six of whom were Ro positive. Three of the 198 patients met the ACR criteria for SLE, one of whom was Ro positive. Some of these patients may have had cutaneous LE rather than PLE, as histopathologic examination of lesions from six of these patients revealed vacuolar changes of the basal keratinocytes. However, none of these patients developed typical SCLE lesions, and PLE lesions can show LE-like histopathology (312 ,313). At times, it can be difficult to differentiate cutaneous LE from PLE. The history of a recurrent photodistributed eruption that begins in spring and improves or resolves during the summer months supports the diagnosis of PLE over cutaneous LE.

Granuloma faciale also can present as indolent, red-brown, to purple facial plaques that can be very resistant to all forms of treatment. Hyperkeratosis, follicular plugging, and atrophy are not seen in this disorder. The epidermis is spared by the histopathologic process seen in granuloma faciale and the pattern of dermal inflammation is quite distinct from that seen in DLE lesions.

Sarcoidosis, Jessner benign-lymphocytic infiltration of the skin, pseudolymphoma of Spiegler-Fendt (syn., Spiegler-Fendt sarcoid), lymphocytoma cutis, angiolymphoid hyperplasia with eosinophilia, lymphoma cutis, lupus vulgaris (314), and tertiary syphilis (315) are other disorders that can clinically simulate some phases of DLE lesions and at times present diagnostic confusion. The histopathologies of these conditions are quite distinct from DLE and each is usually negative for immunoglobulin and complement components at the DEJ upon direct immunofluorescence examination.

Hypertrophic Discoid Lupus Erythematosus

The hyperkeratotic and pseudoepitheliomatous character of hypertrophic DLE lesions can easily be mistaken for keratoacanthoma, squamous-cell carcinoma, prurigo nodularis, or hypertrophic lichen planus (195 ,284).

Lupus Erythematosus Profundus/Panniculitis

The differential diagnosis of patients with lupus panniculitis includes Weber-Christian panniculitis, factitial panniculitis, Talwin-induced panniculitis, pancreatic panniculitis, traumatic panniculitis, morphea profundus, eosinophilic fascitis, sarcoidosis, subcutaneous granuloma annulare, subcutaneous T cell lymphoma, and rheumatoid nodules. Deep excisional biopsy often is required to distinguish LE panniculitis from these other disorders, particularly when classical DLE lesions are not present. One report suggests that the most useful histologic criteria for differentiating LE panniculitis from subcutaneous panniculitis-like T cell lymphoma are the presence of epidermal involvement, lymphoid follicles with reactive germinal centers, clusters of B lymphocytes, mixed cell infiltrate with plasma cells and polyclonal T cell receptor γ gene rearrangement (211).

Mucosal Discoid Lupus Erythematosus

Oral lichen planus presents the closest clinical appearance to that of oral mucosal DLE. A biopsy can be useful to differentiate between these two disorders, but often is not necessary.

Chilblain Lupus Erythematosus

Chilblain LE patients must be distinguished from idiopathic chilblains and the presence of cryoglobulins or cold agglutinins should be ruled out. Chilblain LE patients are more apt to have other evidence of LE (e.g., autoantibodies, DLE, neutropenia), Raynaud phenomenon and their chilblain lesions are more likely to persist into warmer weather months, compared to idiopathic chilblains (288).

Lupus Erythematosus Tumidus

Lesions of LE tumidus can mimic Jessner benign lymphocytic infiltration of the skin, polymorphic light eruption, pseudolymphoma, reticular erythematous mucinosis, DLE, SCLE, scleredema, and erythema figuratum. Microscopically the lesions can look like cutaneous mucinosis.

Management

Acute Cutaneous Lupus Erythematosus

ACLE lesions respond to the more aggressive regimens of systemic corticosteroids and other immunosuppressive agents (e.g., azathioprine, cyclophosphamide) that often are

required to manage the more severe systemic manifestations of LE that often accompany this form of LE-specific skin disease. Increasing evidence suggests that drugs such as hydroxychloroquine can have a steroid-sparing effect on SLE (316). This is likely to be true for ACLE as well. Table 30-5 outlines the treatment of nonacute LE-specific skin disease, which is very similar for subacute cutaneous LE and the various subsets of chronic cutaneous LE.

Subacute Cutaneous Lupus Erythematosus

The management of patients with SCLE should include evaluation to rule out underlying systemic disease at the time of diagnosis, then again at 6- to 12-month intervals, unless the patient develops symptoms that dictate an earlier reassessment (317). The initial evaluation should include a history, review of systems, and physical examination to elicit symptoms and signs of underlying systemic disease (i.e., arthritis, serositis, CNS disease, renal disease). Initial laboratory evaluation should include, at minimum, a complete blood count, platelet count, erythrocyte sedimentation rate, urinalysis, and blood-chemistry profile. Additional determinations that can be of help include C3, C4, and CH_{50}.

Table 30-5: General Treatment Approach to Subacute and Chronic Cutaneous LE

1. Photo-protective measures
a. Physical protection
   Schedule discretionary outdoor activities before 10 am and after 4 pm even on cloudy days since as much as 80% of UV rays penetrate the cloud cover. Limit exposure to reflected UV rays from surfaces such as water, concrete, sand, snow, tile, and reflective window glass in buildings. The window glass in homes blocks some UV rays, especially the sunburning UV rays (UVB). However, considerable amounts of long wavelength UV rays (UVA) may still pass through such glass. Plastic adherent films that can easily be applied to home window glass are available that block all UVB and UVA rays. Clothing can be an excellent form of sun protection. Cover-up with loose fitting and lightweight clothing (long pants and long-sleeved shirt when possible), sunglasses and 4-inch wide brimmed hats. Tightly woven fabric blocks UV rays best. UV protection drops significantly when the fabric becomes wet. Dark colors protecting better than light colors. The average white t-shirt provides a SPF (sun protective factor) of only 6 to 8. Sun protective clothing lines with a rating of SPF 30 or greater are available.
b. Sunscreens
   Sunscreen products (syn. “sunblocks”) should be applied 15 to 30 minutes prior to sun exposure to be most protective. Sunscreen should be reapplied after prolonged swimming or vigorous activity. Water-resistant sunscreens protect skin for 40 minutes of water exposure and waterproof sunscreens protect for 80 minutes. Sunscreen needs to be applied liberally. As much as 1 oz may be needed to cover the entire body. Particular attention needs to be paid to the back of the neck, the ears, and the areas of the scalp with thin hair. Use sunscreens with at least a 30 SPF (sun protection factor). Select a broad-spectrum sunscreen that contains ingredients that effectively block both UVB and UVA rays. Such ingredients include photostabilized forms of avobenzone (Parsol 1789), titanium dioxide, zinc oxide and Mexoryl-conventional. Sunscreen lotions help dry skin and sunscreen sprays work best on the body. Stick type sunscreens can be used on the lips or around the eyes to avoid eye irritation or for maximal protection of the ears. UV light from sunlight exposure causes the skin to produce an important precursor of vitamin D. Adults who use sunscreens daily should consider taking a daily oral supplement of 400 to 800 units of vitamin D. Sunscreen should not be applied to broken skin or rash (allergies to sunscreen ingredients can develop in some people). Keep in mind, sunscreens are not meant to allow individuals to spend more time in the sun than they would otherwise. They are meant to protect the skin while you must be in the sun.
2. Topical or intralesional corticosteroids, tacrolimus and/or pimecrolimus
3. Begin hydroxychloroquine up to 6.5 mg/kg lean body weight/day
a. If smoker, encourage methods for smoking cessation
b. If no response by eight weeks add 100 mg quinacrine/day
c. If no response in 4 weeks switch hydroxychloroquine to chloroquine up to 0.3–2.5 mg/kg lean body weight/day while continuing quinacrine 100 mg/day; if responsive, consider discontinuance of quinacrine to see if needed.
4. If no response after several weeks, try dapsone, a retinoid, clofazimine or gold. (Dapsone might be tried before antimalarials in bullous LE).
5. If the above treatments fail, or could not be tolerated, consider thalidomide or immunosuppressive agents including short-term systemic steroids, methotrexate, azathioprine, mycophenolate mofetil or cyclosporin.
6. Patients with more severe disease may initially be treated more aggressively with immunosuppressive agents, in combination with the antimalarial agents that often take weeks to months before providing beneficial effects 7) Biologics (see text).

The initial management of all SCLE patients should include education regarding protection from sun and artificial sources of UV light, and the elimination of potentially provocative photosensitiving drugs such as hydrochlorothiazide, griseofulvin, and piroxicam if at all possible.

With regards to specific medical therapy, local measures should be maximized first, then systemic agents employed if significant disease activity continues (Table 30-5).

Sun Protection

Patients should be advised to avoid direct sun exposure, particularly during the midday hours and during the summer months when the UV component of sunlight is least attenuated by the atmosphere. A useful rule of thumb is that if one's shadow is longer than one is tall, there is relatively less danger from UV radiation exposure. Tightly woven clothing with vented panels for comfort in hot environments and hats should be worn in conjunction with broad-spectrum sunscreens to achieve maximal shielding from sunlight. Several clothing lines offering maximized UV protection currently are being marketed and are easily accessed through the internet (e.g., Solumbra Ultra Sun Protective Clothing (http://www.sunprecautions.com/); MasqueRays (http://www.sunproof.com/); Sun Protective Clothing (http://www.sunprotectiveclothing.com/)). Such specialty clothing also is marketed for fishermen and those going on safaris.

Patients should select broad-spectrum sunscreens that contain agents that block UVB with a sun protective factor (SPF) of 30 or greater. It has been found that much lower amounts of sunscreens actually are used in real-life situations compared to the amounts employed under lab conditions for determining the SPF rating of a specific sunscreen product. Therefore, when a photosensitive patient uses a SPF 30 sunscreen in real life, they often get a real-life SPF of about 15 or less, the minimum necessary for adequate protection for a LE patient. Sunscreen products containing photostabilized Parsol 1789 (avobenzone), Mexoryl, zinc oxide, or titanium dioxide provide the broadest degree of UVA protection (318) and such products may have added value in SCLE patients (319). Products also should be selected that are most resistant to being washed off by sweating or bathing. Sunscreens should be applied at least 30 minutes before sun exposure and reapplied after bathing or appreciable perspiration. Stick-type sunscreens that are formulated for use on the lips also can be applied around the eyes to avoid the eye irritation that often occurs when other products are applied to this area.

Several reviews addressing comparative sunscreen efficacy have been published (318). A more recent comparison of three different sunscreen combinations showed that some were more effective than others (320). The sunscreen that contained the UVA blockers Mexoryl SX, Mexoryl XL, avobenzone (Parsol 1789), and titanium dioxide in combination with the UVB blocker octocrylene was most effective in protecting 11 of 11 patients. A second sunscreen protected 5 of the 11 patients, and a third protected only 3 of the 11 patients. (The latter two sunscreens did not contain Mexoryl and contained alternative UVB blocking agents other than octocrylene). These results indicate that sun protection may be beneficial to LE patients, and that protection may vary considerably between different sunscreens.

A number of companies offer UV light-blocking films can be applied to home and automobile windows. More information on these products can be obtained through the internet (e.g., Southwall Technologies [http://www.southwall.com/]; North Solar Screen [http://www.northsolarscreen.com/]; UVShield [http://www.uv-shield.com/]). Several of these companies offer films or plastic shields that can be placed over fluorescent light bulbs to block the small but finite amount of UV irradiation that can leak from such sources (321).

Corrective camouflage cosmetics such as Dermablend (Johnson Products [http://www.dermablend.com/]) and Covermark (Covermark Cosmetics [http://www.covermark.com/]) offer the dual benefit of being highly effective physical sunscreens as well as aesthetically pleasing cosmetic masking agents for patients suffering psychologically from therapeutically refractory, chronic, disfiguring skin disease as can result from cutaneous LE.

Local Corticosteroids

Initial treatment usually includes the use of a potent topical corticosteroid like clobetasol propionate 0.05% (Temovate, Glaxo Wellcome), betamethasone dipropionate 0.05% (Diprolene, Schering), halobetasol proprionate 0.05% (Ultravate, Westwood-Squibb), or diflorosone diacetate 0.05% (Psorcon, Dermik). Twice-daily application of these products to lesional skin for 2 weeks followed by a 2-week rest period can minimize the risk of local complications such as steroid atrophy and telangiectasia. Cutaneous LE represents one of the very few clinical situations where such potent topical fluorinated corticosteroids can be recommended for use on atrophy-prone areas such as the face, because the alternatives are unchecked, disfiguring skin disease, or the potential side for effects from systemic therapy. Unfortunately, topical corticosteroids alone do not provide adequate improvement for the large majority of SCLE patients. Most SCLE patients' lesions are too numerous to be managed by intralesional corticosteroid injections and oral corticosteroids should be avoided as long as possible when treating this chronic cutaneous condition.

Topical Pimecrolimus and Tacrolimus

There have been several reports demonstrating benefits of topical tacrolimus (322 ,323 ,324 ,325 ,326 ,327) and pimecrolimus (328) in treating ACLE, SCLE, and DLE. It has been one of the authors' experience (RDS) that these agents tend to be more effective for cutaneous LE that is expressed on facial skin compared to non facial skin, perhaps because of greater ease of percutaneous penetration in facial skin.

Antimalarials

While a number of systemic medications have been reported to be of benefit to SCLE patients, by far the most useful are the aminoquinoline antimalarial agents. The authors as well as

others (329 ,330) have found that approximately 80% of SCLE patients will respond to single-agent or combined antimalarial therapy. The three agents most frequently prescribed for SCLE patients are hydroxychloroquine sulfate (Plaquenil-Sanofi Pharmaceuticals), chloroquine phosphate (Aralen-Sanofi Pharmaceuticals), and quinacrine hydrochloride (Compounding Labs of America) (quinacrine was previously available in the United States under the brand name, Atabrine [Winthrop Labs]). Generally, hydroxychloroquine is best tolerated with the least side effects. The generic form of quinacrine dihydrochloride that has been available from compounding pharmacies in the United States over the past decade appears to be functionally equivalent to Atabrine in the treatment of LE-specific skin disease. A recent article reviewed the use of antimalarial agents in treating cutaneous lupus erythematosus patients (331).

When using either hydroxychloroquine or chloroquine, ophthalmologic examination is required to minimize the risk of retinal toxicity (quinacrine has not been confirmed to be retinopathic). It has been controversial how often ophthalmologic screening exams should be performed. The Physicians' Desk Reference (Physicians Desk Reference. 59th ed. Thompson PDR, 2005; Montvale, NJ.(332) has recommended quarterly examinations, though most clinicians have felt this to be too frequent. Recently the American Academy of Ophthalmology formed a task force to study this issue. The task force recommended that all patients treated with hydroxychloroquine or chloroquine have an exam that includes a funduscopic exam and central visual field testing, during the first year of therapy. Patients at low risk of developing retinal toxicity (patients taking the antimalarials less than 5 years at recommended doses (<6.5mg/kg/day hydroxychloroquine or <3 mg/kg/day of chloroquine) whose initial examination is normal, should have no further special testing for the next 5 years. Patients in the high-risk group (patients who: are older than 60 years, have a high body fat level, are taking higher than recommended doses, or have kidney, liver or retinal disease, or have taken the drug for over 5 years) should have an annual examination (333 ,334). Use of the self-administered Amsler Grid test at home is a useful ancillary measure to help patients detect the earliest evidence of visual-field defects. Retinal changes can become irreversible if not detected early.

Although there have been descriptions in the medical literature of hematological and hepatic toxic effects during HCQ or CQ treatment, some authors stress that pretreatment and follow-up laboratory testing are not necessary, particularly for hydroxychloroquine (335). However, it seems prudent to obtain baseline and periodic blood counts and hepatic function tests, particularly in patients that have pre-existing hepatic or hematologic disease, or in patients at increase risk of developing hematologic or hepatic diseases.

Quinacrine hydrochloride is more likely to induce hemolysis in glucose-6-phosphate dehydrogenase-deficient patients than is hydroxychloroquine or chloroquine (336). Neurotoxicity and muscular toxicity can occur but was much more of a problem in the past when higher daily doses of these drugs were used.

Antimalarial agents can induce a number of dermatologic changes. All can cause a blue-black pigmentation of the skin (particularly in the sun-exposed areas), the palatal mucosa, and the nails. Antimalarials have also been reported to cause lichenoid eruptions that can exacerbate or mimic cutaneous LE lesions (337). Litt's Drug Eruption Reference Manual lists skin conditions (with references) that have been associated with chloroquine and hydroxychloroquine treatment (338). These associations include acute generalized pustulosis, angioedema, bullous eruption, erythema annulare centrifugum, erythema multiforme, erythroderma, exanthems (1% to 5%), exfoliative dermatitis, fixed eruptions, lichenoid eruption, photosensitivity, pigmentation, pruritus, psoriasis and pustular psoriasis, purpura, pustules, Stevens-Johnson syndrome, toxic epidermal necrosis, urticaria, vasculitis, hair bleaching/pigmentation, nail discoloration/pigmentation, gingival/oral pigmentation, and stomatitis. Quinacrine-associated skin conditions have included exfoliative dermatitis, lichenoid eruption, ochronosis, photosensitivity, pigmentation, urticaria, alopecia, and nail/oral pigmentation. Quinacrine frequently causes diffuse yellowing of the skin, sclera, and bodily secretions that are fully reversible on discontinuation of the drug. On occasion, quinacrine and other antimalarials can produce a lichenoid drug reaction that can be the harbinger of severe bone-marrow toxicity (339).

In an average-sized adult, therapy with hydroxychloroquine alone at 400 mg/day initially should be tried. If there is no significant improvement by 2 months, quinacrine, 100 mg/day, can be added to the hydroxychloroquine (340). If the response is inadequate after 4 to 6 weeks of hydroxychloroquine plus quinacrine, chloroquine 250 mg/day can be substituted for the hydroxychloroquine while continuing the quinacrine (an occasional cutaneous LE patient will respond better to chloroquine than hydroxychloroquine). Once disease activity is controlled, the hydroxychloroquine can be decreased to 200 mg/day for maintenance. The mechanisms by which antimalarials might provide therapeutic benefit have been reviewed elsewhere (341).

Two studies have confirmed earlier suspicions that smoking may interfere with the efficacy of antimalarials in treating DLE and SCLE (140 ,141). Both authors have witnessed dramatic improvement in DLE skin lesions in antimalarial-resistant patients soon after they have quit smoking. Therefore, one has additional reasons to encourage cutaneous LE patients to quit this dangerous habit.

Dapsone

Diaminodiphenylsulfone (Dapsone, Jacobus Pharmaceutical Co.) is best for treating the occasional patient having LE-nonspecific vesiculobullous skin lesions that can occur in SLE patients (“bullous SLE”) (342). Within days, 100 mg/day of this drug can provide significant improvement. Hematologic, renal, and hepatic toxicity can occur with this drug and

requires frequent monitoring. The authors have had relatively little positive experience in treating SCLE patients with this agent, although others have reported benefit in isolated cases within a few weeks after starting therapy (296). A topical version of Dapsone (Aczone, 5% gel, QLT USA, Inc, Fort Collins, CO) has recently been approved by the Food and Drug Administration (FDA) for treating acne. It will be interesting to see if it provides benefit when used “off-label” to treat cutaneous LE.

Retinoids

The synthetic retinoids isotretinoin (Accutane, Roche Laboratories), etretinate (Tegison, Roche Laboratories), and acitretin (Soriatane, Roche Laboratories) at approximately one half to 1 mg/kg/day have been shown to significantly improve SCLE lesions (343 ,344). (Please note that etretinate is no longer available, and has been replaced by acitretin.) These agents also have been advocated for hypertrophic DLE (345). The great potential for teratogenic effects with the retinoids make it imperative that fertile females are using contraceptive techniques according to guidelines set forth specifically for patients on retinoids. The new IPLEDGE program has recently been instituted to more closely monitor patients, and help prevent pregnancies (http://www.ipledgeprogram.com/). A common dose-related side effect is mucocutaneous dryness. It is advisable to have patients use sunscreens judiciously while being treated with these agents to minimize their tendency to aggravate photosensitivity. Drug-induced hepatitis and hypertriglyceridemia can occur with these agents and require periodic laboratory evaluation. On occasion, these drugs also can induce bony changes consistent with the diffuse idiopathic skeletal hyperostosis (DISH) syndrome.

Clofazimine

Crovoto reported the successful use of clofazamine (Lamprene, Geigy Pharmaceuticals) in a patient with annular SCLE in 1981 (346). He used a dose of 100 mg/day and noted clearing of the lesions within a few weeks. At this dosage, clofazimine generally is well tolerated, though gastrointestinal intolerance can be a problem. At higher doses, clofazamine rarely has been reported to precipitate in mesenteric arteries resulting in major abdominal catastrophes such as splenic infarction (347). A pink to brownish-black skin pigmentation develops in most patients on long-term clofazimine therapy. This pigmentation resolves over months to years after discontinuing the drug. Similar discoloration of bodily secretions also frequently occurs.

Thalidomide

Thalidomide, 50 to 200 mg/day, can be very effective in otherwise-refractory SCLE and DLE (32 ,348 ,349 ,350 ,351 ,352 ,353 ,354). Generally, about 75% of cutaneous LE patients will respond to antimalarial monotherapy or combination therapy. It now appears that thalidomide can produce good-to-excellent results in 75% of antimalarial-refractory cutaneous LE. Thalidomide is thought to be beneficial from its TNF-α antagonistic effects, although there is also indirect evidence that it might impede UVB-related mechanisms (355).

Because of its notorious teratogenicity (356), special precautions should be taken when prescribing thalidomide. It is available in the United States under the brand name Thalomid (Celgene Corporation). Prescribing physicians and dispensing pharmacies first must register with Celgene Corporation. Once this has been accomplished, Celgene Corporation will send the physician specially developed materials (System for Thalidomide Education and Prescribing Safety [STEPS]) to educate patients to help them avoid birth defects.

Thalidomide can produce irreversible sensory neuropathies in treated patients (356 ,357). A recent study found that cutaneous LE patients receiving a higher dosage of thalidomide had no greater therapeutic response or increased risk of developing neurotoxicity, compared to patients receiving lower doses (358). Another recent study found peripheral neuropathy developed in 50% of 14 cutaneous LE, after a median time of 14 months. No correlation between the occurrence of peripheral neuropathy and cumulative thalidomide dose could be made (359). Baseline and periodic peripheral nerve conduction studies (i.e., measurements of sensory nerve-action potential amplitudes) have been recommended in hopes of detecting neuropathy early so that the thalidomide can be discontinued before more severe, irreversible neuropathy develops. The role of nerve conduction studies in monitoring thalidomide-treated patients still is not well defined.

Recently, there have been a number of cases of thromboembolic disease developing in patients within days to months of starting thalidomide, including patients with SLE, SCLE, and DLE (360 ,361). Some have advised checking antiphospholipid antibodies and plasma homocysteine levels in LE in patients before starting thalidomide as the presence of these antibodies or high homocysteine levels might put thalidomide-treated LE patients at increased risk of thrombotic events, including pulmonary embolism (360 ,362). It is advisable to avoid using thalidomide in patients at increased risk for developing thrombosis until more is learned. The role for prophylactic anticoagulation therapy in patients treated with thalidomide has not yet been well defined. Other thalidomide side effects include a transient myoclonic jerking reactions of the extremities, neutropenia, and secondary amenorrhea as a result of ovarian failure (363).

Gold

Oral gold (auranofin [Ridaura, Connetics Corporation]) or parenteral gold (aurothioglucose [Solganal, Schering]) therapy has been successfully used in those cutaneous LE patients whose disease is resistant to the less toxic forms of therapy (364). Gold frequently has mucocutaneous toxicity and less commonly has hematologic, renal, and pulmonary toxicity that may require its discontinuance.

Interferon-a

Earlier clinical observations had suggested that endogenously produced interferon might be of benefit in SCLE (365). Recombinant interferon (IFN)-α 2A (Roferon-A, Roche) has been used to treat four SCLE patients (366). The dosage ranged from 18 to 120 × 10^6 units injected weekly for 4 to 13 weeks. Two patients had a complete response, one had a partial response, and one patient had no response to treatment. All three patients that responded to treatment later relapsed 4 to 12 weeks after treatment was stopped. Others have noted similar effects (367). Intralesional IFN-α has been reported to be of value in DLE (368). However, the risk of inducing or exacerbating systemic autoimmune reactions, including SCLE, probably outweighs any benefits of IFN-α in this setting (101).

Systemic Corticosteroids and Other Immunosuppressive Agents

Systemic corticosteroids and cytotoxic agents are reserved for patients with more severe disease who have failed the less toxic forms of therapy discussed above. A patient occasionally may be encountered whose disease is so severe that these more potent agents may be used earlier in the disease course, even before the patient is given a complete trial of the less toxic agents.

Methylprednisolone given in “pulse doses” (1 g intravenously for three consecutive days) has been reported to provide improvement in SCLE patients with systemic LE (369). Anecdotally, cyclophosphamide and methotrexate (329 ,370 ,371) as well as azathioprine (372) have been suggested to be of benefit in refractory SCLE. Because of the potential for severe immunosuppression, risk of cancer induction, bone marrow, and mucous-membrane toxicity, these agents should be reserved for patients with severe disease and used only as a last resort in patients with severe cutaneous LE alone. More recently several case reports have indicated that mycophenolate mofetil can be beneficial in treating SCLE (373 ,374 ,375). However, a more recent study found that seven SLE patients with refractory cutaneous lesions, including ACLE, SCLE, DLE, and chilblain LE, were not very responsive to mycophenolate mofetil (376). Dosages ranged between 2 and 3 g and were generally well tolerated.

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) treatment has been successful in treating cutaneous LE, however, the high cost of this treatment, and its potential side effects should make this treatment reserved for more severe cases unresponsive to more conventional agents (377 ,378).

UVA-I Phototherapy

Preliminary animal work has suggested that UVA might dampen the autoimmune abnormalities in experimental SLE (379). Additionally, work from two centers has suggested that SCLE patients might actually benefit from very low doses of whole-body UVA-I (340 to 400 nm) irradiation (380). However, the true value that this somewhat controversial form of treatment will ultimately play remains to be confirmed by controlled studies in larger groups of patients. Caution should be taken in interpreting these data in view of the increasing evidence that UVA (381), including long-wave UVA (193), can play an exacerbating role in the cutaneous manifestations of SLE. Recent experimental evidence in a murine model of SLE argues for further caution in this area (382).

Other Treatments

A number of other therapies have been reported to be beneficial in treating cutaneous LE including extracorporeal photochemotherapy (383), cefuroxime axetil (384), CD4 monoclonal antibody (385) and hormonal treatment, though these reports have been mostly anecdotal and have not been well corroborated. Although there has been a case report of a RA patient with SCLE clearing the skin lesions after etanercept (386), as mentioned earlier, this agent may also induce SCLE. The more recently developed biologic agent rituximab (Rituxan), a recombinant monoclonal antibody that inhibits CD20 expressing B cells, has shown to be beneficial in some patients with SLE and cutaneous LE (387 ,388). Although there is reason to suspect that new biologic agents involved in blocking antigen presentation (alefacept [Amevive] and efalizumab [Raptiva]) may provide benefit in treating cutaneous LE, the high cost these medications make them less than attractive for this purpose.

Table 30-5 presents a suggested treatment approach for subacute and chronic cutaneous LE. These recommendations are based upon the authors' personal experience pertaining to the relative efficacy and safety of this group of drugs.

Chronic Cutaneous LE

The initial approach to the medical management of CCLE is basically the same as that described above for SCLE lesions (Table 30-5). For the most part, both forms of cutaneous LE respond similarly to medical therapy. Local therapy, especially intralesional corticosteroids (e.g., triamcinolone acetonide suspension, 3 to 5 mg/mL for the face with higher concentrations allowable elsewhere), often is more useful in the management of DLE compared to SCLE because one often is treating fewer and smaller lesions in a DLE patient compared to a SCLE patient. Additionally, we, and others (342), have been particularly impressed that combination antimalarial treatment (hydroxychloroquine or chloroquine plus quinacrine) has been more effective in a number of our DLE patients who did not respond to hydroxychloroquine alone. As stated earlier, two studies have indicated that smoking may interfere with the efficacy of antimalarials in treating DLE and SCLE (140 ,141). These findings, and the fact that both authors have witnessed dramatic improvement in DLE skin lesions in antimalarial-resistant patients soon after they have stopped smoking, make it advisable for

all cutaneous LE smokers to quit smoking. It is possible that the dramatic effects that cigarette smoking can have on hepatic microsomal enzyme induction (389) could alter the metabolism of the aminoquinoline antimalarials so as to blunt their effect on cutaneous LE. Alternatively, compounds present in cigarette smoke might be capable of directly exacerbating LE-specific cutaneous inflammation.

As with SCLE, drugs such as dapsone (390), retinoids (390), clofazimine (391), thalidomide (353 ,354 ,392), gold (365), methotrexate (393 ,394 ,395), and azathioprine (390), can be of value in DLE, and other forms of CCLE, when antimalarials have failed. Azathioprine has been advocated for those patients having antimalarial-resistant palmar/plantar DLE (372). One report has demonstrated improvement in DLE from topical tazarotene (396). More recently several case reports have indicated that mycophenolate mofetil can be beneficial in treating DLE, LE profundus, LE tumidus, and (397 ,398) and chilblain LE (397 ,399).

Several other treatments have been reported to be beneficial in treating DLE including imiquimod (400), phenytoin (401), sulfasalazine (402), extracorporeal photochemotherapy (403), vitamin E (reviewed in 404), and cyclosporin A (405) though most have not been well substantiated.

Additional reports have demonstrated improvement in DLE lesion erythema and telangiectasias with pulsed dye (406 ,407) and argon lasers (408).

Once scarring develops within a DLE lesion, little can be done to reverse this process. Although plastic surgery repair of disfiguring lesions of DLE has produced disappointing results, probably a result of the resultant trauma inducing the Köebner or isomorphic phenomenon (409), resurfacing measures by dermabrasion (410), CO_2 laser (411) and erbium:YAG lasers (412) have provided benefit in some patients. It will be of interest to learn if the newly developed nitrogen plasma skin resurfacing technique (Rhytec, Inc., Waltham, MA) offers similar benefit. Hair transplantation, and autologous fat transplantation might occasionally be safely carried out in areas of scarring that are devoid of active inflammation (413). There is some evidence to suggest that the risk of disease reactivation is lessened if the patient is concurrently on medical therapy (e.g., antimalarials) to blunt the Köebner response. The best overall strategy for the management of DLE, however, is always one of early, aggressive, medical management to suppress the inflammatory disease process before scarring and alopecia have developed.

Once DLE lesions have progressed to the point of irreversible dystrophic scarring, the use of properly personalized and applied corrective camouflage cosmetics (e.g., Covermark [http://www.covermark.com/], Dermablend [http://www.dermablend.com/]) and properly fitted hair pieces can offer temporary refuge from the devastating emotional impact that this disfiguring disease process can produce. One study found significant improvement in the quality of life in patients using corrective cosmetics to improve the appearance of their disfiguring disease (414).

Hypertrophic DLE lesions that do not respond to single-agent or combined antimalarial therapy have been reported to respond to treatment with systemic retinoids such as isotretinoin (Accutane) (345) and acitretin (415). Cryotherapy has also provided benefit for hypertrophic DLE lesions (416), as has thalidomide (417).

Untreated, LE panniculitis/profundus is indolently progressive with ulceration often eventually supervening. Intralesional corticosteroid therapy should be approached with great caution because even this minimal form of trauma can cause LE panniculitis lesions to break down and ulcerate. Even a carefully executed diagnostic skin biopsy can, at times, produce chronic ulceration in these lesions. Most cases of LE panniculitis/profundus can be managed successfully with single-agent or combined antimalarial therapy, however, some will require more aggressive treatment with systemic corticosteroids (200). Thalidomide (418) gold (419), methotrexate (395), and cyclosporin A (420) also have been used in refractory LE panniculitis/profundus.

Chilblain LE lesions are often improved by keeping the affected area warm and dry. Antimalarial therapy is often beneficial, and some patients have benefited from dapsone, prednisone and pentoxifylline (421). Methotrexate (395) and mycophenolate mofetil (422) have been helpful in some cases.

LE tumidus lesions are often responsive to photoprotective measures and oral antimalarial therapy. Topical and systemic corticosteroids and methotrexate have also proven beneficial (224 ,395). There is one case report of successful use of topical tacrolimus in a LE tumidus patient, published in the German language (423).

Prognosis

The risks of developing systemic manifestations of LE have already been discussed under the sections dealing with the relationship between the cutaneous and systemic manifestations of the various types of LE-specific skin disease. In this section, we will deal with the outcome of the skin lesions themselves as well as the available morbidity and mortality data concerning these forms of cutaneous LE.

Acute Cutaneous Lupus Erythematosus

Localized ACLE lesions can wax and wane in parallel with the underlying SLE activity (30), often leaving pigment changes in their wake but not producing atrophic dermal scarring.

The authors are unaware of mortality data associated specifically with ACLE. As previously discussed, ACLE often is considered to be an integral expression of underlying SLE and as such, little effort has been made to determine if such patients fare better or worse than those who do not develop ACLE.

Subacute Cutaneous Lupus Erythematosus

Having been recognized as a distinct disease entity for only 25 years, the long-term outcome associated with SCLE lesions has yet to be determined. In the authors' experience, most patients appear to have intermittent recurrences over long periods of time. Some have unremitting lesions that smolder in the same location for years. A superficial form of atrophy has been noted to develop in the lesions of several such patients (personal observation) (Fig. 30-16). Other patients appear to enjoy long-term, if not permanent, remissions of their skin disease.

In short-term follow-up studies, approximately 15% of the SCLE patients studied by one of the authors (RDS) developed ACLE lesions and evidence of active SLE including lupus nephritis (164). This subgroup of patients was marked by the presence of high-titer ANA, leukopenia, and/or anti-dsDNA (234). An interim report from a long-term follow-up study involving the original cohort of SCLE patients at University of Texas Southwestern Medical Center in Dallas, Texas, indicates that the relatively mild disease course that these patients enjoy over the short term appears to hold for longer periods of time (up to 20 years) (424). One of the authors (RDS) is aware of only one death directly attributable to SLE in over 130 SCLE patients that he examined personally (personal unpubl. observation).

More recent reports of the epidemiology of additional SCLE cohorts from different parts of the world have supported the view that this is a subset of LE patients with relatively low risk of developing life-threatening forms of SLE (232 ,425). Additional long-term follow-up studies will be required to determine the true risk of severe systemic disease in patients presenting with SCLE skin lesions.

Chronic Cutaneous Lupus Erythematosus

Left untreated, the majority of patients with classical DLE lesions tend to suffer an indolently progressive disease that can spread to produce large areas of dystrophic cutaneous scarring and scarring alopecia that can be disabling emotionally if not physically. In one recent series of 86 chronic cutaneous LE patients having a mean disease duration of 15.1 years, 57% had some form of destructive or deforming scarring, whereas 35% had pigmentary disturbances (426). Spontaneous remission is observed occasionally (228) and the disease activity can recrudesce within the site of older inactive lesions. Squamous-cell carcinoma occasionally develops in chronic smoldering DLE lesions (Fig. 30-40) (427).

Death from SLE disease activity is distinctly uncommon in patients who present initially with localized DLE. As previously discussed, generalized DLE does carry a higher risk of associated SLE activity.

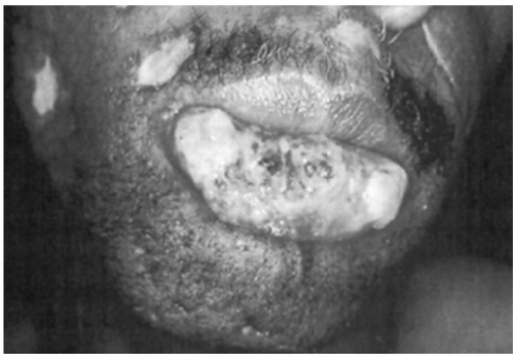

Figure 30-40. Squamous-cell carcinoma developing in the discoid lupus erythematosus lesions of the lower lip.

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Chapter 31
Lupus-Nonspecific Skin Disease

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The cutaneous manifestations of lupus erythematosus have significant diagnostic and prognostic potential. Lupus-specific skin diseases such as acute cutaneous lupus erythematosus (ACLE) and discoid lupus erythematosus (DLE) are seen exclusively in patients with a lupus diathesis, and share specific histopathologic features. When present, such lesions provide important clues about visceral manifestations of systemic lupus erythematosus (SLE). For example, an exacerbation of ACLE almost always suggests potentially aggressive organ-threatening visceral disease. Additionally, generalized DLE (DLE lesions both above and below the neck) indicates a patient who typically meets ACR criteria for SLE although the specific clinical features of the systemic illness can be mild.

A large number of cutaneous lesions that are found in LE patients are not specific for LE, that is, they also occur in patients that don't have or never develop LE (Table 31-1) and do not share the histologic features of LE-specific skin disease. Such skin lesions are usually found in the context of SLE or a significant risk thereof. Nonspecific skin findings in LE include vasculitis, photosensitivity reactions, alopecia, Raynaud phenomenon, livedo reticularis, soft-tissue calcification, bullous lesions, urticaria, cutaneous mucinosis, skin necrosis, ulcerations, and nail changes.

As with some forms of LE-specific skin disease (e.g., ACLE), the presence of nonspecific skin lesions may be an indicator of underlying SLE activity. Parodi et al. applied the Systemic Lupus Activity Measure (SLAM), which is used to evaluate systemic disease activity, to 176 patients with skin manifestations of LE. The most frequent skin lesions found in patients who had higher activity scores were photosensitivity, Raynaud phenomenon, oral ulcers (all lupus nonspecific lesions) in addition to cicatricial alopecia (1). Similarly, a Yugoslavian group of investigators led by Zecevic evaluated 66 patients with the SLE Disease Activity Index (SLEDAI) and found that patients with LE-nonspecific skin lesions had significantly increased disease activity compared to those with LE-specific lesions (2). Additionally, Vila et al. studied patients with incomplete lupus and discovered that patients who had photosensitivity, oral ulcers, Raynaud phenomenon, as well as serologic abnormalities and a malar rash, were more likely to evolve into complete SLE (3). Herein we review this important group of cutaneous manifestations of LE, which may have equal if not more importance than LE-specific skin lesions in regards to correlation with systemic disease activity.

LE-Nonspecific Lesions as Classification Criteria for SLE

Some LE-nonspecific conditions have high prevalence rates in SLE (i.e., photosensitivity, oral ulcerations, alopecia, Raynaud phenomenon) and have previously been or are currently included in the ACR criteria for classification of SLE.

Photosensitivity

Patients with SLE often have photosensitivity reactions. Indeed, the presence of sensitivity to ultraviolet radiation (UVR) is one of 11 American College of Rheumatology (ACR) criteria for the diagnosis and treatment of lupus erythematosus (4).

Photosensitivity is defined clinically as an abnormal response to UV (5). These responses include exaggerated sunburn reactions that do not necessarily show the characteristic LE-specific histopathology, although LE-specific skin lesions can often be induced after UVA and/or UVB light exposure (6 ,7). Some patients with SLE merely exhibit burning, stinging, and/or redness after exposure to low levels of UVR (i.e., decreased minimal erythema dose). Such photosensitivity may be induced by UVA and/or UVB radiation, or possibly even fluorescent lights under special circumstances (8). Some patients even report that systemic disease symptoms, including weakness, fatigue, and joint pain, are increased by sun exposure (9). However, for ethical reasons this has not been examined experimentally in humans and reliable animal models of the systemic component of lupus photosensitivity are not available. The clinician should be wary of diagnosing photosensitivity based on history alone, and other causes and/or mimics of photosensitivity such as medication-induced photosensitivity and rosacea should be excluded.

Photosensitivity seems to correlate strongly with the presence of Ro/SSA antibody. In one study, patients with photosensitivity had higher titers of anti-Ro antibody than those without photosensitivity (10). Ioannides et al. found that Ro and La antigen expression in skin biopsy specimens was 4- to 10-fold higher in patients with LE with photosensitivity than in those patients with LE without photosensitivity (11), however this work has not been independently confirmed. Chapter 29 presents a complete review of the pathogenetic basis of lupus photosensitivity.

Table 31-1: Classification of LE Nonspecific Skin Disease (Modified from the Gilliam Classification Scheme)

I. LE NONSPECIFIC CUTANEOUS LESIONS THAT SERVE AS CLASSIFICATION CRITERIA FOR SLE
- a. Photosensitivity
- b. Mucosal ulceration
- c. Alopecia
- d. Raynaud phenomenon

II. LE NONSPECIFIC CUTANEOUS VASCULAR REACTIONS
- a. Vasculitis
  - i. Small vessels
    1. Dependent palpable purpura
    2. Urticarial vasculitis
  - ii. Medium and large vessels
    1. Purpuric plaques with stellate or retiform borders with or without cutaneous necrosis and ulceration
    2. Subcutaneous nodules that with or without ulceration
- b. Vasculopathies
  - i. Ischemic
    1. Raynaud phenomenon
  - ii. Thromboembolic
    1. Associated with antiphospholipid antibodies
       - A. Livedo reticularis
       - B. Superficial thrombophlebitis
       - C. Cutaneous ulcers
       - D. Purpura/ecchymoses
       - E. Subungual splinter hemorrhages
    2. Not associated with antiphospholipid antibodies
       - A. Resulting from monoclonal cryoglobulins
         - i. Purpura/ecchymoses
         - ii. Hemorrhagic skin necrosis
         - iii. Ulceration
       - B. Resulting from cholesterol embolization
         - i. Livedo reticularis
         - ii. Purpuric infarction of toe tips and/or fingertips
       - C. Resulting from calciphylaxis
- c. Other cutaneous vascular reactions
  - i. Urticaria
  - ii. Periungual telangiectasia
  - iii. Palmar erythema
  - iv. Erythromelalgia

III. OTHER LE NONSPECIFIC CUTANEOUS LESIONS
- a. More common
  - i. Cutaneous mucinosis
  - ii. Calcinosis cutis
  - iii. Nail changes
  - iv. Cutaneous manifestations of overlapping autoimmune disorders
  - v. Acquired SLE-associated bullous dermatoses
    1. EBA-like bullous dermatosis of LE
    2. Dermatitis herpetiformis-like bullous dermatosis of LE
    3. Pemphigus erythematosus
    4. Bullous pemphigoid
    5. Porphyria cutanea tarda
- b. Less common
  - i. Lichen planus
  - ii. *Acanthosis* nigricans
  - iii. Erythema multiforme
  - iv. Cutis Laxa
  - v. Eruptive dermatofibromas
  - vi. Acquired ichthyosis
  - vii. Interstitial granulomatous dermatitis/palisaded neutrophilic granulomatous dermatitis

Photosensitivity appears to be a relatively sensitive indicator of SLE. Chien et al. found photosensitivity in 90% of 80 Taiwanese children with SLE (10). Sanders et al. found an abnormal reaction to UVR and visible light in 93 of 100 patients with cutaneous lupus and/or SLE (12). Other studies cite between 50% and 79.5% incidence rates of photosensitivity in patients with SLE (13 ,14). In a large epidemiologic study, Cooper et al. found that photosensitivity occurs less often in African-American patients (15). Nonetheless, because of the frequency of photosensitivity in SLE, all patients should be taught the importance of protecting skin from UVR. See Chapter 30 for recommendations regarding UV protection.

Mucosal Ulceration

Oral ulceration is commonly found in patients with LE, and represents one of the 1982 revised ACR criteria for the classification of SLE (4). Oral ulcers are present in roughly 25% to 45% of SLE patients (14 ,16) and up to 25% of DLE patients (17). Although these lesions can show LE-specific histopathologic changes on biopsy, particularly in DLE patients, they often are nonspecific. Available incidence/prevalence data does not separate between LE-specific and LE-nonspecific oral ulceration. Nonetheless, oral ulcerations are likely an indicator of disease activity, and in one study they were only seen in patients with active SLE (16). Yell et al. assessed mucocutaneous findings in 73 patients with SLE, and found that 31.5% had a history of mouth ulceration. Of these, 15% noted ulcers at the onset of their disease. Four percent had ulceration of the palate, and 4% had buccal ulceration, whereas an additional 4% had lip lesions (14). Another study noted 6% of SLE patients with lip lesions (17).

The location and asymptomatic nature of LE-related oral ulcers can help differentiate them from other types of oral ulcers such as aphthous stomatitis, lichen planus, herpes simplex, or drug-induced ulcers (e.g., methotrexate or gold). LE-related ulcerations are more apt to be on the hard palate (Fig. 31-1). One study, conducted by Urman et al., found them on the hard palate in 89% of 182 SLE patients. These authors also observed that oral ulcers were asymptomatic in 82% of patients (18). By contrast, non-LE-related oral ulcers are usually quite painful. LE-related oral ulcers often improve with treatment of other systemic or cutaneous manifestations of LE. Symptomatic lesions may also be treated topically with various agents alone or in combination, including topical corticosteroids, tetracycline, diphenhydramine, and lidocaine hydrochloride (Xylocaine Viscous).

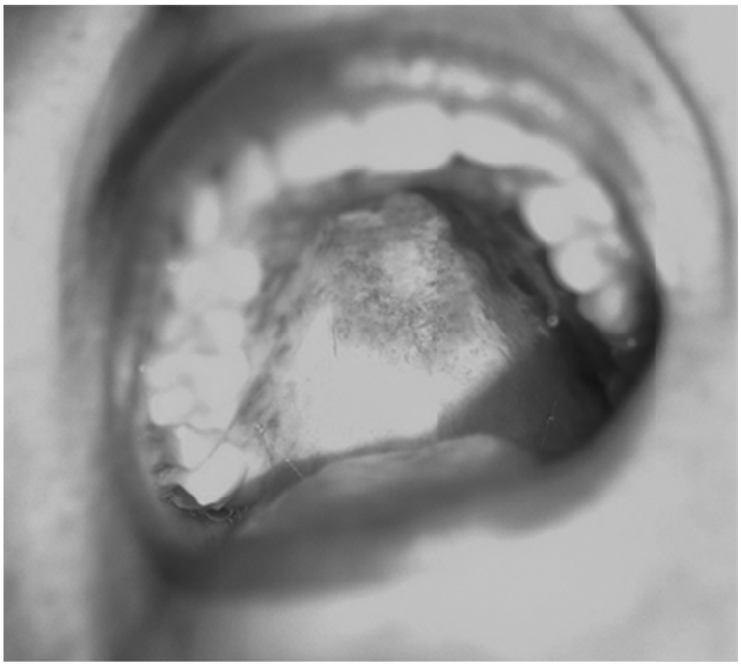

Figure 31-1. (See color plate.) Ulceration on hard palate in a patient with SLE.

Nasal and genital ulcerations are also seen in SLE and appear to occur during periods of disease activity. Burge et al. looked at mucosal involvement in 121 patients with either SLE or CCLE, and found that nasal ulcerations were present in 7 (6%). Vulvar lesions were present in 2/121 patients (17). Nasal ulcerations are often painful, and may even progress to ulceration of the nasal septum. Nasal ulceration and perforation have been reported in association with flaring systemic disease (19).

Alopecia

Alopecia is a common finding in lupus patients, as is suggested by its inclusion in the original 1971 preliminary criteria for the classification of SLE. Because of low sensitivity and specificity, it was not incorporated into the 1982 revised criteria. Alopecia in lupus can have a considerable psychosocial impact (20).

There are several different types of hair loss in patients with SLE. Some are associated with scarring of the scalp's skin, whereas others are not.

Scarring alopecia frequently occurs in DLE lesions (see Chapter 30). This should be distinguished from the diffuse nonscarring alopecia that characterizes LE-nonspecific hair loss. LE-nonspecific alopecia has been referred to as “lupus hair” and presents as course, dry hair that has increased fragility. It often results in broken hairs and may be more prominent over the frontal hairline (Fig. 31-2). Dubois noted

such “lupus hair” in 6% of 520 SLE patient (21). This likely has considerable overlap with a diffuse nonscarring alopecia caused by telogen effluvium, which involves a synchronization of hair into the telogen (resting) phase with subsequent hair shedding, and typically follows by 2 to 3 months of an acute illness, such as a systemic lupus flare, delivery of a pregnancy, or other types of physical stress. Alopecia areata is another cause of nonscarring alopecia and is seen in increased frequency in patients with lupus and other autoimmune illnesses (22). Its pathogenesis, histology, and course are distinct from LE nonspecific alopecia (“lupus hair”). Lastly, medication-induced hair loss, as may be seen with medications commonly used in lupus patients such as cyclophosphamide and methotrexate, should also be considered in the differential diagnosis of LE-nonspecific alopecia.

Figure 31-2. (See color plate.) Lupus hair in a patient with active lupus nephritis and low complements.

As with photosensitivity and oral ulceration, assessing prevalence and incidence of LE-nonspecific alopecia is difficult, since most studies do not clearly delineate between scarring alopecia as a result of chronic DLE and LE-nonspecific hair loss. Saurit et al. found that alopecia was the most prevalent nonspecific mucocutaneous manifestation in 77 patients with SLE, affecting nearly 60% of patients. Similar to photosensitivity, diffuse alopecia was more frequent among patients with active SLE (23). Another study performed by Wysenbeek found alopecia in 54% of 74 SLE patients (24,24a). The two studies that specifically separate nonscarring alopecia cite slightly lower incidence rates. Cardinali et al. found that 18 of 58 patients with SLE (31%) had nonscarring alopecia, and confirmed the finding of occurrence in the active phase of SLE (25), whereas another group of investigators in the United Kingdom reported LE-nonspecific nonscarring alopecia in 40% of 73 SLE patients (14).

LE-nonspecific alopecia is typically self limited. The hair usually returns to normal after the underlying SLE activity diminishes. There may be some shortening of the time to regrowth of hair with use of topical minoxidil 2% (26).

Additionally, lupus patients can develop common forms of nonscarring alopecia that are experienced by individuals not having lupus. For example, a male lupus patient can develop typical male pattern balding (androgenetic alopecia) that is unrelated to his lupus. Additionally, a female lupus patient is also at risk for developing a closely related pattern of androgenetic alopecia.

Raynaud Phenomenon

Raynaud phenomenon was included in the original American Rheumatism Association classification criteria set for SLE but was removed when this criterial set was revised in 1982. Raynaud phenomenon will be discussed below as a type of cutaneous vascular reaction pattern.

Cutaneous Vascular Reactions

Reactions that involve the cutaneous vasculature are important to recognize in patients with SLE as they can frequently indicate underlying systemic vascular pathology. In a discussion of cutaneous vascular reactions that can be seen in lupus patients, it is crucial to differentiate between vasculitis (inflammatory vascular wall injury) and several types of vasculopathy. In this context, the term “vasculopathy” is defined as vascular wall narrowing resulting in ischemia or noninflammatory vascular lumen occlusion resulting from thromboembolic disease. Vasculitis is caused by primary inflammation (usually immune-complex-mediated) of vessel walls with secondary occlusion of lumina with fibrin. Vasculopathic processes are multifactorial with some being caused by vascular lumen narrowing (e.g., Raynaud phenomenon/systemic sclerosis) and others being caused by occlusion with bland thrombi in the absence of primary vascular wall inflammation (e.g., hypercoagulable state resulting from antiphospholipid antibody production) (27). There are many causes of vasculopathy (Table 31-2) in patients with lupus. LE-nonspecific vasculopathy is most commonly the result of antiphospholipid antibodies. The cutaneous vasculitides and vasculopathies may present with similar clinical findings, but are important to distinguish from one another, since they have different treatments (i.e., anticoagulation in thromboembolic disease and immunosuppressives/glucocorticoids in vasculitis).

Vasculitis

Vasculitis is typically classified based on the size of the affected vessel (28). In SLE, all sizes of vessels may be affected (29). Leukocytoclastic vasculitis (LCV) (syn. hypersensitivity vasculitis, cutaneous small vessel vasculitis) affects postcapillary venules and presents as palpable petechial lesions on dependant areas (Fig. 31-3). When LCV presents in patients with SLE, other causes of LCV such as medications and infections should be excluded before a diagnosis of primary lupus-associated LCV is assigned. Involvement of medium and/or large vessels may present as purpuric plaques with stellate or retiform borders with or without cutaneous necrosis and ulceration, or subcutaneous nodules (Fig. 31-4). Occasionally lesions may not be purpuric, but rather may resemble urticaria.

So-called urticarial vasculitis is not uncommon in SLE and may be distinguished from urticaria by presence of individual lesions greater than 24 hours, tenderness, and possibly the presence of residual pigment or bruising upon resolution of the urticarial component, and histologic changes of vasculitis (Fig. 31-5). Cutaneous vasculitis may be treated with colchicine, diaminodiphenylsulfone (Dapsone), glucocorticoids, or immunosuppressive medications. Compression stockings play a vital role in preventing recurrences. Vasculitis is discussed in further detail in Chapter 36 .

Table 31-2: Potential Causes of Vasculopathy in SLE

1. Lupus anticoagulant/antiphospholipid antibodies
2. Cholesterol emboli
3. Infectious emboli (endocarditis, etc.)
4. Calciphylaxis
5. Protein C, protein S deficiency
6. Antithrombin III deficiency
7. Heparin/warfarin necrosis
8. Prothrombin 2021A mutation
9. Homocystinemia
10. Cryoglobulinemia
11. Cryofibrinogenemia
12. Disseminated intravascular coagulation
13. Purpura fulminans (meningococcemia)

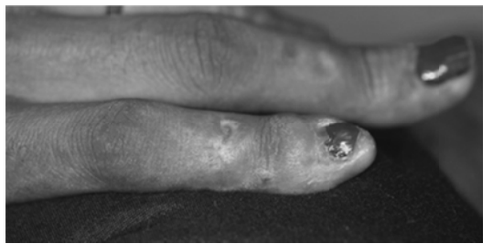

Figure 31-3. (See color plate.) Palpable purpura on the lower extremities in a patient with leukocytoclastic vasculitis and SLE.

Vasculopathy—Ischemic

Raynaud Phenomenon

Rheumatic disease–associated Raynaud phenomenon is associated with intimal hyperplasia of digital arterioles resulting from poorly understood nonvasculitic immunologic processes. With progression of the intimal hyperplasia, the vascular lumen is narrowed resulting in decreased blood flow and distal ischemia. This ischemia is accentuated to the point of clinical symptoms by cold and stress-induced reflexes that induce peripheral vasospasm.

Like photosensitivity, oral ulcers, and alopecia, Raynaud phenomenon has been clearly documented as a cutaneous finding that strongly correlates with underlying systemic lupus erythematosus. Cardinali et al. found that Raynaud phenomenon was found in 39.6% of patients with SLE (25). Additionally it has been shown that Raynaud phenomenon seems to herald a worse prognosis and is associated with higher disease activity scores (1 ,3). The presence of Raynaud phenomenon in patients with SLE may correlate with an increase in DLE lesions on the hands (Fig. 31-6). Skin changes that can be seen in association with chronic severe Raynaud phenomenon include focal ulcerations on the fingertips and periungual areas that result in pitted scarring upon resolution, prominent nailfold capillary ectasia and drop out, punctate cuticular hemorrhage due to incompetent nailfold capillaries, fingertip tuft atrophy, digital calcinosis, and pterygium inversus unguium, A more complete discussion of Raynaud phenomenon can be found in Chapter 36 .

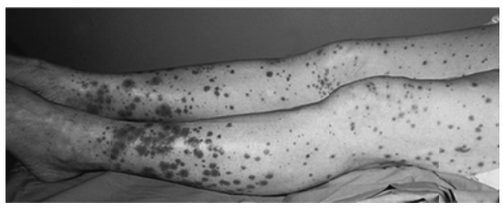

Figure 31-4. (See color plate.) Medium-sized vessel vasculitis with large retiform purpura and smaller ulcerations in an SLE patient.

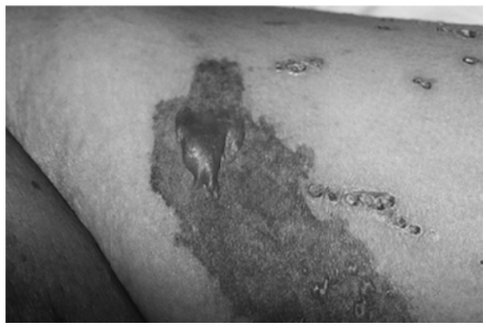

Figure 31-5. (See color plate.) Urticarial vasculitis in a patient with SLE and flaring lupus nephritis.

Vasculopathy—Thromboembolic

Associated with Antiphospholipid Antibodies

Patients with SLE and antiphospholipid antibodies frequently present with cutaneous symptoms. These include livedo reticularis, thrombophlebitis, retiform purpuric plaques, which may later become necrotic and ulcerate, lower extremity ulcers, purpura, ecchymoses, painful skin nodules, and subungual splinter hemorrhages (25 ,30 ,31). Lesions are often present in acral locations, since smaller vessels are more likely to become occluded. Both venous and arterial vessels may be involved, and may result in digital infarcts and even gangrene. Other rarer skin changes associated with antiphospholipid antibodies include atrophie blanche-like lesions (painful, ivory colored stellate scars on the lower extremities), Degos-like lesions (small, porcelain-white circular atrophic lesions with peripheral erythema and telangiectases) and lesions of primary anetoderma (focal loss of dermal elastic tissue, resulting in localized areas of flaccid

or herniated saclike skin (32 ,33). Scheinfeld et al. describe a patient with reticulate and stellate acral pigmentation who had high titers of circulating anticardiolipin antibodies (34).

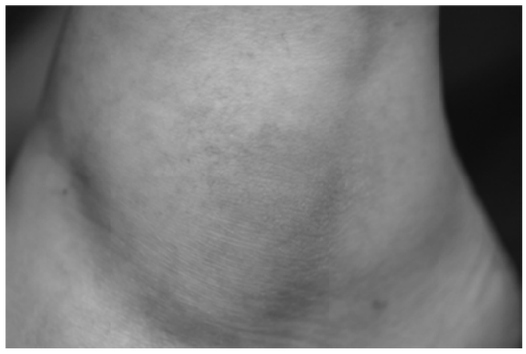

Figure 31-6. (See color plate.) Patient with Raynaud's phenomenon, tapered fingers, and LE-specific skin lesions on the fingers.

Livedo reticularis is clinically manifested as net-like, blanchable, complete or incomplete, red-purple rings on the extremities and less commonly on the trunk resulting from impeded flow of blood through vessels. Some data argue that the incomplete or broken form of livedo reticularis (i.e., livedo racemosa) has a stronger association with the thromboembolic clinical manifestations of the antiphospholipid antibody syndrome than the complete ring variety. The presence of livedo reticularis may be an indication of risk for pregnancy loss.

Sneddon syndrome is characterized by widespread livedo reticularis and ischemic cerebrovascular disease often accompanied by labile hypertension. Antiphospholipid antibodies are seen in at least 41% of Sneddon syndrome patients, and some consider it to be a variant of SLE with antiphospholipid antibodies (35). Livedo reticularis frequently accompanies purpuric skin lesions in vasculopathic conditions (Fig. 31-7).

Not Associated with Antiphospholipid Antibodies

Skin lesions resulting form cutaneous vascular occlusion can be seen in lupus patients for reasons other than phospholipids antiphospholipid antibody-induced thrombosis. An example would be the presence of cryoglobulins. Cryoglobulins have been observed in approximate 25% of various SLE cohorts. Monoclonal cryoglobulins tend to precipitate in cutaneous vessels producing overlying purpura/ecchymosis, hemorrhagic skin necrosis, and ulceration. Mixed cryoglobulins, especially in the context of concurrent hepatitis C virus infection, produce a small vessel cutaneous vasculitis, typically dependent palpable purpura, rather than primary vessel lumen occlusion.

SLE patients are at risk for premature, severe atherosclerosis. Cholesterol crystals can release from atherosclerotic plaques spontaneously or after intravascular procedural manipulation and embolize to smaller vessels producing livedo reticularis as a result of the impedance of blood flow to the overlying skin. (Livedo reticularis is a cutaneous vascular pattern resulting from slowed blood flow that can be seen in a number of clinical settings other than SLE.) When cholesterol crystals embolize to terminal arteries in the digits, purpuric infarction of the tips of toes and/or fingertips can result. This can be confused with SLE vasculitis or antiphospholipid antibody-associated vasculopathy affecting digital vessels.

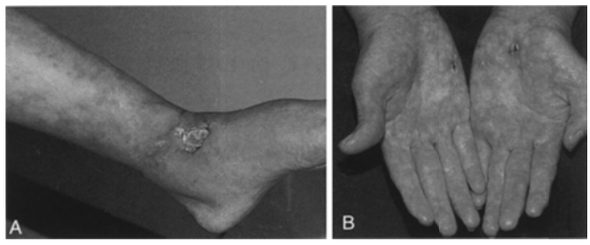

Figure 31-7. (See color plate.) **A**, Livedo reticularis and ulceration of the leg in a patient with systemic lupus erythematosus and high-titer IgG anticardiolipin antibodies. **B,** Similar changes on the hands of the same patient.

Skin necrosis also can result from calciphylaxis in SLE patients with renal failure. These lesions typically present with painful indurated areas of cutaneous hemorrhage that rapidly become necrotic and ulcerate. Radiographic or histopathologic evidence of cutaneous calcification can help diagnose this disorder. It is important to remember that cutaneous necrosis, and ulcerations also can be induced by certain medications (e.g., propylthiouracil, warfarin, hydroxyurea).

Other Cutaneous Vascular Reactions

Urticaria

Urticaria is sometimes associated with LE, and is thought to be a manifestation of the disease process' immune dysregulation. One study found chronic urticaria in 44% of 73 SLE patients (14). This study also reported that some patients noted worsening urticaria with sun exposure. Urticaria typically presents with an acute onset of edematous erythematous papules and plaques (wheels) that itch. It must be differentiated from urticarial vasculitis (see above). Urticarial vasculitic lesions are more likely to be painful, nonblanching (e.g., purpuric), and remain in the same location for at least several days, whereas urticarial lesions tend to be pruritic, blanching, and more evanescent (typically less than 24 hours). A skin biopsy can be helpful in differentiating between the two.

Urticaria is commonly caused by medications, and LE-nonspecific urticaria that occurs merely as part of the patient's lupus diathesis should be considered a diagnosis of exclusion. Additionally, chronic infections and even underlying malignancies may induce urticaria, particularly in treatment resistant cases. Urticaria is often associated with thyroid autoimmunity, which is seen frequently in patients with SLE. Verneuil and colleagues reported that the frequency of antithyroid antibodies was 26.7% in patients with urticaria, as opposed to only 3.3% in normal controls (39). Thus, laboratory studies for antithyroid peroxidase and antithyroid microsomal antibodies may be helpful in evaluating the cause of urticaria in lupus patients.

Treatment of urticaria includes discontinuing any suspected triggering drugs or foods, in addition to the use of antihistamines and other antipruritics. A recent study documented efficacy of hydroxychloroquine in ameliorating symptoms of urticaria (40).

Palmar Erythema

Macular erythema over the hyper- and hypothenar eminences has been reported in lupus patients. One study documented that 4% of a group of 73 patients with SLE had chronic palmar erythema (14). Reticulated palmar

erythema can also be a sign of vasculopathy associated with antiphospholipid antibodies (Fig. 31.7-B).

Periungual Telangiectasia

Dilated capillaries of the nailfolds have been found in LE patients but less frequently than in patients with dermatomyositis (including clinically amyopathic dermatomyositis) or systemic sclerosis. Dilated capillary loops (megacapillaries), and capillary loop dropout are the hallmarks of “scleroderma-pattern” capillaroscopic changes, however, when seen in SLE patients, this pattern of nailfold changes appears to correlate strongly with Raynaud phenomenon. Furtado et al. found that such nailfold telangiectasias in SLE patients were associated with anti-U1RNP antibodies (36). They found no correlation with anticardiolipin antibodies. In another study, telangiectasia and erythema of the proximal nailfold were found in 76% of patients who had both DLE and SLE, but none in patients with DLE in the absence of SLE, suggesting that this is a rather sensitive indicator for systemic disease activity (25).

Erythromelalgia

Erythromelalgia (erythermalgia) is characterized by intense burning pain in the feet and hands, accompanied by local macular erythema and warmth. It differs from Raynaud phenomenon in that it worsens with exposure to heat instead of cold. It may be seen as an isolated clinical entity or in association with several different underlying illnesses, including SLE. It appears to be caused by microvascular arteriovenous shunting. Erythromelalgia that occurs in association with SLE is well documented, and should be included in the list of nonspecific vascular cutaneous reactions in SLE (37 ,38).

Other LE-Nonspecific Skin Lesions

Cutaneous Mucinosis

Small amounts of mucin deposition are sometimes found in LE-specific skin lesions, particularly in biopsies from chronic cutaneous LE lesions. However, some LE patients will manifest papulonodular cutaneous lesions with abundant amounts of mucin in the absence of the classic vacuolar changes in the basal layer of the epidermis or perivascular and perifollicular inflammation seen in LE-specific skin disease (41 ,42 ,43 ,44). Such lesions may be differentiated from the tumid subtype of chronic cutaneous lupus, and appear as indurated erythematous papules, nodules, or plaques, typically on the trunk and/or arms (Fig. 31-8). Histopathologic examination of these lesions reveals diffuse dermal mucin deposits. Some of these patients have LE-specific lesions elsewhere.

The pathogenesis of these lesions remains uncertain, although one report found that fibroblasts isolated from an LE patient with cutaneous mucinosis produced larger amounts of glycosaminoglycan than normal fibroblasts, and the production of this glycosaminoglycan was stimulated by the patient's serum (45). Similar lesions also have been reported in patients with dermatomyositis and scleroderma (46). There have been anecdotal reports of massive cutaneous (46) and periorbital (47) mucinosis in LE. One LE patient presented with nodular cutaneous mucinosis that 3 years later developed into atrophie blanche-like skin lesions (48), with evidence of an underlying vasculopathy, as has been found in other cases (42). Cutaneous mucinoses are often responsive to antimalarial or prednisone therapy (41).

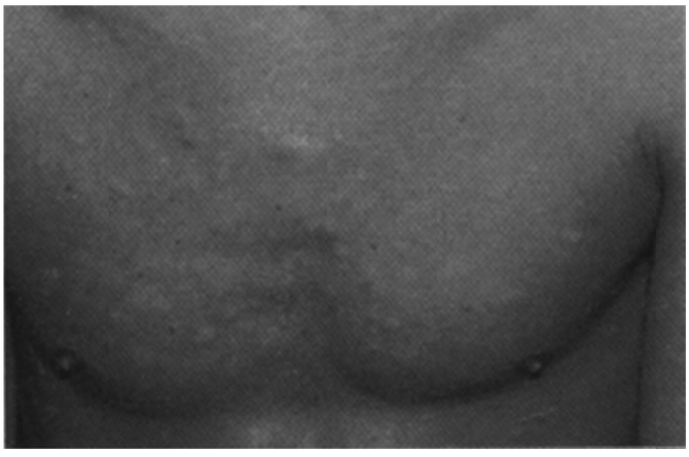

Figure 31-8. (See color plate.) Papular and nodular dermal mucinosis in a patient with systemic lupus erythematosus, including pleural effusions and glomerulonephritis.

Calcinosis Cutis

Calcinosis cutis is less common in SLE than it is in juvenile dermatomyositis and systemic sclerosis, with prevalence rates of 17% according to one study (49 ,50). Calcinosis in SLE has occurred in the setting of normal calcium metabolism and renal function. Calcinosis cutis because of secondary dystrophic calcification also has been reported in SCLE (51), DLE, and especially in lupus panniculitis (52 ,53). Calcification is commonly found on the extremities and buttocks, is often asymptomatic, and is sometimes found as an incidental radiologic finding (49). Calcification in SLE may be periarticular, within joints or muscles, or in the subcutis. Sometimes the overlying skin can ulcerate and the calcified material can be extruded as a white toothpaste-like or pebble-like material. Patients with superficial lesions should protect these areas from trauma with padded bandages. Some improvement of calcinosis cutis has been noted anecdotally in patients treated with aluminum hydroxide (54), calcium-channel blockers (55), colchicine (49), probenecid (56), low-dose warfarin (57), and bisphosphonates (49), although no controlled trials have been performed. Surgical treatment has been reported to provide additional benefit in some cases (58 ,59).

Calciphylaxis presents in patients with chronic renal failure as painful, indurated plaques, ecchymoses, ulceration, and eschar formation. Calcium is deposited in the walls of blood vessels and fibrosis and thrombosis occurs consequently, with secondary ischemia and necrosis of tissues.

Prognosis is poor, with mortality rates between 60% and 80%. Although the cause is unknown, altered calcium-phosphorous metabolism has been implicated. Patients have been treated with parathyroidectomy with variable results (58). Calciphylaxis has been reported in patients with lupus and end-stage renal disease (60 ,61). It has recently been reported that chelation therapy with intravenous sodium thiosulfate has been of benefit to several calciphylaxis patients (62 ,63). If this form of treatment is confirmed to be of value in calciphylaxis, it would be interesting to know whether it might be of therapeutic benefit in cutaneous calcinosis resulting from SLE and related entities such as dermatomyositis and systemic sclerosis.

Nail Changes

A number of nail changes have been noted in LE patients. One study reported nail changes in 31% of 165 SLE patients, the most common change being onycholysis (64). Tosti has noted that patients with SLE may have many nonspecific nail changes including altered keratinization of the nail matrix leading to leukonychia, nail pitting or ridging, and onycholysis or onychomadesis (65). One study reported finding diffuse, dark blue-black nail dyschromia in 52% of 33 African-American SLE patients, apparently from increased melanin deposition (66). Red lunulae have been reported in LE (67 ,68). One study found red lunulae in 11 of 56 (20%) patients with SLE or cutaneous LE, usually in association with periungual erythema or chilblains lupus lesions (68). DLE may affect the nail bed and nailfold and produce marked subungual hyperkeratosis or longitudinal melanonychia (69).

Cutaneous Manifestations of Overlapping Autoimmune Disorders

LE patients often may present with clinical features that overlap with other autoimmune disorders such as Sjögren syndrome and systemic sclerosis. It is therefore not surprising that LE patients sometimes exhibit cutaneous manifestations of other autoimmune disorders, including sicca signs and sclerodermatous skin changes, such as sclerodactyly or morphea (70 ,71). Anti-U1RNP antibodies have been found frequently in sera from patients with SLE-systemic sclerosis overlap syndrome. A recently described disorder called nephrogenic fibrosing dermopathy may mimic scleroderma and should be considered in lupus patients with renal disease who develop rapidly progressive sclerotic skin lesions (Fig. 31-9) (72 ,73). Rheumatoid nodules have been reported in patients with lupus and may or may not be associated with concomitant rheumatoid arthritis (74 ,75).

Acquired SLE-Associated Bullous Dermatoses

Confusion persists concerning the nosology and classification of the bullous skin changes that can occur in LE (76). LE-specific skin diseases (ACLE, SCLE, and DLE) may all manifest with secondary bullae as a consequence of intense injury at the dermoepidermal junction (77) (see Chapter 30). A second group of blistering skin lesions in SLE comprises disorders in which autoantibodies directed at skin antigens lead to blister formation. Since these do not have the specific LE histology, they have been referred to as LE nonspecific bullous lesion (78). The entity commonly described as “bullous SLE” represents an example of such lesions. Active SLE patients occasionally will develop a severe, generalized vesiculobullous eruption that resembles dermatitis herpetiformis (DH) or epidermolysis bullosa acquisita (EBA) (79) (Fig. 31-10). The histology of these lesions shows marked neutrophilic infiltration with papillary microabscess formation similar to DH and the inflammatory variant of EBA. The direct immunofluorescence findings, however, are more consistent with those seen in SLE, which is the primary argument in classifying these lesions as a unique entity, rather than an overlap between EBA or DH and SLE. Autoantibodies against type VII collagen (the EBA antigen), a normal constituent of anchoring fibrils of the sublamina densa zone, are present in some such patients (79). This type of lesion occasionally can represent the initial manifestation of SLE in adults and children and often occurs in the context of very

active SLE including lupus nephritis. The rather vague term “bullous SLE” often has been used to describe such lesions (79), however, we feel that the more descriptive terms “DH-like bullous dermatosis of LE” or “EBA-like bullous dermatosis of LE” are more appropriate, because other forms of bullous skin lesions can occur in SLE patients. Dapsone is a very effective treatment for this subset of disorders. Methotrexate and other immunosuppressives have been utilized as well (80 ,81).

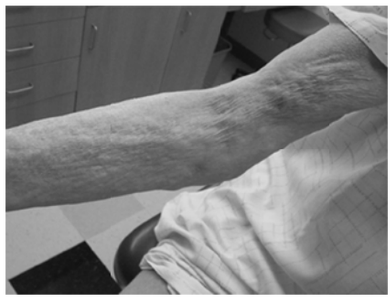

Figure 31-9. (See color plate.) Nephrogenic fibrosing dermopathy in a patient on renal dialysis for SLE-induced end stage renal disease.

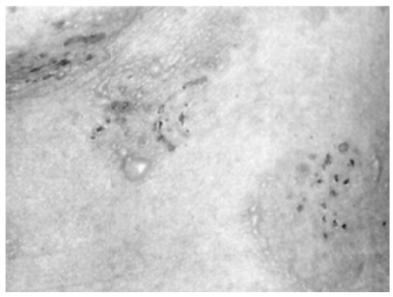

Figure 31-10. EBA-like bullous dermatoses of LE in patient with active membranoproliferative glomerulonephritis.

Examples of bullous skin diseases that have been linked anecdotally to cutaneous LE and SLE include bullous pemphigoid (82), dermatitis herpetiformis (83), porphyria cutanea tarda (84), and pemphigus vulgaris (85). In many of these reports, it is not clear whether the bullous skin changes are the result of the LE autoimmune process or develop as a mere chance occurrence in patients who also have LE. Pemphigus erythematosus also has been linked to LE. Patients with pemphigus erythematosus whose skin lesions are typically accentuated in sun exposed areas often have immunological evidence of LE-like autoimmunity, circulating antinuclear antibodies and immunoglobulin/complement deposition at the dermal-epidermal junction (DEJ) (i.e., the Senear-Usher syndrome) (86). However, such patients rarely develop significant clinical manifestations of SLE.

Less Common LE-Nonspecific Skin Diseases

There is some controversy over whether lichen planus occurs simultaneously in some cutaneous LE patients or whether some cutaneous LE lesions are mimicking lichen planus (87). In one case, lesions of SCLE appeared to evolve into those of lichen planus (88). One study concluded that some LE patients do, in fact, have both skin disorders, based on clinical, histopathologic, and immunopathologic findings (89). The histopathology of lichen planus can sometimes mimic that of LE-specific skin lesions so that it may not always be easy to differentiate between the two when the clinical appearance of the skin lesions is not classic for either.

Acanthosis nigricans has been reported in SLE in the absence of glucose intolerance (90), as well as in the presence of anti-insulin receptor antibodies (Type B insulin resistance). Acanthosis nigricans also has been associated with lupoid hepatitis (91).

LE patients may develop erythema multiforme from drugs or other causes. It is important to remember that sometimes SCLE can mimic erythema multiforme and one can question if the initial report of Rowell syndrome (e.g., erythema multiforme along with chilblains and DLE plus anti-Ro/La autoantibody production) might have been SCLE rather than erythema multiforme (92).

Cutis laxa (acquired loose skin due to destruction of elastic fibers) and anetoderma (focal loss of dermal elastic tissue, resulting in localized areas of flaccid or herniated saclike skin) can occur in lupus patients. Both have been reported to occur as a consequence of previous inflammation with LE-specific skin lesions, and in the absence of such lesions (Fig. 31-11). There has been speculation that loss of elastic fibers may be the result of infiltrating lymphocytes and/or circulating antibodies in patients with SLE (93). Anetoderma has also been associated with the antiphospholipid antibody syndrome.

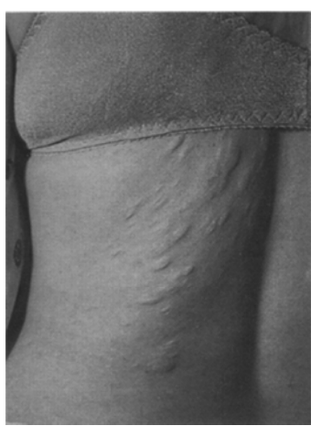

Figure 31-11. (See color plate.) Anetoderma in a patient who had typical discoid lupus erythematosus lesions on other parts of her body.

Multiple eruptive dermatofibromas (small, firm, benign fibrohistiocytic cutaneous neoplasms) have been described to occur in patients with SLE (94).

Acquired ichthyosis, which is characterized by symmetric fish-like scaling of the skin, may present in patients with SLE. A case reported by Tlacuilo-Parra et al. resolved with treatment for SLE (95).

Over the past decade, a spectrum of aseptic dermal granulomatous histopathologic changes referred to as interstitial granulomatous dermatitis/palisaded neutrophilic granulomatous dermatitis has been increasingly described in the skin of patients having autoimmune connective tissue diseases including lupus erythematosus (96 ,97), rheumatoid arthritis (98), antiphospholipid syndrome (99), and nonspecific arthritis (100). Additionally, this histopathologic reaction pattern has been described in other dermatologic settings as well, including drug reactions (angiotensin-converting enzyme [ACE] inhibitors, calcium channel blockers [CCBs], beta-blockers, lipid-lowering agents, antihistamines, anticonvulsants, antidepressants) (101), other hypersensitivity reactions (soy) (102), and paraneoplastic syndromes (lymphoma) (103). Also, a reaction that was first thought to represent interstitial granulomatous dermatitis/palisaded neutrophilic granulomatous dermatitis was subsequently shown to be a reaction to occult cutaneous infections (atypical mycobacteria, histoplasmosis, and HIV) (104 ,105 ,106). This histopathologic reaction pattern has been described in the context of a number of different syndromic designations

(arthritis and interstitial granulomatous dermatitis [Ackerman syndrome], interstitial granulomatous dermatitis with cords, interstitial granulomatous dermatitis with arthritis, interstitial granulomatous dermatitis with plaques and arthritis, rheumatoid papules, Churg-Strauss granuloma, cutaneous extravascular necrotizing granuloma, superficial ulcerating rheumatoid necrobiosis, and palisaded neutrophilic and granulomatous dermatitis of immune complex disease). The pathophysiology of this reaction pattern has been speculated to relate to immune complex deposition.

The interstitial granulomatous dermatitis/palisaded neutrophilic granulomatous dermatitis histopathologic pattern has been associated with a number of different types of cutaneous lesions including red to violaceous papules, plaques, subcutaneous nodules, and rope-like cords. The papules, plaques, and subcutaneous nodules have typically been described in a bilaterally symmetrical distribution on the trunk and/or extremities favoring the skin folds. The rope-like cords that occur in this setting are typically unilateral and have a predilection for the lateral chest wall and axilla. Such lesions can simulate a superficial thrombophlebitis, however, veins are not affected. This histopathologic pattern has also been associated with generalized pruritic scaly eruptions and pigmented purpura (97 ,98 ,99 ,100).

There has been limited success in treating interstitial granulomatous dermatitis/palisaded neutrophilic granulomatous dermatitis lesions with hydroxychloroquine, dapsone, systemic corticosteroids (107).

Dermatoses in SLE not Related to Lupus

Greenwald's law of lupus states that SLE is often falsely accused of causing anything and everything that might happen to a patient subsequent to the diagnosis of SLE (108). Therefore, one must keep in mind that not everything that appears in the skin of a patient having a diagnosis of LE is necessarily a direct cause of LE.

A patient with SLE who presents with skin lesions may have (1) LE-specific skin disease (e.g., ACLE); (2) LE-nonspecific skin disease (e.g., urticarial vasculitis); (3) lesions resulting from SLE-induced internal organ damage (e.g., calciphylaxis); or (4) lesions that occur as a consequence of medical therapy required for SLE (e.g., adverse drug reactions, cutaneous infections). Additionally, patients may present with common dermatoses that are not at all connected to LE or its treatment (e.g., androgenetic alopecia, seborrheic dermatitis, seborrheic keratoses, acne rosacea, allergic contact dermatitis from poison ivy exposure). One study of skin disease in SLE patients found that rashes unrelated to LE autoimmunity were more common (69%) than rashes were related to LE autoimmunity (42%). Nonlupus rashes included rosacea, seborrheic dermatitis, acne, xerosis, facial telangiectasia, and eczema, among others (109). This highlights the need for accurate diagnosis of skin lesions in patients with SLE.

Drug-Related Skin Disorders

Drugs that are administered to LE patients not infrequently induce skin lesions. In a study of 84 consecutive patients with SLE, 6 were noted to have a drug eruption (109). A wide variety of skin lesions can be induced by medications, including SCLE, which is discussed in detail in the previous chapter. Many nonspecific skin lesions that are associated with LE can also be caused by medications, including photosensitivity reactions, alopecia, vasculitis, urticaria, bullous skin lesions, and erythema multiforme. *The Drug Eruption Reference Manual,* which is updated annually by Jerome Z. Litt, is a useful resource that lists the cutaneous side effects of numerous medications that have been cited in the literature (111).

Drug reactions may mimic lupus-specific skin disease, as is seen with medications that induce a photodistributed rash. These include antihypertensives (thiazide diuretics, ACE inhibitors, and CCBs), sulfa-drugs, and nonsteroidal anti-inflammatory drugs (NSAIDS). Toxic epidermolytic necrolysis (TEN) is often very difficult to distinguish from generalized blistering ACLE. TEN more often results from exposure to a number of medications including anticonvulsants, antibiotics, allopurinol, and sulfonamides, among others.

Antibiotic allergy, especially to sulfonamides has been reported as both a predisposing factor and as an exacerbating agent in SLE. Petri and Allbritton compared 221 patients with SLE to age-and sex-matched controls. Allergy to penicillin/cephalosporin, sulfonamides, and erythromycin was significantly more common in exposed patients with SLE than in exposed controls (110). Antibiotic rashes most commonly produce a morbilliform or measles-like eruption that typically occur 3 to 7 days after initiation of the antibiotic therapy. Indeed, this type of skin reaction is the most common presentation of drug hypersensitivity in general (112).

NSAIDs frequently are associated with pruritus, urticaria, and/or edema. Photosensitivity reactions, oral ulcerations, alopecia, purpura, erythema multiforme, TEN, vasculitis, exacerbation of psoriasis, and fixed drug eruptions also have been associated with the use of these agents.

Systemic corticosteroids are commonly associated with acneiform lesions, ecchymoses, and stria formation. Facial erythema, acanthosis nigricans, hypertrichosis, black hairy tongue, and steroid-withdrawal panniculitis are less frequently associated with the use of these agents. Depigmentation, atrophy, and skin necrosis have occurred at corticosteroid injection sites. The immunosuppressive effects of these medications can result in bacterial, viral, and fungal skin infections.

Antimalarials can cause ecchymotic blue-black pigmentary changes in the skin, particularly on photo-exposed areas, oral mucosa, nails, and anterior shins (Fig. 31-12). Bleaching of body hair also can be seen. Quinacrine can cause a yellow discoloration of the skin, sclera, and body secretions and might be misdiagnosed as jaundice. Quinacrine also can cause lichenoid reactions (thickening of the skin often with increased pigmentation and

accentuated skin markings typically associated with pruritus) (Fig. 31-13). The pigmentary changes usually will resolve within several months after stopping the antimalarial agent; however the pigmentation of the oral mucosa may be irreversible.

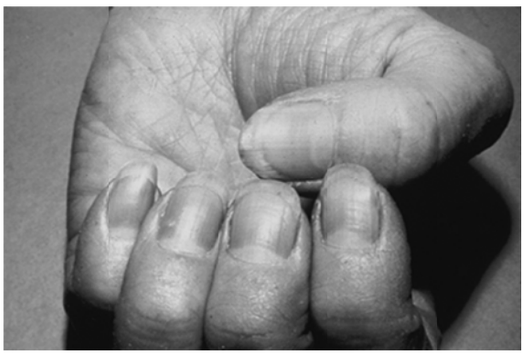

Figure 31-12. (See color plate.) Hydroxychloroquine hyperpigmentation involving the nails and oral mucosa.

Immunosuppressive drugs may cause adverse drug reactions as well. Cyclophosphamide has been reported to induce neutrophilic eccrine hidradenitis, a self-limited skin condition causing painful cutaneous nodules, characterized by an inflammatory cell infiltrate involving the eccrine sweat glands (113). Methotrexate can induce oral ulcerations, TEN, and acral erythema (114).

Both parenteral and oral-gold preparations can cause pruritus, stomatitis, exfoliative dermatitis, lichen planus-like, and pityriasis rosea-like skin reactions. Chrysiasis is the term that refers to the slate-gray pigmentary skin change that occurs in gold-treated patients. The dose-dependent color change is most marked in sun-exposed areas and may be permanent. Gold compounds also may cause drug-induced cutaneous LE, alopecia, purpura, vasculitis, erythema nodosum, and brown-nail discoloration.

Physicians should always consider the possibility that a drug might be inducing cutaneous disease in patients that present with skin problems. A history that a drug was recently started a few weeks before a skin change was noted might implicate an offending medication. However, it is important to realize that some drug-induced skin conditions do not become apparent until after months, and sometimes years of treatment with a particular medication. Delayed cutaneous hypersensitivity reactions to phenytoin sodium (Dilantin) and a number of other drugs characteristically appear at 3 to 6 weeks following initiation of the drug. Delayed drug-induced multiorgan hypersensitivity syndrome (DIDMOHS) (115) and drug rash with eosinophilia and systemic symptoms (DRESS) (116) are synonymous terms that have been used to refer to this characteristic pattern of drug hypersensitivity. Additionally, hydroxyurea-induced dermatomyositis-like skin disease might become apparent only after the patient has been treated with hydroxyurea for several years (117 ,118).

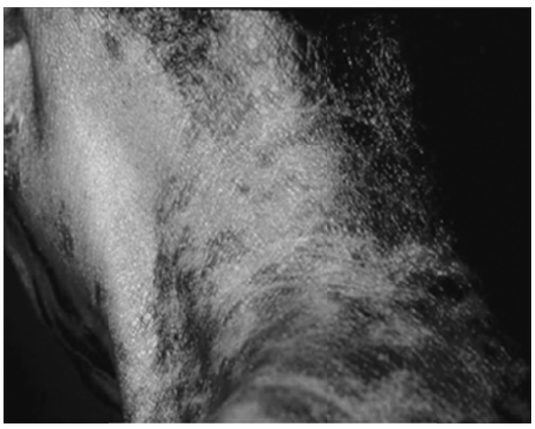

Figure 31-13. (See color plate.) Lichenoid drug eruption from hydroxychloro-quine.

Cutaneous Infections

Infections cause 25% to 50% of morbidity in SLE patients, and major infections are important causes of hospitalization (119 ,120). Patients with SLE develop cutaneous infections as the result of immune dysregulation and/or treatment with immunosuppressive medications. Skin infections were found to be one of the most common types of infection in SLE patients in a review by Juarez et al. (120).

Not only is cutaneous infection with *Staphylococcus aureus* common, but it may eventuate in staphylococcal bacteremia and even death in immunocompromised SLE patients (121). Staphylococcal skin infections include impetigo, folliculitis, furunculosis, pyoderma, and cellulitis. Cutaneous streptococcal infections, including cellulitis from streptococcus pyogenes and *Streptococcus pneumoniae*, have been described in lupus patients (122 ,123). Mortality from bacterial infections was found to correlate with hypocomplementemia (121). One group of authors concluded that seemingly minor bacterial infections of the skin in patients with SLE should warrant aggressive antimicrobial treatment, especially when there is complement deficiency (122).

Atypical mycobacterial infections are a frequent cause of morbidity in SLE patients. Cutaneous infections with *Mycobacterium kansasii*, *M. marinum*, *M. haemophilum*, *M. chelonae*, and *M. fortuitum* have been reported. Presentations varied, and included erythematous papules, plaques, cellulitis, panniculitis mimicking lupus profundus, ulcerative subcutaneous nodules, abscesses, and vasculitis (Fig. 31-14). In a few cases hematogenous dissemination occurred (124 ,125 ,126 ,127 ,128 ,129).

Herpes zoster is the most frequent viral infection in lupus patients and occurs mainly in those with previous histories of nephritis, hemolytic anemia, thrombocytopenia, and previous use of cyclophosphamide (119). Localized herpes zoster is the most common clinical presentation, and is typically seen in patients with inactive disease, who take less

than 20 mg/day of prednisone. Disseminated herpes zoster (affecting two or more noncontiguous dermatomes) and bacterial superinfection may occur and are related to the use of high doses of corticosteroids or immunosuppressants, especially mycophenolate mofetil and cyclophosphamide (119). Recurrent and disseminated herpes simplex infection may be a problem for patients with SLE, again especially those treated with medications like mycophenolate mofetil (130 ,131). Disseminated viral infections are classically characterized by painful or pruritic vesicles on erythematous bases, often with central umbilication. Any lupus patient with blisters should have a Tzanck preparation or direct fluorescent antibody exam from a representative lesion. Patients with herpes zoster who are immunosuppressed may require treatment with intravenous acyclovir in addition to holding immunosuppressive medications.

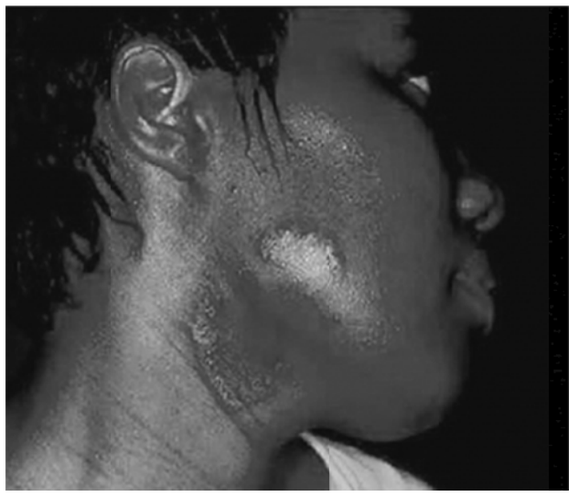

Figure 31-14. (See color plate.) Mycobacterium chelonae infection in a male with SLE on immunosuppressive medication.

Norwegian (crusted) scabies is a severe skin infestation which has been described in patients who are immunosuppressed, including those with SLE (132). Patients typically present with intensely pruritic, widespread crusted erythematous plaques, which may mimic hypertrophic DLE, psoriasis, or even impetigo. Mite counts are very high and the diagnosis is easily confirmed with a skin scraping. Treatment with both topical and oral antiparasitic agents (i.e., permethrin and ivermectin) is usually required.

Cutaneous fungal infections can occur frequently in SLE patients, especially in those receiving high doses of corticosteroids (Fig. 31-15). Oral candidiasis is the most common infection (119). Onychomycosis from candida species, as well as from dermatophytes, has a higher prevalence in SLE than in controls (133 ,134). Cutaneous deep fungal infections such as coccidioidomycosis, histoplasmosis, cryptococcosis, and chromoblastomycosis have presented in patients with SLE particularly those on immunosuppressive medications (135 ,136 ,137 ,138). Such infections typically present as erythematosus papules, nodules, plaques, or cellulitis-like changes. Rarer infections, which include those caused by *Penicillium marneffei,* subcutaneous phaeohyphomycotic cyst as a result of *Exophiala jeanselmei,* and primary cutaneous mucormycosis may present significant morbidity in lupus patients (139 ,140 ,141). Patients with SLE, a fever and new skin lesion(s) should have a biopsy, including tissue sent for both routine histologic evaluation with special stains for organisms, and culture directed at detecting common bacterial as well as mycobacterial and fungal infections.

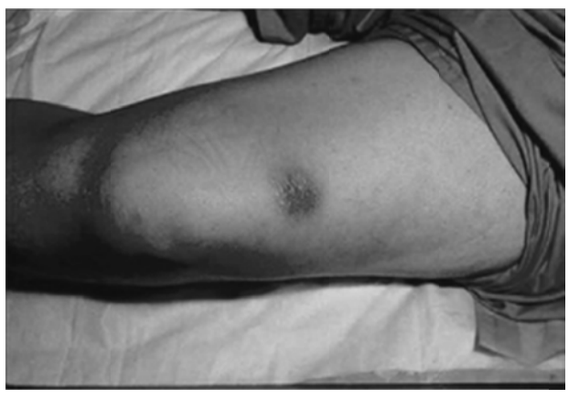

Figure 31-15. (See color plate.) Dermatophyte infection in an SLE patient mimicking DLE.

The Skin Biopsy

As this and the previous chapter have emphasized, the skin biopsy plays a central role in the diagnosis, classification, and management of lupus skin disease. In the ideal world, a fully trained dermatologist would be available to help evaluate, biopsy, and comanage any lupus skin lesion about which a rheumatologist or primary care physician might have questions. However, in the United States there is a growing dermatology manpower problem and American dermatology is drifting away from a focus on clinical issues relating to complex medical dermatology problems such as cutaneous LE. Therefore, for the rheumatologists and primary care physicians who do not have ready access to a dermatologist, we offer the following comments pertaining to skin biopsy techniques.

There are three types of skin biopsy techniques commonly used in dermatology: punch excision, shave excision, and scalpel excision. The shave excision technique is used predominately for suspected cutaneous malignancies in which only a superficial biopsy specimen containing predominately epidermal and superficial dermal tissue is required for diagnosis. This technique is not adequate for obtaining the deeper tissue necessary for an appropriate evaluation of cutaneous LE lesions. When one is interested in the subcutaneous pathology such as in suspected lupus profundus/panniculitis, a full-thickness wedge excision obtained by scalpel followed by suturing is the best way to ensure an adequately deep sample of subcutaneous tissue plus overlying dermis and epidermis. However, since the epidermis and dermis are the most frequently affected components of the skin in most types of LE skin lesions, a punch type excisional biopsy is the technique most commonly used in LE patients.

The punch biopsy technique itself is a very straightforward and can be performed by any physician or a physician assistant (142). It can be relatively painless when performed under local anesthesia accomplished by using lidocaine (Xylocaine) buffered with sodium bicarbonate delivered with a 30- or 32-gauge needle. The small linear scar left behind after a 3- or 4-mm punch biopsy defect has been sutured can be virtually invisible after healing. Facial skin heals exceedingly well in this regard. On less cosmetically sensitive areas of skin, the biopsy defect can be left open to heal by secondary intention with little risk. Thus, there should be no hesitation in obtaining biopsy specimens from facial skin lesions if this information might be of help in the diagnosis or management of an LE patient.

More important than the technical aspects of a skin biopsy is the skill and experience that the pathologist who examines the biopsy specimen has in evaluating inflammatory skin disease. Just as muscle pathology is a highly specialized area of pathology, so is dermatopathology. Every effort should be made to ensure that a biopsy from an inflammatory skin lesion is reviewed by a board-certified dermatopathologist. The value of the dermatopathologic evaluation can be enhanced by providing the dermatopathologist relevant clinical and laboratory information concerning the patient when submitting the biopsy specimen.

Take Home Points

- Patients with SLE may present with the more common lupus nonspecific skin lesions: photosensitivity, mucosal ulceration, alopecia, and Raynaud phenomenon. These correlate with SLE disease activity and may be used as a type of biomarker in most patients.
- Patients with SLE often have lupus-nonspecific cutaneous vascular reactions. These include true vasculitis of any sized vessel, and thrombotic vasculopathies, of which there are several different causes, including thrombosis related to antiphospholipid antibody syndromes. Disease presentations range from palpable purpuric lesions, urticarial lesions, eschar formation, and ulceration, to gangrene. Activity of the cutaneous vascular lesions often correlates with SLE disease activity.
- Patients with SLE have other less commonly encountered lupus nonspecific skin diseases which may cause significant morbidity. These include, cutaneous mucinosis, nail changes, bullous dermatoses, lichen planus, acanthosis nigricans, erythema multiforme, cutis laxa, eruptive dermatofibromas, and acquired ichthyosis.
- The clinician should exclude drug-induced dermatoses when a patient with SLE presents with skin lesions that are atypical or are discordant to the clinical context. Drug-induced skin lesions can simulate both LE specific skin disease (e.g., subacute cutaneous LE) and lupus non-specific skin disease (e.g., palpable purpura because of small-vessel cutaneous leukocytoclastic vasculitis).
- The clinician should exclude cutaneous infections when a patient with SLE presents with skin lesions. Such lesions might represent primary cutaneous infections or be a reflection of systemic infection with opportunistic organisms.
- Many patients with skin lesions and SLE may benefit from dermatologic referral and possibly skin biopsy.

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Section V
Clinical Aspects of Lupus

Chapter 32

The Clinical Presentation of Systemic Lupus Erythematosus

Daniel J. Wallace

Before the description of the lupus erythematosus (LE) cell by Hargraves et al. (1) in 1948, systemic lupus erythematosus (SLE) was considered to be a rare, fulminant disease occurring in young women, with classic rash and a fatal termination in months. The illness now is conceptualized as a chronic disorder of a pleomorphic nature. No classic pattern exists, and the diagnosis must be based on an overall view of the clinical picture with the aid of serologic and laboratory studies and, if required, biopsies or other diagnostic procedures. This chapter presents an overview of the clinical presentation of SLE; the following chapters detail its involvement in various organ systems.

History

The author commences the interview by asking the patient how they feel, and a social, occupational, and emotional history and listing of current medications precedes the review of systems along with eliciting prior therapeutic interventions and workups. All of the items in Tables 32-1 and 32-2 should be covered. The physician should inquire specifically about the effects of sun exposure on the patient's skin, as well as probe for constitutional symptoms of fatigue, fever, weight loss and aching. Queries include what time of day is associated with stiffness (e.g., morning stiffness for lupus, afternoon for fibromyalgia) and aching, and which joints hurt the most. Early symptoms and signs can be subtle. Up to 10% of healthy individuals have a positive antinuclear antibody, and two thirds who are told by a doctor they have lupus do not fulfill established criteria for the disease (2). It is often useful to have patients fill out a detailed medical history questionnaire at the time of their first visit, which should take less than 30 minutes to complete. This allows detection of subtle psychosocial or sensitive issues that otherwise would not be found. Having patients write down what bothers them, in their own words, can be revealing. Filling out a questionnaire also decreases the possibility that important information might be inadvertently omitted.

Chief Complaint

Many variations are seen in the presenting complaint that brings a patient to the physician. Diagnosis is rendered difficult by the protean manifestations of the disease. The classic presentation of butterfly rash and arthritis in a young woman occurs in a minority of patients. Initially, any system may be affected and heal; months or years later, the same or another system may become involved. Table 32-3 lists the chief complaints noted at the initial diagnosis of SLE in several large studies. The two major areas of involvement are joint and cutaneous systems, followed by the nonspecific complaints of fatigue, fever, and malaise. The presentation of 101 children who were followed at the Mayo Clinic was similar to that of adults (4). Additionally, Ropes (5) found that joints were the first system to be involved in 27% of 142 patients, followed by fever, weight loss, and malaise (25%), and then skin rash (20%). Grigor et al. (6) followed 50 patients with lupus; the initial manifestations were arthritis or arthralgia (62%), cutaneous (20%), fever and malaise, thrombocytopenia, hemolytic anemia, and neuropsychiatric symptoms (4% each), and recurrent thrombophlebitis (2%). If present, organ-system damage evolves early. Rivest et al. followed 200 lupus patients and showed evidence for organ-system damage a mean 3.8 years after disease onset (7). In the <LUpus in MInority populations: NAture vs. nurture (LUMINA) cohort of 471 patients, the first American College of Rheumatology (ACR) criteria fulfilled were arthritis (34.5%), photosensitivity (18.2%), and positive antinuclear antibody (14.2%) (8).

Variations in Clinical Presentation

The presentation and clinical characteristics of SLE are presented in detail in the following chapters. Age and sex may cause variations in the appearance of the disease; these distinctions are summarized here. Chapter 49 covers features of incomplete lupus, other rheumatic diseases, and undifferentiated connective-tissue disease.

Table 32-1: Cumulative Percentage Incidence of SLE Manifestations

Manifestations	Harvey et al. (54) 1954(105 cases)	Dubols and Tuffanelli (55) 1964 (520 cases)	Estes and Christian (79) 1971 (140 cases)	Fries and Holman (42) 1977 (193 cases)	Hochberg et al. (14) 1985(150 cases)	Pistiner et al. (3) 1991 (464 cases)	Eurolupus (69) 1992 (704 cases)	GLADEL Cohort (67) 2003 (1214 cases)
I. Systemic Sx								
A. Fever	86	84	-	55	-	41	52	57
B. Weight loss	71	51	-	31	-	-	-	27
II. Musculoskeletal								
A. Arthritis and arthralgia	90	92	95	53	76	91	84	93
B. Subcutaneous nodules	10	5	11	-	12	-	-	
C. Myalgias	-	48	-	42	-	79	-	18
D. Aseptic bone necrosis	-	5	-	-	24	5	-	1
III. Cardiorespiratory								
A. Cardiomegaly	15	16	-	10	-	-	-	17
B. Pericarditis	45	31	19	6	23	2	-	3
C. Myocarditis	40	8	8	-	-	12	-	
D. Cardiac failure	8	5	11	-	-	3	-	
E. Systolic heart murmur	44	20	-	38	-	12	-	
F. Diastolic heart murmur	-	1	-	2	-	1	-	
G. Libman-Sacks valvulitis	32	-	-	-	-	1	-	
H. Hypertension	14	25	46	-	-	25	-	27
I. Pleurisy	56	45	48	41	57	31	-	22
J. Pleural effusion	16	30	40	16	-	12	-	
K. Lupus pneumonia	22	1	9	-	-	6	-	3
IV. Cutaneous-vascular								
A. Skin lesions, all types	85	72	81	67	-	55	-	
B. Butterfly area lesions	39	57	39	10	61	34	58	61
C. Alopecia	3	21	39	45	45	31	-	58
D. Oral/nasal ulcers	14	9	7	18	23	19	24	42
E. Photosensitivity	11	33	-	-	45	37	45	56
F. Urticaria	7	7	13	-	-	4	-	
G. Raynaud	10	18	21	17	44	25	34	28
H. Discoid lesions	-	29	9	10	15	23	10	12
V. Nervous system								
A. CNS damage, all types	-	26	59	-	39	-	27	26
B. Peripheral neuritis	-	12	7	-	21	5	-	1
C. Psychosis	19	12	37	-	16	5	-	4
D. Seizures	17	14	26	8	13	6	-	8
VI. Ocular lesions								
A. Cytoid bodies	24	10	-	-		4	-	
B. Uveitis	-	1	-	2	-	1	-	1
VII. Genitourinary								46
A. Proteinuria/abnormal sediment	65	46	53	47	-	31	39	7
B. Nephrotic syndrome	-	23	26	-	13	14	-	
VIII. Gastrointestinal								
A. Dysphagia	6	2	-	-	-	8	-	
B. Severe nausea	14	53	-	36	-	7	-	
C. Diarrhea	8	6	-	25	-	8	-	
D. Ascites	-	11	9	-	-	-	-	1
E. Abdominal pain	10	19	16	34	-	1	-	
F. Bowel hemorrhage	5	6	-	6	-	1	-	
IX. Hemic-lymphatic								
A. Adenopathy	34	59	36	23	-	10	12	15
B. Anemia (<11 g)	78	57	73	38	57	30	-	-
C. Hemolytic anemia	-	-	12	-	8	8	8	12
D. Leukopenia (<4,500)	-	43	66	35	41	51	-	42
E. Thrombocytopenia (<100,000)	26	7	19	-	30	16	22	19
X. Serologic								
A. Hypoalbuminemia	58	32	77	-	30	-	-	
B. False + VDRL	15	11	29	-	26	-	-	
C. LEPREP	82	82	78	-	71	42	-	
D. ANA	-	-	87	95	-	96	96	98
E. Low C3	-	-	-	40	59	39	-	49
F. Anti-DNA	-	-	-	39	28	40	78	71
G. Anti-Sm	-	-	-	26	17	6	10	51
H. Anti-SSA (Ro)	-	-	-	-	32	19	25	48
I. Anti-RNP	-	-	-	-	34	14	13	
J. Anticardiolipin	-	-	-	-	-	38	-	

CNS, central nervous system; VDRL, Venereal Disease Research Laboratory (a syphilis test).

Table 32-2: Cumulative Percentage Prevalence of 16 Clinical and Laboratory Manifestations in 2,000 Lupus Patients

(Attributable to lupus during the course of their disease; in published studies since 1975 collected by the author)

Positive ANA	97%
Arthritis, arthralgia, or myalgia	80%
Skin changes	71%
Low complement	51%
Cognitive dysfunction	50%
Fever	48%
Elevated anti-dsDNA	46%
Leukopenia	46%
Pleuritis	44%
Proteinuria	42%
Anemia	42%
Antiphospholipid antibodies	35%
Elevated gamma globulin	32%
Pleural or pericardial effusion	12%
Central nervous system vasculitis	12%
Adenopathy	10%

Table 32-3: First System Involved as Determined by History (%)

Manifestation	Dubois and Tuffanelli (50) (520 cases)	Harvey et al. (49) (105 cases)	Haserick (69) (275 cases)	Larsen et al. (70) (200 cases)	Norris et al. (5) (101 cases)
Arthritis and arthralgia	46	47	55	59	48
Discoid lupus	11	0	4	0	13
Butterfly area eruptions and blush	6	20	17	14	-
Eruptions on other parts of body (nonspecific dermatitis)	2		0		
Fever	4	2	0	1	24
Fatigue, malalse, weakness	4	17	0	0	
Renal involvement	3	5	3	6	-
Pleurisy	3	5	2	2	-
Edema and anasarca	1	0	0	0	2
Positive STS	2	5	8	4	-
Cervical adenopathy	2	0	0	0	-
Anemia	2	4	0	0	-
Raynaud's phenomenon	2	3	5	1	-
Myalgia	2	0	0	0	-
Photosensitivity reaction	1	4	4	0	-
Pericarditis	1	1	0	2	-
Pleural effusion	1	0	2	0	-
Epilepsy	1	0	3	0	-
Generalized adenopathy	1	2	0	1	-
Purpura	-	-	-	-	9
Mouth ulcers	-	-	-	-	3

STS, biologic false positive for syphllis.

Proto or Latent Lupus

When does lupus begin? Sixteen healthy Finnish women who stored sera in connection with a maternity welfare program developed SLE years later. Ten (62.5%) were ANA positive at the time of collection, compared with 6% of controls (9). 130 individuals diagnosed with SLE while serving in the U.S. military had previously donated blood to the Army/Navy Serum Repository. ANAs, as well as other autoantibodies, were positive an average of 3.3 years prior to diagnosis (10).

A 1981 survey of our 609 private patients who were diagnosed between 1950 and 1980 revealed a 4.1-year interval between the onset of symptoms and the diagnosis of SLE (11). Our survey of 464 patients with idiopathic SLE who were seen between 1980 and 1989 documented a 2.1-year interval (3). Is the disease changing, or have increased physician awareness and newer diagnostic and serologic testing made it possible to diagnose SLE earlier? In the 0- to 19-year age group, it took only a mean of 3 months to make the diagnosis; in patients older than 60 years, the more subtle presentation of idiopathic SLE took 3.2 years to detect, on average. One third of 44 patients

who were referred to our group with a positive ANA to rule out lupus but who did not fulfill ACR criteria for the disease at the time of the visit had clear-cut SLE 6 months later (12). Urowitz et al. identified 22 patients with a constellation of features that were suggestive of SLE but who did not fulfill ACR criteria (13). Over a 5-year observation period, seven patients (32%) evolved into SLE. Few had organ-threatening disease, and no predictive factors distinguished the 7 who developed SLE from the 15 who did not. Hochberg et al. reported a 1-year interval among 150 patients who were seen between 1980 and 1984 (14). The Eurolupus cohort of 1,000 patients reported a mean 2-year period between the onset of symptoms and diagnosis in 1993 (15). Improved diagnostic techniques have allowed for more rapid diagnosis. In Limerick, Ireland, the mean time from symptoms to diagnosis has decreased from 102.8 months to 11.5 months between the 1970s and 1990s (18). By 2004, the interval had dropped among the LUMINA group to a mere 9 months (8).

In summary, nonorgan-threatening lupus, especially in older patients, can be difficult to diagnose initially; additionally, it may be difficult to determine when SLE started.

Lupus in Children

See Chapter 53 , Systemic Lupus Erythematosus in Childhood and Adolescence.

Late-Onset Lupus

Defined as disease with onset in patients over the age of 50 years, late-onset lupus often is insidious (19), with a polymyalgia-like or rheumatoid arthritis-like pattern (20), and it may be difficult to distinguish from autoimmune thyroiditis (4) or primary Sjögren syndrome (21). It comprises 6% to 20% of all cases (22) and includes more men than any other group, except young children (Table 32-4). The clinical course generally is benign, and the disease is more easily controlled with medication. One of our patients had active disease at age 90 (23), and onset has been reported at age 92 (24). The relatively small number of patients with late-onset lupus in large, published series led Ward and Pollison (25) to perform a meta-analysis based on nine studies of its clinical and laboratory features. Their 1989 effort compared 170 late-onset patients with 1,612 younger-onset patients. They concluded that the older-onset subset had more serositis, interstitial lung disease, Sjögren syndrome, and anti-La/SSB. A significantly lower incidence of alopecia, Raynaud phenomenon, fever, lymphadenopathy, hypocomplementemia, and neuropsychiatric disease was present (26). Only one of the reviewed studies was population based (27). These findings have been confirmed among European (15 ,16 ,17) and Asian populations (28 ,29 ,30 ,31 ,32). In a consortium of lupus centers, 86 patients with onset at 55 years of age or older were compared with 155 who experienced onset younger than age 40 (33). The former group had more cardiovascular, ocular, and musculoskeletal disease, and greater tissue damage, but this could not necessarily be attributed to lupus per se. Hence, this group has a lower 5- and 10-year survival than younger lupus.

Table 32-4: Sex Ratios by Age at Age of Onset or First Diagnosis of SLE

Age at Onset or First Diagnosis (yr)	Female:Male Ratio
0–4	1.4:1
5–9	2.3:1
10–14	5.8:1
15–19	5.4:1
20–29	7.5:1
30–39	8.1:1
40–49	5.2:1
50–59	3.9:1
60 and above	2.2:1

Additional reports have suggested that HLA-DR3 is more common in whites with late-onset lupus (14), autoantibodies other than ANA are much less common in late-onset patients (34 ,35), serositis is the most frequent presenting feature (36 ,37), late-onset lupus is more frequent in whites than African Americans (38), and African Americans with late-onset lupus have a worse prognosis than whites with late-onset disease (39). Braunstein et al. (40) evaluated the joint films of 24 patients with SLE onset older than age 50 years. No differences were noted in the amount of osteoporosis, erosions, or soft-tissue calcification, but soft-tissue swelling was significantly increased in the older group.

In summary, most studies have shown that patients with SLE onset older than age 50 years have more serositis, pulmonary parenchymal disease, and Sjögren syndrome, less central nervous system involvement and nephritis, and a better lupus prognosis.

Male Lupus

Although males constitute only 4% to 18% of those with SLE, their clinical presentation is similar to that observed in women. Hochberg et al. (14) noted a mean age of onset in men of 40.4 years (vs. 31.8 in women) among a 150-patient cohort. Except for a statistically significant increase in peripheral neuropathy, no other clinical, laboratory, or human leukocyte antibody (HLA) phenotypic differences, could be found.

If we restrict our review to studies including at least 30 males, Ward and Studenski (41) compared 62 men with 299 women who were followed at Duke University between 1969 and 1983; 23 clinical and laboratory variables were analyzed. The only significant differences were

more seizures and renal disease in the men. Pistiner et al. (3) compared 125 clinical and laboratory parameters among 30 men and 434 women who were seen in our office between 1980 and 1989. Only four significant differences were observed ($p < 0.01$): men had less alopecia and fibromyalgia but more nephritis and hypocomplementemia. Fries and Holman (42) observed that men had more anti-DNA and skin disease; Urowitz et al. (43) found that men had more pleuritis but less photosensitivity, alopecia, and thrombocytopenia. The 92 males in the Eurolupus cohort (15) had more arthritis and less serositis but no other differences. This includes 30 males who were studied in more detail by Font et al. (44) in Spain. Sixty-one Chinese male patients with lupus had less arthritis and leukopenia and anti-Ro(SSA) than matched females (45). Forty-one males followed by the Hopkins Lupus Cohort had more thrombotic episodes, lower complement, more hemolytic anemia, and seizures (46). Seventy-two males in Taiwan had more renal and skin diseases than females but less arthritis and adenopathy (47). Fifty-nine Danish males had more serositis, nephropathy, and atherosclerotic complications than 454 females (48). Alarcon-Segovia et al. in Mexico City compared 107 males with 1,209 females (49). They had a higher prevalence of anti–double-stranded (ds)DNA antibodies, renal disease, and vascular thromboses and took higher doses of corticosteroids. Another Mexican survey and a study of 30 male Turks (compared with 100 females) with SLE also confirms this (50 ,51). Black males may have a more aggressive presentation of lupus (52). The reader is referred to Isenberg's critical review of the so-far-difficult search for unifying features for male lupus (53).

In summary, male lupus is at least as severe or more severe than female lupus.

Constitutional Symptoms

The generalized symptoms of fever, weight loss, malaise, and fatigue do not fit into any organ-system classification; therefore, they are discussed here.

Fever

Fever secondary to active disease was recorded at some time in 86% of Harvey's patients who were seen in the early 1950s (54), in 84% of Dubois' 520 patients between 1950 and 1963 (55), in 55% of 193 patients at Stanford University in the 1960s and 1970s (42), 52% of the Eurolupus cohort (15), and in 41% of Wallace's 464 patients seen between 1980 and 1989 (3). This consistent, decreasing trend probably reflects better understanding of the disease and the greater availability of nonsteroidal anti-inflammatory drugs (NSAIDs). No fever curve or pattern is characteristic. SLE can present with fever as its sole manifestation (56). Active SLE can result in temperatures as high as 106°F (41°C). It can be difficult to distinguish the fever of SLE from that caused by complicating infections. A review of 617 patients with fever of unknown origin from five classic studies found SLE to be the cause in 0% to 5% of cases (57). Most recently, in 2003 an autoimmune etiology was found in 17% of 192 patients with a fever of unknown origin (out of 290) where a source was identified. Eight (4%) had SLE (58).

Fever in SLE results from endogenous pyrogens that are produced largely by polymorphonuclear leukocytes and monocyte/macrophages. These include tumor necrosis factor α; interleukin (IL)-1, -2, and -6; interferons, and arachidonic acid products. IL-1, in particular, promotes the release of arachidonic acid and, ultimately, prostaglandin E2 (PGE_2). PGE_2 is known to exert a direct pyrogenic effect on the hypothalamic thermoregulatory center (59 ,60).

Stahl et al. (61) at the National Institutes of Health (NIH) studied 106 hospitalized patients with SLE. In 63 patients (38%), 83 febrile episodes were recorded; 60% resulted from SLE activity, 23% from infection (bacteremia was present in one half of these and was fatal in one third), and 17% from other causes (primarily postoperative fevers and drug reactions). The single most useful feature identifying infection was the presence of shaking chills. Leukocytosis (especially in the absence of steroid therapy), neutrophilia, and normal anti-DNA levels also were helpful. The SLE components associated with fever were dermatitis, arthritis, and pleuropericarditis. Although often associated with SLE, a daily low-grade fever, up to 100°F (37.8°C), may be overlooked unless patients are specifically asked to check their temperatures daily. Inoue et al. (62) followed 49 patients with SLE through 74 febrile episodes and found that 25 episodes were secondary to infection. Discriminant analysis showed that 95% of 74 febrile episodes could be correctly classified as to the cause of fever when a combination of white blood cell count (low with SLE, normal to high with infection) and α_2-globulin levels (high with SLE, normal with infection) are used as variables. A close correlation between interferon-α (but not IL-1 or tumor necrosis factor) and degree of fever was observed in 25 untreated patients with SLE (63).

Temperature elevation in an illness that is characterized by a high incidence of this finding should not prevent the physician from carefully searching for other causes. The frequent occurrence of infection in patients with SLE often warrants blood cultures, urine cultures, and chest radiographs. Urinary tract infections are common in young women, who may be asymptomatic. Opportunistic infections and drug reactions should be considered. The cause of the temperature elevation should be investigated before suppressing this helpful clinical finding by administering or increasing the dose of salicylates, NSAIDs, or corticosteroids.

In summary, fever can be a manifestation of lupus, medication, or infection. All fevers, especially in those already on corticosteroids or immune suppressives, warrant prompt attention and aggressive management.

Anorexia and Change in Weight

Anorexia and an insidious onset of weight loss over a period of months were noted in 51% of patients in the Dubois series (55), in 71% of patients in Harvey et al. (54), in 31% of 193 patients followed by Fries and Holman (42), in 63% of Rothfield's 209 patients (64), and in 35% of children with SLE (65). It was a clinical finding in 9% of 704 European patients with SLE who were evaluated at a routine visit (66 ,67). The degree of weight loss almost always is less than 10% and most immediately precedes the diagnosis of SLE. Weight gain in SLE usually results from nephrotic syndrome, ascites, and adjunctive therapies (e.g., antidepressants) or corticosteroid treatment.

Malaise and Fatigue

A sense of malaise and fatigue is common in patients with SLE, especially during periods of disease activity. They feel tired and achy but initially find difficulty in pinpointing the problem. Low-grade fevers, anemia, or any source of inflammation can result in fatigue, as can emotional stress, fibromyalgia, or depression. The administration of certain cytokines, such as IL-2 and interferon-α induce fatigue (68), and SLE is associated with cytokine dysregulation. Rothfield (64) noted moderate to severe fatigue in 81% of 209 patients, and Fries and Holman (42) noted fatigue in 82% of 193 patients. Fifty-nine patients who were followed at the NIH filled out a fatigue questionnaire (69). Their mean fatigue severity (on a scale of 1 to 7) was 4.6. Of these, 53% reported fatigue to be their most disabling symptom, although it did not correlate with any laboratory measure. Three surveys published in the late 1990s suggested that fibromyalgia was the most important component of fatigue in lupus patients (70 ,71 ,72). Omdal et al. associated depression and hysteria with fatigue among 57 lupus patients but did not screen for fibromyalgia (73). No correlations with serum cytokines, antiphospholipid antibodies or other disease characteristics were noted (74). Among 120 lupus patients, those with fatigue tended to be less fit and have reduced exercise capacity andmuscle strength (75 ,76). Nor surprisingly, disease activity correlates with fatigue in groups where more serious lupus is treated (75 ,77).

Goldenberg's review of the literature suggests that the prevalence of fatigue in patients seen in primary-care settings ranges from 14 to 25% and costs $1 billion a year in diagnostic evaluations in the United States (78). (See Chapter 57 , Principles of Therapy and Local Measures, for a discussion of the management of fatigue.)

In summary, most lupus patients complain of fatigue. The most common associations are inflammation, fibromyalgia and psychosocial stressors, deconditioning, and the effects of medication.

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Chapter 33

The Musculoskeletal System

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Joints, muscles, and their supporting structures are the most commonly involved system in systemic lupus erythematosus (SLE), affecting 53% to 95% of patients (see Chapter 32 , Table 32-1). Kaposi (1), in 1872, first described the joint manifestations, and the reader is referred to a historical review (2). Most commonly, musculoskeletal symptoms are the chief complaint in lupus (see Chapter 32 , Table 32-2), and articular pain is the initial symptom in 50% of patients (3). The biochemical mediators of joint inflammation in SLE and the pathophysiology of this arthritis have not been well studied. This chapter will review these issues.

Joints: Symptoms, Signs, Deformity, and Radiographic Findings

The chief joint manifestations are stiffness, pain, and inflammation. The pattern of arthritis is recurrent, often evanescent, but can be deforming. Morning stiffness occurs in 46% to 73% of patients (4 ,5 ,6). Fries and Holman (7) found arthritis in 53% of their 193 patients, nodules in 3%, wrist swelling in 31%, metacarpophalangeal (MCP) swelling in 31%, and proximal interphalangeal (PIP) swelling in 40% at any point. Grigor et al. (8) described nondeforming arthritis or arthralgia in 88% of their patients, deforming arthritis in 10%, erosions in 6%, avascular necrosis in 6%, myalgias or myositis in 32%, and tendon contractures in 12%. Petri (9) has associated musculoskeletal damage in SLE with blacks and those of lower socioeconomic status.

Areas Affected

All major and minor joints may be affected, including the wrists, knees, ankles, elbows, and shoulders, in that order of prevalence (6). Most patients with SLE eventually develop some PIP and MCP involvement, and complaints of joint pain without objective physical findings for long periods may be noted. Once symptoms of discomfort became objectively apparent, morning stiffness characteristic of typical rheumatoid arthritis (RA) usually is seen. Marked, diffuse puffiness of the hands also often occurs. Stress fractures caused by corticosteroid-induced osteoporosis can produce swelling and mimic synovitis (10).

Hands and Wrists

Persistent, rheumatoid-like deformities may occur in the hand, as they did in 35% of patients observed by Dubois and Tuffanelli (4), with thickening of the PIP joints, ulnar deviation, and subluxation. Armas-Cruz et al. (11) found similar changes in 22% of 108 patients. Those changes often may appear insidiously over the course of many years while the patient is in an apparent clinical remission. The primary lesion appears to be inflammation involving synovial tissues, with minimal or belated destruction of cartilage and bone.

Hand deformities can include ulnar deviation and subluxation, swan-neck deformities (in 3% to 38%), and subluxation of the thumb interphalangeal joints (5 ,12 ,13 ,14 ,15 ,16). Erosions are rare (seen in approximately 4%). Some authors have noted positive correlations among deforming arthritis, Sjögren syndrome, anti-SSA, C-reactive protein, and the presence of rheumatoid factor (12 ,15 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24) and negative correlations with hypertension (25).

Jaccoud reversible, subluxing, nondeforming arthropathy is seen in 3% to 43% of patients with SLE (13 ,14 ,16). Table 33-1 summarizes the differential diagnosis between SLE and RA of the hand, and Figure 33-1 demonstrates some of the abnormalities mentioned here. In one report, Jaccoud arthropathy was found in 43% of 939 patients with SLE and is associated with a benign prognosis (26).

Whether or not primary antiphospholipid syndrome without SLE is associated with an inflammatory arthritis is the subject of some debate (27). A Dutch group correlated Jaccoud arthropathy with anticardiolipin antibodies, fetal loss, and thrombosis (28). Joint ligamentous laxity with consequent hypermobility is present in half with SLE (29). It does not require surgical treatment, because grip strength is usually intact. Dray et al. (30) treated 10 patients with subluxation excision, MCP arthroplasties, and joint stabilization by ligamentous reconstruction; unfortunately, 70% of the tendon relocations failed to maintain correction. An excellent review of hand surgery consideration in SLE has been published (31).

Table 33-1: Comparison of Hand Involvement in Systemic Lupus Erythematosus and Rheumatoid Arthritis

Parameter	SLE	RA
Raynaud's phenomenon	Approximately 30%	Rare
Joint pain	Mild	May be severe
Recurrent synovitis	In 10–30%	Common
Joint deformity	Caused by loss of soft-tissue support	Caused by loss of soft tissue support and articular surface destruction
Thumb IP joint hyperextension	Not associated with MP joint flexion contracture	Often associated with MP joint flexion contracture
Ulnar drift of fingers	Almost always reversible	Often irreversible, with subluxed MP joints
Wrist	May be lax; normal function	Often subluxed, with carpal bone destruction
Erosive changes	Rare	Common
Cause of deformity	Uncertain; occurs after supporting soft tissue structures are weakened	Synovitis, pannus formation; cartilage and bone destruction

IP, interphalangeal; MP, metacarpophalangeal.

All Hand Imaging Studies

Several reports have examined the radiographic findings of the lupus hand in detail. Weissman et al. (32) evaluated 59 patients, and 34 demonstrated abnormalities. Of these, 10 had acral sclerosis (these probably had mixed connective tissue disease), 7 had alignment abnormalities, and 1 showed an erosion. Leskinen et al. (33 ,34) reviewed joint radiographs of 124 patients with SLE, and cystic bone lesions were found in 51 (41%). Most were subchondral and located in the small joints of the hands and feet, and a vasculitic cause was proposed. In a multicentered European study, 10 of 60 lupus patients with erosive disease (vs. 28% without) had anti-RA33 antibodies ($p < 0.05$) (35). About half with SLE demonstrate evidence for inflammatory arthritis of the hands as determined by uptake on a technetium (Tc) bone scan (36). Ten of 37 Spanish SLE patients who fulfilled the inflammatory arthritis ACR criteria for lupus had imaging abnormalities; most of which was soft tissue swelling (37). MR imaging of the hands were more sensitive than conventional radiography in assessing soft tissue pathology and bony alterations in 14 German SLE patients (38). Twenty-six wrists of SLE patients were ultrasonographically compared with 15 healthy controls. Synovitis (42%), synovial proliferations (19%), effusion (25%), and erosions (4%) were found (39).

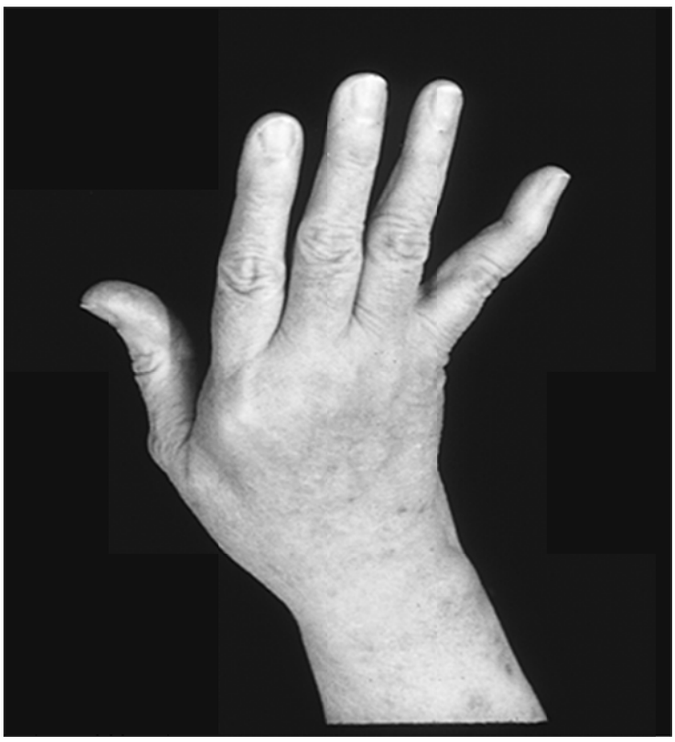

Figure 33-1. Jaccoud's arthropathy.

Rarely, a resorptive arthropathy resembling the opera-glass hand syndrome has been reported (40 ,41), as has periosteal elevation secondary to ischemic bone disease or, perhaps, vasculitis (42).

Knees

Jaccoud-like arthropathy (i.e., reversible subluxation) has been observed in the knees (43 ,44 ,45). Deep venous thrombosis (DVT) in a patient with antiphospholipid antibodies may be difficult to differentiate from a Baker cyst, both of which are observed in SLE (46). At least one case of chondrocalcinosis involving the knee joint associated with SLE has been reported (47), and it certainly is more common.

Feet

Mizutani and Quismorio (48) noted hallux valgus, metatarsophalangeal subluxation, hammertoes, and forefoot widening without erosions or cystic changes in patients with SLE. This is similar to the Jaccoud type of arthropathy in the hands. Other studies have confirmed these findings (49). The deformities result in painful bunions and callosities, and several podiatry publications have reviewed the issues of proper foot care in those with SLE (50 ,51).

Neck

Several cases of atlantoaxial subluxation have been reported (52 ,53). One report postulated that patients who are treated with corticosteroids have increased ligamentous laxity, which promotes the rupture of ligamentous and capsular supporting structures (53), but it seems equally likely that laxity of ligaments in the neck have the same physiologic bond as laxity in any other joint. In another report, 5 of 59 patients with lupus had atlantoaxial subluxation. All were asymptomatic. Subluxation was associated with longer disease duration, Jaccoud arthropathy, and chronic renal failure (52).

Sacroiliac Joint

Several studies of almost 100 patients suggested that over half of all patients with active SLE have radiographic sacroiliitis or increased uptake on joint scanning. I believe this figure is too high. Seronegative spondyloarthropathies were excluded, and most patients had no sacroiliac symptoms (54 ,55 ,56 ,57 ,58 ,59). A French survey of 41 SLE patients found radiographic unilateral sacroiliitis in 6 (15%). Only 1 individual was symptomatic (60).

Hips

Although hip synovitis is not uncommon, any complaints of isolated severe hip pain should lead one to suspect avascular necrosis (discussed below).

Shoulders

Jaccoud arthropathy can be found in the shoulders (61).

Temporomandibular Joint

Jonsson et al. (62) evaluated temporomandibular joint (TMJ) involvement in 37 patients with SLE and compared them to a control group of 37 healthy age- and sex-matched individuals. Of those with SLE referred to an oral surgeon, 59% had severe past TMJ symptoms (vs. 14% of controls), and 14% had present severe symptoms (vs. 3% of controls). Clinical examination revealed TMJ signs, such as clicking, crepitation, jaw fatigue or stiffness, facial pain, tenderness to palpation, pain on movement of the mandible, locking, or dislocation, to be present in 41% (vs. none of the controls). Additionally, of the patients with SLE, 30% had abnormal radiographs (vs. 9% of controls) that included condyle flattening and osteophytes, 11% had erosion, confirming other reports (63 ,64), and 72.5% had renal disease (vs. only 27.5% of the non–TMJ-involved patients with SLE). Although symptoms may be referred to this joint, limitation of the ability to open the jaw is rare unless coexistent scleroderma is present (65 ,66).

Synovial Histopathology

SLE is characterized by a mild to moderate inflammatory synovitis that is similar in character but less angry-appearing than that in RA (11 ,67). Bywaters (68 ,69) reported the presence of chronic synovitis with a fibrotic process in SLE in patients with Jaccoud-type deformities. Only three groups have examined the synovial histopathology of SLE in any detail.

Goldenberg and Cohen (70) studied 13 patients with lupus: 92% had synovial membrane hyperplasia, 100% had microvascular changes, 83% had surface fibrin deposits, and most had a perivascular infiltrate. Several of the biopsy specimens were indistinguishable from those of RA.

Labowitz and Schumacher (20) studied synovial biopsy specimens from seven patients; superficial fibrin-like material was seen in four, and focal or diffuse synovial lining proliferation in six. Five had a primary perivascular inflammatory reaction with predominantly mononuclear cells, and vasculitis was noted in only one patient. Synovial and vascular lesions were found in two patients, who had no objective signs of joint inflammation. On electron microscopy, fibrin was noted in three of four specimens. Type A (i.e., phagocytic), type B (i.e., synthetic), and intermediate cells were seen, but there was no clear predominance. Two patients had platelets and fibrin-like material obliterating small-vessel lumens. Vascular endothelial inclusions of a viruslike type were observed in two patients.

Natour et al. (71) reviewed 30 knee synovial biopsies. The most frequent findings were synoviocyte hyperplasia, minimal inflammation, edema, congestion, vascular proliferation, fibrinoid necrosis, intimal fibrous hyperplasia, and fibrin on the synovial surface.

In conclusion, the synovial histopathology of SLE does not appear to be specific, and it cannot clearly be differentiated from that of RA. Despite the extensive connective tissue change, little cartilage and bone destruction seems to occur. This gross finding tends to separate the deforming arthritis associated with SLE from that typically seen in RA. Large synovial cysts are uncommonly reported in patients with SLE (72 ,73 ,74).

Synovial Fluid

Ropes (75) found that the volume of accessible synovial joint fluid (Table 33-2) ranged from 5 to 1,500 mL in 133 patients. It was unusually clear, but occasionally hemorrhagic, and could be a transudate or an exudate. Pekin and Zvaifler (76) reported synovial fluid findings in 26 patients with SLE, and viscosity was uniformly good. The white count was less than 2,000/mL in 19 and exceeded 10,000/mL in only 2 patients. Granulocytes were always less than 50%, and the synovial fluid complement level was normal in 11. Of the 26 patients, 10 were classified as having noninflammatory transudates. Most had nephrotic syndrome. The exudates had a high protein content but variable complement levels. Serum to synovial fluid complement

ratios generally were elevated in contrast to the total protein or immunoglobulin G (IgG) ratios, suggesting consumption of complement at the synovial level.

Table 33-2: Characteristics of Synovial Fluid in SLE

Clear, yellow, normal viscosity, good mucin clot
White blood cell count: 2,000 to 15,000/mL, with primarily lymphocytic predominance
Low-titer ANAs may be present; LE cells occasionally are seen
Glucose level normal
Protein levels normal or increased
Complement levels normal or decreased

ANAs, antinuclear antibodies; LE, lupus erythematosus.

Schumacher (77) and Schumacher and Howe (78) examined synovial fluid from 17 patients with SLE. All had a white count lower than 40,000/mL. In comparing SLE with RA, Hollander et al. (79) reported a mean white count of 5,000/mL for SLE (vs. 15,000 for RA) with 10% neutrophils (vs. 50 for RA) and a good mucin clot and high viscosity (vs. a poor mucin clot and low viscosity for RA). Pascual (80) found that 14 patients with SLE had a synovial fluid counts ranging from 3,000 to 19,300/mL, and Hasselbacher (81) confirmed the increased viscosity in SLE synovial fluid and generally found low C3 complement levels. Cell counts in joint fluids must be interpreted cautiously, because analysis of the same fluid is subject to a great deal of variability among laboratories (82).

Secondary joint infection or avascular necrosis of bone may occur infrequently in SLE. It should be suspected when a localized effusion persists despite antiinflammatory therapy (83 ,84 ,85 ,86).

Lupus erythematosus (LE) cells can be found in vivo in synovial fluid (83). They were present in six of nine patients in one report (87), in 8 of 17 fluids examined by Schumacher (77), and by Schumacher and Howe (78) with electron microscopy, in 44% of 18 patients (88), in 2 of 14 patients examined by Pascual (80), and in two of three patients with drug-induced lupus (89). Rarely, RA cells (i.e., ragocytes) may be found in SLE synovial fluid (90 ,91).

Antinuclear antibodies (ANAs) are difficult to measure in synovial fluid, which must be treated with hyaluronidase before analysis. ANAs are present in approximately 20 of synovial fluid samples from patients with either RA or SLE (20 ,92 ,93 ,94), irrespective of serum levels. The synovial fluid of those with drug-induced lupus is similar to that reported for idiopathic SLE (89). Lipid synovial effusions rarely occur (95). Table 33-2 summarizes these observations.

On electron microscopy, Schumacher et al. (78 ,96) found meshworks or tubuloreticular structures in monocytes in seven patients. LE cells contained distinct chromatin filaments.

In summary, the synovitis of SLE is usually a bland, milder inflammatory process than rheumatoid arthritis that is deforming in a minority of cases.

Subcutaneous Nodules

Described by Hebra (97) and Kaposi (1) in 1872, subcutaneous nodules are present in 5% to 12% in the large series listed in Table 32-1 (Chapter 32) and in 2% to 10% in other series (98 ,99 ,100). Most occur in small joints of the hand, but nodules as large as 2 cm in diameter frequently occur on the extensor tendons of the hand and wrist. They may be exceedingly tender and often are transitory. Hoarseness secondary to rheumatoid-like vocal cord nodules has been described (101). These nodules are associated with patients who have SLE and rheumatoid-like arthritis and positive rheumatoid factor and rarely are seen without these features (102 ,103 ,104).

Histologically, subcutaneous nodules are granulomas and need to be differentiated from lupus panniculitis (profundus) (105), erythema nodosum, and a benign mesenchymoma. In children, it is important to distinguish them from granuloma annulare. Several reviews of the histology of the nodules in SLE have appeared (98 ,106 ,107). Vascular damage, with structural disarray in areas having collagen degeneration, is found, along with fibrinoid deposits and lymphocytic infiltrates in vessel walls. This microvasculopathy is similar to that observed in rheumatoid nodules.

Tendinitis, Tendon Rupture, Joint Laxity, and Carpal Tunnel Syndrome

Synovitis can induce a carpal tunnel syndrome, which may be the initial manifestation of idiopathic SLE or drug-induced lupus (108 ,109). Numerous reports and reviews have been published regarding tendon ruptures in SLE (110 ,111 ,112 ,113 ,114 ,115). The following conclusions can be derived: (1) almost all occur in weight-bearing areas, especially in tendons about the knee (65%; most are infrapatellar) and ankle (Achilles tendon; 27%); (2) an increased association with trauma, males, long-term oral steroid administration, intra-articular injections, Jaccoud deformity, and/or long disease duration is noted; and (3) most patients are in clinical remission at the time of rupture.

A definitive diagnosis can be made with magnetic resonance imaging (MRI) (116 ,117). Tendon biopsy specimens reveal degeneration, mononuclear infiltration, neovascularization, and vacuolar myopathy (118). One group has correlated hyperparathyroidism (especially in patients with severe renal disease) and hydroxyapatite and urate crystal deposition in the knee tendons, with resulting ligamentous laxity (119 ,120). Pritchard and Berney (113) observed 4 cases of tendon rupture (all patellar) in 180 patients followed over a 10-year period.

The Beighton criteria for joint laxity (hypermobility) is significantly more often fulfilled in SLE compared with controls, especially in those with longer standing disease (121).

Carpal tunnel syndrome was found in 48 (11%) of 436 patients with SLE seen at the University of Pittsburgh between 1972 and 1990 (122).

Calcinosis and Soft Tissue Swelling

Commonly observed in scleroderma, dermatomyositis, and crossover syndromes, soft-tissue calcifications rarely are seen in SLE (123 ,124). Calcinosis has been associated with trauma, pressure points, the TNF-α–308A allele, and subcutaneous edema. Lesions contain deposits of IL-6, IL-1, TNF-α, macrophages, osteocalcin, and bone matrix proteins (125 ,126).

They were observed radiographically in 9 of 130 patients in one study in which SLE was not adequately defined (127). Otherwise, approximately 30 case reports have appeared in the literature. Calcinosis universalis has been noted (128), and deposits of calcium phosphate in muscle, subcutaneous nodules, and periarthritis can occur in those with discoid or systemic lupus (129 ,130 ,131). Literature reviews are available (132 ,133). Periarticular radiographic calcification was present in 13.5% of 52 SLE patients, and was more common in patients taking diuretics (134). Diltiazem, which is a calcium channel blocker, is the probable treatment of choice (135 ,136). Aluminum hydroxide may be helpful (137), although surgical removal may be necessary (138 ,139). Soft tissue edema without calcinosis has been reported (140).

Chondritis

See Chapters 39 and 40 .

Osteoporosis

See Chapter 64 .

Costochondritis

Patients with SLE frequently complain of discomfort at the costochondral junctions. Esophageal spasm, angina pectoris, and pericarditis must be ruled out.

Myalgia, Myositis, and Myopathy

Generalized myalgia and muscle tenderness (Table 33-3), most marked in the deltoid areas and quadriceps (proximal muscles), are common during exacerbations of the disease and have been observed in 40% to 80% of patients (4 ,7 ,141). Muscle perfusion is diminished in SLE, either because of “Raynaud” of the muscles or accelerated atherogenesis (142).

Inflammatory myositis involving the proximal musculature occurs in 5% to 11% of patients (141 ,142 ,143 ,144 ,145) and can be confirmed by muscle biopsy, electromyographic (EMG) studies, and elevation of the serum creatine phosphokinase (CPK) or aldolase levels. Myoglobin levels also may be increased (145). The myositis responds to steroid therapy.

The differential diagnosis of proximal muscle weakness is a common problem in the management of patients with SLE. An inflammatory myositis must be differentiated from a drug-induced myopathy (glucocorticoid or antimalarial). Muscle enzyme levels are only elevated in the former group, but many untreated patients with SLE and myalgias have normal muscle enzyme levels. Frequently, generalized weakness is so prominent that some patients initially are diagnosed as having dermatomyositis (146). Inflammatory myositis can develop at any time during the course of the disease (147 ,148 ,149 ,150 ,151 ,152 ,153 ,154). Three large groups of dermatomyositis/polymyositis patients demonstrated a 7%, 4%, and 1% concurrence of SLE (155 ,156 ,157). The skin lesions of dermatomyositis/polymyositis also can appear in patients with SLE (150 ,151).

Table 33-3: Lupus Myositis and Myopathy in SLE

Myalgias occur in 40% to 80% of patients with SLE and are most marked in proximal muscles; weakness is a common symptom
Inflammatory myositis (often with an increased CPK level) has a cumulative incidence of 5% to 11%
Steroid- and antimalarial-induced myopathies must be excluded
EMG and muscle biopsy findings range from the normal to the classic patterns seen in dermato/polymyositis
Myalgias without myositis may respond to salicylates, NSAIDs, antimalarials, or 20 mg/d of prednisone or less
If diagnostic criteria are met for both SLE and dermato/polymyositis, treatment with 1 mg/kg/d of prednisone should be initiated

CPK, creatine phosphokinase; EMG, electromyogram; NSAIDs, nonsteroidal antiinflammatory drugs.

Tsokos et al. (158) evaluated 228 patients with SLE at the National Institutes of Health (NIH). Of these, 18 (8%) had prominent muscle disease. The CPK level was elevated in 1 patient, and the aldolase level was higher in 11. In 72%, myositis was concomitant with disease onset. No evidence of myocarditis was found, and all responded to 20 mg or less of prednisone daily. Very low CPKs may correlate with increased extra-muscular active lupus (159).

Foote et al. (160) followed 276 patients with SLE at the Mayo Clinic, and 11 met the diagnostic criteria for dermatomyositis/polymyositis. All were female, with a mean age of 29 years. In contrast to the NIH findings, the onset of myositis in these patients occurred 13 years after the onset of SLE. Also, Raynaud phenomenon was more prevalent in this group. The patients were treated with 30 to 60 mg of prednisone daily, and 1 was given azathioprine. After 4 years, 2 were dead and 6 asymptomatic. Isenberg et al. (161) compared 11 patients with SLE and myositis with 19 who had polymyositis. Over a 7-year period, both groups had chronic, relapsing courses and no differences could be noted. Lupus patients tended to have more cutaneous and articular disease (162).

Rhabdomyolysis has never been reported in SLE (163).

Two cases of inclusion body myositis in SLE have been found (164 ,174).

Electromyography

The principal EMG findings in polymyositis and dermatomyositis are (a) spontaneous fibrillation; (b) positive or sawtooth potentials; (c) small-amplitude, complex, polyphasic, or short-duration potentials; and (d) salvos of repetitive, high-frequency potentials. In patients with SLE, the EMG findings range from normal to those of classic dermatomyositis/polymyositis. Only four studies, however, have examined EMG findings in patients with SLE.

O'Leary et al. (165) found nonspecific EMG abnormalities in two of nine patients with SLE but in all with dermatomyositis. Erbsloh and Baedeker (166) performed EMGs on 15 patients with muscle symptoms, and their main findings were a decrease in mean potential duration to only 54% of normal, an increase in the mean phase frequency, and a corresponding decrease in the phase quotient. In only one patient were the findings normal. Tsokos et al. (158) performed EMGs on 8 of their 18 patients with SLE and myopathy. Of these, 5 had normal findings, neuropathy was demonstrated in 1, and the classic polyphasic peaks seen in polymyositis were noted in 2. In a study of 35 unselected patients with SLE, EMGs were suggestive of a myopathy in 23 (167). Also, 34 Norwegian patients with SLE underwent extensive neurophysiologic testing: 62% complained of muscle weakness, and 33% had any EMGs and 21% any nerve conduction abnormalities, neither of which correlated with symptoms (168).

Depending on various parameters, such as observer interpretation, whether an outpatient or inpatient population was used, and whether patients had muscle symptoms, between 22% and 90% of patients with SLE have abnormal EMGs (3 ,158 ,165 ,166 ,167).

Muscle Biopsies

Muscle biopsy findings (Fig. 33-2) range from normal to interstitial inflammation, fibrillar necrosis, degeneration, and vacuolization with fibrosis as a late occurrence seen with dermatomyositis/polymyositis. First used in the 1930s (165 ,169), muscle biopsies can be helpful in distinguishing inflammatory from drug-induced myopathies, as well as in determining reversibility based on the degree of fibrotic changes.

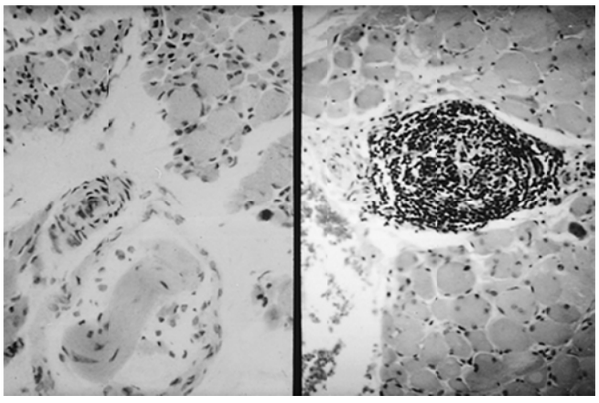

Figure 33-2. Lupus myositis.

Klemperer et al. (169) noted an inflammatory infiltrate in 5 of 30 cases of SLE, and Madden (170) noted the same in 6 of 21 biopsy specimens. The changes were nonspecific. Erbsloh and Baedeker (166) performed biopsies on 16 patients: 2 demonstrated impressive round cell infiltration, and 12 had parenchymal damage that occasionally included necrosis, along with a sparse interstitial infiltrate. Tsokos et al. (158) performed biopsies of 11 of 18 patients with SLE and myopathy: a mixed cellular, perivascular, and inflammatory reaction with interstitial inflammation without much muscle fiber degeneration was noted in 6 patients, and 5 had nonspecific changes or atrophy without inflammation.

Pearson (171) described muscle biopsies in 20 patients with various forms of SLE and noted rare, nonspecific fiber degeneration and interstitial myositis in 10 patients, no change in 3, and minor or insignificant muscle fiber vacuolization in 7 (4 taking steroids and 3 taking antimalarials). The vacuolar lesions were variable in extent and degree, sometimes being focal and scanty and on other occasions appearing widespread and extensive in individual fibers. These changes did not correlate with CPK levels, clinical measures of strength, or steroid administration. No vacuolar myopathy was noted among 16 biopsy specimens at the University of Toronto (172) and was seen in only 1 of 12 cases in another report (173). In the last few years, some centers have started performing less-invasive needle quadriceps biopsies. Lim et al. (175 ,176) also evaluated needle biopsy specimens from 55 patients with SLE. In these studies, lymphocytic vasculitis correlated with high sedimentation rates, inflammatory arthritis, and Sjögren syndrome. Subclinical skeletal muscle lymphocytic vasculitis was present in 10 of 22 patients with active lupus and was not seen with inactive disease. Staining muscle tissue for immune fluorescence revealed evidence for deposits in 37% of 132 European patients (177).

Three studies have included ultrastructural examinations. Oxenhandler et al. (178) evaluated the immunopathologic characteristics of lupus myopathy in 19 patients. Type I fibers predominated in 44%, and type II fiber atrophy was seen in 33%. Eight patients had an inflammatory myositis. Immunoglobulin or complement staining was seen in 13 patients in sarcolemmal-basement membrane areas, 5 had myofibrillar IgG, 5 showed vascular immunoglobulin or complement deposits, and IgG-containing globules were seen in 10. The University of Toronto group (172) emphasized the universality of immunoglobulin deposition in 16 SLE muscle biopsies, despite the rarity of concurrent inflammation. Finol et al. (173) emphasized the presence of muscle atrophy, microtubular inclusions, and a bland mononuclear cell infiltrate in 12 biopsy specimens; necrotic changes were only present in 1 patient, who had an elevated CPK level. This group confirmed earlier suggestions by Norton et al. (179 ,180) that the microvascular circulation of skeletal muscle is decreased

because of capillary basement membrane thickening. Pallis et al. (181) extended these findings and correlated thickening with impaired pulmonary gas exchange (as measured by diffusing capacity) and improvement with steroid therapy. Immunocytochemical studies have suggested increased levels of vascular cell adhesion molecule-1 (VCAM-1) in SLE muscle inflammatory infiltrates (182).

In summary, lupus myositis is mild and uncommon; drugs used to manage SLE can also damage muscles. Myalgias without frank myositis is often observed, and this may coexist or need to be differentiated from coexisting fibromyalgia.

Avascular Necrosis of Bone

Prevalence

First reported in SLE by Dubois and Cozen (183) in 1960, avascular necrosis (AVN), also known as aseptic necrosis or ischemic necrosis of bone, was observed in 26 (6) of Dubois' 520 patients (4), in 25 of Wallace's 464 patients (184), and in 24% to 30% of 150 and 103 patients at Johns Hopkins, which is an AVN referral center (185 ,186). Gladman et al. observed symptomatic AVN in 12.8% of 744 lupus patients followed at the University of Toronto between 1970 and 1985 (187). Other studies have noted AVN in 4% to 9% of patients with SLE (188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198). Asymptomatic AVN was noted on MR imaging in 6 of 30 Greek patients with primary antiphospholipid syndrome (199). AVN is a major source of morbidity and alteration in the quality of life in young women with lupus. This section reviews the pathophysiology, diagnostic testing, clinical presentation, and associations of AVN, as well as its treatment (Table 33-4). The reader is referred to an excellent review of 524 published studies on the subject (200).

Table 33-4: Avascular Necrosis (AVN) of Bone in SLE

AVN occurs in 5% to 10% of patients
Most cases are associated with corticosteroid administration; the remainder probably are induced by Raynaud's phenomenon, a small-vessel vasculitis, fat emboli, or the antiphospholipid syndrome
MRI is the diagnostic method of choice; CT and bone scans are less accurate and do not pick up preradiographic lesions as well; the radiographic appearance can be classified into four stages
Multiple sites can be affected; the femoral head, tibial plateaus, and humeral head are the most common
An association exists between AVN and Raynaud's phenomenon, increased steroid dosage, and duration of treatment
Treatment includes limiting weight-bearing, administration of antiinflammatory analgesics, and core decompression for stage I and II lesions; reconstructive surgery usually is required for stage III and IV disease

Pathophysiology and Classification

The usual mechanism is death of subchondral bone, resulting in osseocartilaginous sequestration with adjacent secondary osteosclerosis. The term osteochondritis dissecans has been used to refer to small areas of this process, such as disease involving a segment of femoral head or condyle.

The initial pathologic lesion probably is obliteration of the blood supply of the epiphysis, followed by reactive hyperemia, which is seen on the radiograph as osteoporosis. At this stage, the necrotic bone is radiographically demarcated from viable bone, because the dead tissue does not take part in the decalcification. By contrast, the necrotic area appears increased in density compared with the osteoporotic bone around it. During the healing stage, as new blood vessels grow in and bone repair occurs, the newly formed bone is soft. With continued pressure on the surface, flattening may occur (e.g., on the medial and superior aspects of the femoral head). These irregularities in the contour of the articular surfaces cause definite and consistent adaptive changes that are manifested later as degenerative arthritis.

AVN can have various types of causes (201 ,202): posttraumatic, nontraumatic, or idiopathic. Fractures, microfractures, or dislocations may cause AVN. Nontraumatic causes include embolic factors (e.g., as in sickle cell anemia, thalassemia, alcoholism, pancreatitis, and decompression states), small-vessel changes (e.g., as in SLE, polyarteritis, or Fabry disease), and deposition ischemic necrosis (e.g., increased lipocytes caused by steroid therapy, Gaucher disease, or Cushing disease). Renal transplant patients who receive pulse steroids and high doses of steroids are especially susceptible. Conditions that are associated with idiopathic AVN include gout, pregnancy, prolonged immobilization, cytotoxic therapy, hyperparathyroidism, familial tendencies, lymphoma, metastatic carcinoma, and degenerative arthritis.

In SLE, Raynaud phenomenon, vasculitis (203 ,204 ,205), fat emboli (206 ,207 ,208), corticosteroids, and defects in fibrinolysis (209) can induce ischemia that results in bony necrosis (210). Zalvaras and colleagues have correlated abnormal values of protein C, protein S, von Willebrand factor, lipoprotein A; factor V Leiden, 677C to T methylene-tetra-hydrofolate reductase, and prothrombin gene mutations with AVN (211 ,212 ,213). Eighty-two percent of 45 patients with AVN at Johns Hopkins had at least one procoagulant, which was significant compared with 30% in controls (214).

That no cases of AVN were recorded before 1960 indicates the importance of the introduction of corticosteroids in the 1950s in regard to our perception of this entity.

Ficat and Arlet (215) have classified AVN using a radiographic scale. In stage 0, only hemodynamic changes have taken place. The patient is asymptomatic, and routine radiographs are normal. In stage I, minimal pain with mild restriction of motion may be present. Stage II is characterized by a dull, aching pain on weightbearing, decreased range of motion, and a slight limp. Radiographs demonstrate

diffuse osteoporosis, sclerosis, or cyst formation. By the time that stage III is reached, advanced radiographic changes are evident, and the patient has taken a quantum leap in restricted movement and pain. Subchondral bone collapse (the crescent sign) with normal joint space is present radiographically. Stage IV represents end-stage disease with osteoarthritis, as seen on the radiograph. Most patients are symptomatic and require surgery.

Diagnostic Techniques and Hemodynamic Studies

MRI can detect AVN months to years before it is evident on routine radiographs (Fig. 33-3). This has made earlier, noninvasive methods of detecting AVN obsolete. MRI should be ordered for a patient with SLE who has hip pain and a normal radiograph if glucocorticoid therapy is being given. Plain radiographs are often unreadable and subject to marked observer variations (Fig. 33-4) (216). Gallium scans (193) and technetium scans with pertechnetate or sulfur colloid (217 ,218 ,219 ,220) generally are accurate but can be subject to false-positive or false-negative interpretation. Scintigraphic images of radiographically negative osteonecrosis tend to contain a photopenic zone, whereas more advanced lesions have increased uptake (221). Several studies have compared bone scanning with MRI (222 ,223 ,224 ,225 ,226 ,227); the latter has better specificity and sensitivity, but both procedures can yield false-positive findings. Although computed tomography (CT) with multiplanar reformation (228) can be useful, MRI derives the same, or more, information with less effort and without exposing the patient to radiation.

On MRI, the reactive interface between live and dead bone at the periphery of AVN lesions has a characteristic double-line sign on T2-weighted images (229). A low-intensity band on T1-weighted images is an early, specific finding for AVN (230). Early changes at the femoral head can demonstrate bone marrow edema (231), and premature conversion to fatty marrow is evident in younger patients. In AVN, the femoral neck usually is surrounded by joint fluid. These findings have been confirmed in patients with SLE (222 ,225 ,232 ,233). Interestingly, the advent of MRI has allowed investigators to detect cases of incidental, asymptomatic, and nonprogressive AVN (259).

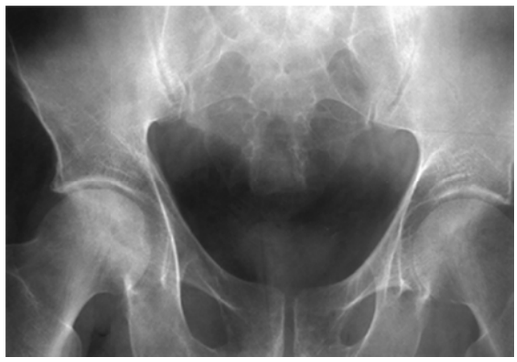

Figure 33-3. Avascular necrosis of the hip: plain film.

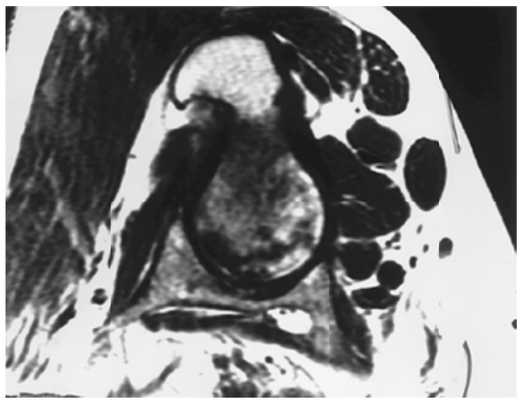

Figure 33-4. Avascular necrosis: T1-weighted magnetic resonance imaging.

Zizic et al. (234 ,235) at Johns Hopkins used invasive techniques to obtain more information about the hemodynamics of AVN. They found increased baseline bone marrow pressure in most patients with SLE after carrying out a stress test (a greater than 10 mm Hg elevation after instillation of 5 mL of physiologic saline into the femoral head). Almost 90% of patients had abnormal intraosseous venography, as characterized by incomplete filling of the main extraosseous veins, diaphyseal reflux, and stasis of contrast material. Venography was abnormal in all stages of AVN, including preradiologic ones. In a follow-up study (236), bone marrow pressure, saline stress test, and/or ischemic intraosseous venography results were abnormal in 254 of 259 ischemic bones (98%) so evaluated, and AVN was detected in 93% of 55 radiologically normal bones. Using these parameters, 36 of 48 joints on the contralateral asymptomatic side had abnormalities, and 15 patients (42%) developed AVN over a 47-month follow-up period. Thus, hemodynamic measurements are of predictive value in identifying the at-risk joints (237), but this procedure is not widely available. Femoral head perfusion is decreased in lupus in general without AVN as a result of greater marrow fat content (238).

Clinical Associations in SLE

In SLE, AVN tends to occur at multiple sites. At the NIH, 90% of 31 cases were polyarticular, and 84% were symmetric (239). Of Dubois' 26 patients, 22 had bilateral femoral head involvement, 1 had unilateral involvement, and 3 had affected knees (240). Zizic et al. (241 ,242 ,243) reported that 91 cases involved the femoral head and 83 occurred in

multiple sites. Reports have appeared of 6, 8, 13, and even 28 different sites in a single patient (244 ,245 ,246 ,247 ,248). Although the hip is the most common site, AVN can involve other joints and often results in a delay in diagnosis. Three of 11 University of Connecticut patients studied by Urman et al. (195) had AVN of the wrist. Most studies have recorded a mean onset of AVN in the fourth decade of life, with an average SLE disease duration of 4 to 7 years (106 ,249).

The diagnosis of AVN should be considered in any patient with SLE who has persistent pain in one or a few joints without evidence of disease activity in other systems, especially if glucocorticoids have been given. The association between vasculitis and AVN in the absence of steroid administration is well documented (203 ,204 ,205). Many studies have addressed the role of corticosteroids in inducing AVN in those with SLE, but early studies were hampered by control groups in which AVN was excluded based on normal radiographs (249 ,250). It has been suggested that increased doses of steroids (especially in the first year of treatment) and the duration of steroid therapy are correlated with a greater risk of AVN in SLE patients (241 ,242 ,251 ,252 ,253). Bolus steroids were not similarly associated (254 ,255). These dose-time relationships were confirmed in a meta-analysis of 22 papers (most patients without SLE) by Felson and Anderson (256), although this confirmation has been challenged (257). The clinical associations observed by our group are shown in Table 33-5 (258). Mok et al. (259) performed a similar survey and found many of the same associations.

SLE also has additional unique features that might predispose a patient to the development of AVN. Zizic (262) and Smith et al. (194) have both commented on the comparative rarity of AVN in steroid-dependent populations of patients with asthma, dermatologic disorders, and inflammatory bowel disease. AVN was found to be associated with Raynaud phenomenon, central nervous system (CNS) lupus, vasculitis, myositis, peripheral neuropathy, and elevated sedimentation rates (251 ,260 ,261). Others have only been able to suggest the association with Raynaud phenomenon (191 ,194).

Does the presence of antiphospholipid antibodies predispose the patient to AVN? Since Asherson et al. (263) originally made the suggestion based on older and less accurate methods of detection, two studies have concurred with this finding (219 ,259) and seven have not (198 ,238 ,258 ,264). The Hopkins cohort has published studies with conflicting results (186 ,265 ,282). It is possible that antibodies to annexin-V may be important (266), and an excellent critical review of this controversy has been published (267).

Treatment

Small areas of AVN can remain asymptomatic or heal spontaneously. Rare reports of spontaneous regression have appeared (268), but most patients with a clearly established diagnosis experience progressive disease if they are treated nonoperatively. Conservative management often is a holding action (especially in the hips) and consists of analgesics, nonsteroidal antiinflammatory drugs (NSAIDs), and limited or nonweightbearing for several months (269). Alcohol use should be discouraged, and efforts to decrease steroid doses should be attempted. Early stage disease may improve spontaneously if the necrotic area is small. Among 99 AVN patients followed in Toronto with 217 affected joints, there was no increase in mortality but increased disability (187).

A number of surgical techniques have been used successfully with AVN of the femoral head, including drilling or core decompression, free bone grafts (cortical or osteochondral allograft), vascularized bone grafts (muscle pedicle graft, free vascularized fibular graft, vascular anastomosis), osteotomy (varus or valgus angulation, rotation), and joint reconstruction (femoral head or total hip replacement) (269 ,270 ,271). Other joints are approached in a conceptually similar manner. The discussion here is limited to results that have been reported for SLE.

Early series documented excellent results with total hip replacement (272 ,273). Between 1971 and 1982, 39 of 43 prosthetic hip replacements performed at the Mayo Clinic on patients with SLE were for stage III or IV AVN (274). Of these, 29 patients had conventional hip replacements, and 14 had bipolar endoprosthetic replacements. Over a 66-month follow-up period, all were rated as having good or excellent results. Complications included delayed wound healing (15%) and superficial wound infection (10%). In our group, all seven patients who underwent total hip arthroplasties with at least a 10-year follow-up reported excellent results (258). Success rates are equal to patients with AVN and rheumatoid arthritis (277). Other groups also have reported greater than 90% long-term success rates (275 ,276 ,277). Joint arthroplasty in SLE has not been associated with increased rates of infection or lupus flare risks (278). Total knee arthroplasty for corticosteroid associated AVN yielded excellent results in only 11 of 25 patients treated at Johns Hopkins (186).

Whereas few disagree about the management of stage III and IV AVN, the indications for core decompression in earlier stages are controversial. First reported by Zizic et al. (234 ,280) as removal of an 11-mm diameter core of bone from the central axis of the femoral head, success was claimed in treating stages I, II, and III disease. Two studies of SLE failed to confirm these findings (175 ,214), however, and our results at UCLA Medical Center have been mixed. Zizic's (262) review of the literature (including other diseases) suggests a 77% success rate for preradiologic disease and a 52% success rate for stage II AVN. At Johns Hopkins, 68% of 31 patients undergoing core decompression for AVN required total hip replacements over a mean 12-year follow-up period (279). The coring procedure probably is not indicated for patients with stage III or IV disease.

In summary, AVN is a painful and debilitating complication found in 5% to 10% of SLE patients. It is usually associated with corticosteroid use, may be associated with a coagulopathy and responds to surgical interventions.

Table 33-5: Comparisons between Systemic Lupus Erythematosus Patients with and without Avascular Necrosis (AVN)

Characteristic	Percentage[a] of Patients with AVN (*n* = 26)	Percentage[a] of Patients without AVN (*n* = 462)	*p* value[b]
Female	96.15	93.29	
Hispanic	7.69	7.61	
Black	19.23	10.85	
White	57.69	72.67	
On dialysis	11.54	5.15	
Living in 1991	95.15	95.02	
Mean age at diagnosis (years)	26.15	31.47	0.02
Mean follow-up (years)	9.28	5.94	
History of			
Fevers	53.85	41.34	
Arthritis	88.46	91.56	
Myalgias	76.92	79.44	
Pericarditis	23.08	11.90	
Hypertension	46.15	24.03	0.02
Pleural effusion	26.92	11.06	0.03
Pleuritic pain	34.62	30.30	
Skin rashes	73.08	52.60	
Raynaud's	26.92	26.42	
Headache	26.92	30.30	
Cerebritis	26.92	9.74	0.01
Nephritis	57.69	25.16	0.04
Thromboemboli	15.38	7.14	
Anemia	50.00	29.69	0.047
Hemolytic anemia	21.74	7.26	0.03
Leukopenia	50.00	50.55	
Thrombocytopenia	24.00	15.63	
Laboratory test results of			
Positive ANA	95.83	95.74	
Decreased C3	36.00	38.41	
Positive anti-DNA	40.00	40.05	
Positive anti-RNP	25.00	14.54	
Positive RA latex	23.52	22.62	
Positive anti-Ro (SSA)	19.05	17.76	
Anticardiolipin antibody	30.77	38.07	
Elevated sedimentation rate	65.22	54.38	
Treatment history of			
Trial of NSAIDs	88.46	70.77	
Rx hydroxychloroquine	44.00	61.76	
Use of systemic steroids	84.62	76.25	
High-dose oral steroids	50.00	35.75	
Pulse IV steroids	20.00	11.26	

[a]Percentage unless otherwise noted (ie, years).
[b]A *p* value of <.05 is considered to be statistically significant. Only significant *p* values are listed.
ANA, antinuclear antibody; C3, third component of complement; RN, ribonucleoprotein; RA, rheumatoid arthritis; Ro (SSA), Sjögren's syndrome A; NSAIDs, nonsteroidal antiinflammatory drugs; IV, intravenous.
From ref. 241.

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Chapter 34

Cardiovascular Manifestations of Lupus

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Cardiovascular Manifestations of SLE

The cardiovascular (CV) system shares the same vulnerability as other systems to the immunologically mediated processes in SLE that result in organ damage and dysfunction. Manifestations of cardiovascular disease (CVD) from SLE range from asymptomatic findings to life-threatening disease, and include pericardial disease, myocardial dysfunction, conduction system abnormalities, valvular heart disease, and premature atheromatous disease with its associated morbidity and mortality. Some of these emerging comorbidities are a consequence of better disease therapy; half a century ago SLE patients with organ-threatening disease did not live long enough to experience these manifestations. Advances in technology have provided the means for earlier and better detection of these conditions, but we are still in the process of delineating their mechanisms and designing directed interventions toward their reversal and prevention.

Pericardial Disease

Epidemiology and Pathogenesis

Pericarditis is the most common clinically diagnosed CV manifestation of SLE. The prevalence of pericardial disease is reported from 16% to 61%, depending on the criteria chosen to establish the diagnosis (1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12) (Table 34-1). Echocardiographic evidence of pericardial thickening or effusion is detected in 18% to 54% of SLE patients (4 ,5 ,6 ,7 ,8 ,9 ,10 ,11), whereas pericardial disease at autopsy has been reported in up to 61% (1). Symptomatic pericarditis is less common but has been estimated to occur in about 25% of patients with SLE.

Clinical Presentation and Diagnosis

Symptoms of acute pericarditis include positional chest pain, often worse when supine or with inspiration (pleuritic), and located in the precordium or substernal region. Other associated symptoms include, fever, dyspnea, and tachycardia. Clinical signs may include decreased heart sounds, a pericardial rub, hypotension, or pulsus paradoxus (an exaggeration of the normal respiratory variation of the pulse, becoming weaker with inspiration and stronger with expiration). Diagnostic testing that may be useful in substantiating the diagnosis of acute pericarditis includes electrocardiography (ECG), chest radiography, and echocardiography. ECG findings in acute pericarditis include PR segment depression and widespread, upwardly concave ST segment elevation. T-wave inversions usually occur several days into the clinical course. Electrical alternans and reduced QRS voltage are most often seen when large effusions are present. Chest radiographs may reveal an enlarged cardiac silhouette when an effusion is present, and pericardial fat lines may be visible. Echocardiography is the most common method used to confirm the diagnosis of pericardial disease because of its high sensitivity and specificity. Additionally, this noninvasive technique can grossly estimate the amount of pericardial fluid, and also identify findings such as the loss of collapse of the inferior vena cava during inspiration and the right ventricle during diastole that are indicative of pericardial tamponade. In some patients, right heart catheterization is necessary to confirm a diagnosis of tamponade. Computerized tomography (CT) and magnetic resonance imaging (MRI) studies are frequently utilized in the evaluation of chronic pericardial disease, and may be helpful in identifying pericardial thickening and constrictive pericarditis.

From a practical standpoint, the diagnosis of pericarditis is most often made clinically, based on characteristic symptoms and physical examination findings. However, other disorders can mimic acute pericarditis and should be considered in this setting, including pneumonia, pulmonary embolism, infection, myocardial infarction (MI), and aortic dissection.

Treatment

Symptomatic pericarditis may respond to therapy with nonsteroidal anti-inflammatory drugs (NSAIDs), particularly in mild cases. However, if the symptoms are severe or refractory to NSAIDs, corticosteroid therapy (20 to 40 mg/day prednisone or equivalent) is usually effective. Pericardiocentesis and/or pericardial window is necessary in the setting of a pericardial effusion that causes hemodynamic compromise or in patients with severe refractory symptoms associated with the effusion. Pericardial effusions in SLE are generally inflammatory, with an elevated leukocyte count and a predominance of polymorphonuclear leukocytes. The fluid is exudative, with elevated protein levels and normal to low glucose. Effusions may be serosanguineous or overtly

hemorrhagic, and may test positive for antinuclear (ANA) and double-stranded DNA antibodies, and LE cells. It should be noted that ANA testing of pericardial fluid may be positive in other etiologies of pericarditis, such as tuberculous and neoplastic disease, which prohibits these autoantibodies from providing significant diagnostic utility (13 ,14).

Table 34-1: Pericardial Disease in SLE

Author	Year	Patient #	Prevalence (%)	Diagnostic Modality	Comment
Griffith/Vural (1)	1951	18	61	Autopsy	
Dubois/Tuffanelli (2)	1964	520	31	Not available	
Bulkley et al. (3)	1975	36	53	Autopsy	
Doherty et al. (4)	1985	50	42	Echo	
Badui et al. (5)	1985	100	39	Echo	Pericarditis or effusion
					Consecutive Female Patients
Klinkhoff et al. (6)	1985	47	21	Echo	
Crozier et al. (7)	1990	50	54	Echo	Consecutive patients
Nihoyannopoulos et al. (8)	1990	93	20	Echo	
Sturfelt et al. (9)	1992	75	19	Echo	
Jouhikainen et al. (10)	1994	74	22	Echo	Consecutive patients
Kalke et al. (11)	1985	54	18	Echo	
Houman et al. (12)	2004	100	16	Not available	Retrospective analysis

Pericardial effusions may also occur in SLE patients from other comorbid conditions, including uremia, nephrotic syndrome, hypothyroidism, and infection. In this setting, treatment of the underlying condition is the most appropriate management. Asymptomatic pericardial effusions are frequently detected in SLE, but it is unclear whether these represent a benign manifestation or indicate early disease activity that will progress to clinically manifest disease. There currently are no data to support treatment of asymptomatic, hemodynamically insignificant pericardial effusions in patients with quiescent disease.

Pericardial thickening may occur from recurrent episodes of pericarditis. Although rare, chronic pericardial disease can result in constrictive pericarditis, which may require pericardial stripping.

Prevention

There are no specific measures targeting the prevention of pericardial disease. However, treatment and control of disease activity may help decrease its incidence. In patients with end-stage renal disease, maintaining regular dialysis with good clearance is important in preventing uremia-associated pericardial effusions.

Myocardial Dysfunction

Epidemiology and Pathogenesis

Impaired myocardial function has been reported in 5% to 64% of SLE patients, and is frequently associated with comorbidities such as hypertension and ischemic heart disease (7 ,8 ,11 ,15 ,16 ,17 ,18 ,19) (Table 34-2). Abnormalities in both systolic and diastolic function have been detected, as well as an abnormal response to exercise. del Rio et al. demonstrated that SLE patients have decreased left ventricular ejection time and a longer pre-ejection period when compared with controls (20). One study of 5 SLE women without any clinically evident cardiac disease undergoing cardiac catheterization demonstrated increased wall stiffness, decreased pump function and contractility, and diminished coronary artery reserve (15).

As with pericardial disease in SLE, differences in prevalence of myocardial abnormalities reflect the methods of detection. The studies reported in Table 34-2 include those demonstrating myocardial dysfunction that may arise from various etiologies; autopsy studies are most likely to reliably represent inflammatory disease of the myocardium. Since numerous factors can lead to transient and sustained myocardial depression, it is unclear how often SLE-associated myocarditis is the lone cause of this condition. Wijetunga et al. noted that clinically apparent myocarditis in SLE occurs on average in 9% of patients (combined studies) (19). Autopsy studies attest that subclinical myocarditis may occur more frequently, with a reported prevalence as high as 57% in combined studies from the presteroid era. Since the introduction of glucocorticoid therapy, however, combined series report prevalence as low as 7%. It has been hypothesized that myocarditis in SLE arises from an immune-complex mediated process, in which complement activation and inflammatory cytokine activity orchestrate damage to myocardial vessels, rather than a primary myopathic disorder. Circulating autoantibodies have been examined in the context of myocarditis in SLE, but no consistent or specific association has been found. Myocarditis can result in global myocardial depression from necrosis and

fibrosis of myocytes, or in conduction system disturbances from inflammatory and degenerative changes in conduction tissues.

Table 34-2: Myocardial Dysfunction in SLE

Author	Year	Patient (#)	Prevalence (%)	Diagnostic Modality	Comment
Strauer et al. (15)	1976	5	100	Cath	Decreased contractility, cardiac output, stroke volume, and ejection fraction
Badui et al. (5)	1985	100	14	Echo	Depressed function Consecutive patients
Leung et al. (16)	1990	75	9	Echo	Depressed function Consecutive Patients
Nihoyannopoulos, et al. (8)	1990	93	5	Echo	Regional/global dysfunction
Sasson et al. (17)	1992	35	64/14	Echo	Diastolic dysfunction Active/inactive disease Consecutive patients
Roldan et al. (18)	1996	58	13	Transesophageal echo	Congestive heart failure
Kalke et al. (11)	1998	54	20	Echo	Systolic/diastolic dysfunction
Wijetunga et al. (19)	2002	126	57	Autopsy	Combined studies (1950 to 1960's); myocarditis
Wijetunga et al. (19)	2002	46	7	Autopsy	Combined studies (1970 to 1990's); myocarditis

Although SLE-associated myocarditis may lead to cardiomyopathy and impaired ventricular dysfunction, a number of other more commonly occurring conditions should be considered prior to this diagnosis. Ischemic heart disease, often an afterthought when evaluating premenopausal females, should be considered immediately in the setting of abnormal myocardial function in SLE. Valvular stenosis or insufficiency may also result in dilated, poorly contractile chambers and congestive heart failure. Pulmonary embolism and pulmonary hypertension can lead to impaired ventricular function and right-sided heart failure. Long-standing hypertension is another common cause of ventricular dysfunction.

Chloroquine-associated cardiomyopathy occurs in SLE infrequently. The English literature reports less than 20 cases, with fewer than half of these biopsy-proven (21). Other manifestations of cardiotoxicity from antimalarial agents include atrial and ventricular conduction system disorders. Lysosomal disruption, a mechanism purported to have an important role in the beneficial effects of antimalarial therapy in rheumatic disorders, is considered a possible factor in its associated cardiotoxicity. Animal studies have demonstrated both acute and chronic adverse cardiac consequences of chloroquine use, including depressed contractility, conduction abnormalities, and myocardial necrosis and fibrosis (22 ,23 ,24 ,25).

Clinical Features and Diagnosis

The diagnosis of myocardial dysfunction in SLE is usually considered based on clinical signs and symptoms, but is not one that can be established solely by clinical means. The manifestations of lupus-associated myocardial dysfunction are not specific for lupus, but are reflective of the degree of myocardial dysfunction. Symptoms and signs include fatigue, dyspnea, tachycardia, cough, orthopnea, paroxysmal nocturnal dyspnea, and pleural effusions. The cardiac examination may be unremarkable in patients with mild disease. More severe cases can manifest with jugular venous distention, peripheral edema, murmur, or overt congestive heart failure. Electrocardiographic changes may include nonspecific ST-T changes, premature atrial or ventricular complexes, dysrhythmias, or conduction abnormalities. In lupus myocarditis, patients may present with asymptomatic disease, detected only by ECG changes, or with a clinical picture mimicking acute MI with chest pain, ECG changes, and elevated cardiac enzymes.

Diffuse myocardial dysfunction can result in global chamber dilatation, and may appear as cardiomegaly with or without pulmonary edema on chest radiograph. Echocardiographic findings in myocarditis include prolonged relaxation time, decreased deceleration of early diastolic flow velocity, reduced E/A ratio (filling of left ventricle in early vs. late diastole), and increased chamber size. Echocardiography is a rapid and noninvasive means of diagnosing impaired myocardial function, but other

modalities such as functional nuclear imaging studies and cardiac catheterization can also be useful in diagnosis. Since SLE may be associated with a number of other conditions leading to the generalized impairment of myocardial function, such as hypertension, coronary artery disease (CAD), medication effect, and infection, it may not always be possible to readily identify SLE-associated myocarditis as the culprit of myocardial dysfunction. It is imperative that the underlying etiology of myocardial dysfunction be sought. Heart failure from severe valvular insufficiency would require a different therapeutic approach than heart failure from three-vessel obstructive CAD or from SLE-associated myocarditis.

The presenting signs and symptoms of chloroquine-associated myocardial dysfunction are those of congestive heart failure, with or without associated dysrhythmia. Imaging studies are useful in demonstrating depressed myocardial contractility, but are nonspecific. The small numbers of patients who are affected by chloroquine-associated cardiomyopathy prohibit reliable identification of its risk factors, but analysis of the limited data suggests that its risk factors are the same as for retinal toxicity: older age, higher dose per unit body weight, and duration of therapy (25).

Other means of diagnosing myocarditis are also being investigated, including echocardiographic evaluation of diastolic function (purported to be an early sign of myocarditis), myocardial gadolinium uptake on citrate scintigraphy, and MR imaging to evaluate myocardial enhancement and relaxation (11 ,26 ,27). None of these diagnostic methods are specific for SLE-associated myocardial dysfunction.

Although myocardial biopsy may be supportive of the diagnosis of lupus myocarditis when an immune-mediated process is identified, findings may be nonspecific, demonstrating inflammatory cell infiltration, immunoglobulin deposition, fibrinoid necrosis, or fibrosis and scarring. Myocardial biopsy also is fraught with potential technical problems including small tissue specimen size, sampling error, subjective pathologic interpretation of findings, and no well-established sensitivity and specificity for biopsy findings (28). A study of 845 patients examining the role of myocardial biopsy in diagnosing unexplained cardiomyopathy demonstrated that biopsy provided increased sensitivity over clinical diagnosis (100 vs. 66%), but did not improve specificity (86 vs. 87%) (29). Seven SLE patients were part of this study; in six patients, the diagnosis was made by clinical and laboratory evaluation prior to biopsy (the diagnosis of the seventh patient was also established by an unspecified diagnostic evaluation noted as “other interventions”). Unless circumstances are extenuating, biopsy is not frequently performed for this diagnosis alone. A diagnosis of myocarditis in the setting of myocardial dysfunction is generally reserved for cases where other etiologies have been excluded.

The high frequency of cardiac involvement in SLE from disease-associated processes makes the diagnosis of chloroquine-associated cardiomyopathy one of exclusion, with myocardial biopsy the only means of definitive diagnosis. The histologic abnormalities associated with chloroquine cardiotoxicity include myocyte necrosis and fibrosis, myeloid bodies, curvilinear bodies, and intracytoplasmic vacuoles (30 ,31 ,32). These histopathologic findings were also noted in the animal studies and appeared to be dose-related. The presence of myocyte necrosis and fibrosis implies some permanent consequences to affected tissues; however, patients often have significant clinical improvement and reversal of myocardial dysfunction upon discontinuation of antimalarial therapy. Unfortunately, as with lupus myocarditis, biopsy findings may be nonspecific and fail to establish the suspected agent as the precipitating factor even when this disorder is present. Other more common etiologies, such as ischemic heart disease or infection should be investigated prior to making this diagnosis.

Treatment

The treatment of myocardial dysfunction and heart failure in SLE should be focused upon its cause. For example, dysfunction arising from ischemic heart disease might require medical or operative intervention to improve myocardial oxygen supply and decrease metabolic demand. Treatment of congestive heart failure arising from uncontrolled hypertension would include intensive blood pressure control. Heart failure not readily attributable to other causes and thought to be from SLE-associated disease activity would require immunosuppressive therapy. In patients with irreversible, significant left ventricular failure, anticoagulation therapy may be considered for prevention of thromboembolic disease (33). Moderate to severe left ventricular dysfunction is associated with cardiac dysrhythmias and also with sudden cardiac death (34). Any SLE patient with impaired myocardial function with signs and symptoms of or with an established dysrhythmia should be considered for Holter monitoring to detect potentially lethal arrhythmias.

SLE-associated myocarditis is generally treated with systemic glucocorticoids, usually beginning with high dose or pulse intravenous therapy followed by several weeks of oral therapy, based on clinical response and other disease manifestations. Although no immunosuppressive agents have been studied in controlled trials in SLE myocarditis, case reports of therapy with agents such as azathioprine, intravenous immunoglobulin, and cyclophosphamide suggest that these agents may also be beneficial (35 ,36 ,37). Clinical manifestations usually improve significantly with therapy, and often resolve. In patients with clinical signs and/or symptoms of congestive heart failure, standard therapy, including sodium and fluid restriction, diuretics, inotropic agents (e.g., digitalis), and afterload reducing agents may provide significant abatement of symptoms. However, afterload reduction should be avoided in patients with hemodynamically significant aortic stenosis, and angiotensin-converting enzyme (ACE) inhibitors should be used cautiously in patients with renal insufficiency. Patients with stable myocardial dysfunction (i.e., not in

overt congestive heart failure) may also benefit from the use of beta-blocking agents; these should be initiated at a low dose and under close surveillance. Conduction system involvement associated with myocarditis resulting in dysrhythmias may require anti-arrhythmic therapy, anticoagulation, or, in severe cases, placement of an automated implantable cardiac defibrillator.

Discontinuation of the agent with clinical and echocardiographic improvement suggests, but does not establish, the diagnosis of chloroquine cardiomyopathy. Biopsy may be required in patients with severe myocardial dysfunction, or in those who fail to improve upon discontinuation of the agent.

Prevention

Myocardial dysfunction may arise from numerous etiologies in SLE. Preventative measures should focus on maintaining suppression of disease activity, to diminish the likelihood of immunologic injury to the myocardium and associated structures. Since ischemic heart disease is a common cause of myocardial dysfunction, screening for and intensive management of CV risk factors is important to decrease the risk of ischemia-related dysfunction. The potential benefits of antimalarial therapy in SLE far outweigh the risks of use in the great majority of patients. These agents are a rare cause of this condition, which implies that other more common conditions should be considered first.

Conduction System

Epidemiology and Pathogenesis

Conduction system abnormalities have been described in SLE, affecting from 10% to 74% of patients depending on the definition of abnormality, when the study was performed, and if underlying CVD is present (5 ,9 ,38 ,39 ,40) (Table 34-3). Low grade (first- and second-degree blocks) are more commonly reported, while high-grade or complete heart block occurs rarely. Intraventricular (bundle branch) conduction abnormalities have also been noted. Conduction system abnormalities more often occur in young and middle-aged SLE women, and in patients with a longer duration of disease. Conduction system defects may be transient, or may persist and progress from limited to more widespread involvement.

Table 34-3: Conduction System Abnormalities in SLE

Author	Year	Patient (#)	Prevalence (%)	Diagnostic Modality	Comment
Shearn (38)	1959	73	62	ECG	
Hejtmancik et al. (39)	1964	137	52	ECG	
Badui et al. (5)	1985	100	74	ECG	Any abnormality
Klinkhoff et al. (6)	1985	47	32	ECG	
Logar et al. (40)	1990	67	10	ECG	Conduction defect 6/7 anti-Ro antibody +
Sturfelt et al. (9)	1992	54	17	ECG	Any abnormality

Conduction system involvement in SLE has been attributed to both immune-mediated injury to the conduction tissues and to the small vessels of the myocardium. In patients with CAD, it may be difficult to discern whether the conduction defects arise from ischemic insult or disease-associated inflammation of the myocardium. Although neonatal heart block in children of women with SSA/anti-Ro antibodies is well established, this relationship does not appear to be as strong in adults with the autoantibody. The anti-U1RNP antibody has also been associated with derangements of the conduction system in SLE (41).

Clinical Features and Diagnosis

Patients with conduction system abnormalities may be asymptomatic, or may have symptoms such as weakness, syncope, and heart failure with higher grade or intraventricular blocks. Electrocardiography is the usual means of detecting conduction system abnormalities. Exercise testing may also reveal atrioventricular block in patients with acquired conduction system disease. It is important to evaluate for other causes of conduction system disease, such as drugs or ischemia, before attributing disease to lupus activity alone.

Treatment

Asymptomatic patients with well-controlled disease, a negative evaluation for other etiologies, and low-grade block may only need close observation. Higher-grade block with or without symptoms frequently requires intervention. Long-standing involvement with tissue inflammation and immune complex deposition may lead to conduction system fibrosis, which can be irreversible. Medical therapy or pacemaker implantation may be needed to maintain hemodynamic stability.

Valvular Heart Disease in SLE

Epidemiology and Pathogenesis

Valvular heart disease is prevalent in SLE, with abnormal echocardiographic findings reported in 21% to 61% of patients

(7 ,8 ,9 ,10 ,16 ,18 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49) (Table 34-4). The classic valvular abnormality associated with SLE is Libman-Sacks endocarditis, which is characterized by noninfectious verrucous vegetations that may be found on any or all valves. Libman-Sacks endocarditis most commonly manifests as valvular thickening, noted in 51% of 61 patients in a study of valvular heart disease in SLE (18). An autopsy series of 36 SLE patients noted the presence of Libman-Sacks lesions in 50% of cases (3). Other reported abnormalities that contribute to the prevalence figures in Table 34-1 include valvular stenosis and insufficiency.

Table 34-4: Valvular Heart Disease in SLE

Author	Year	Patient (#)	Prevalence (%)	Diagnostic Modality	Comment
Bulkley et al. (3)	1975	36	50	Autopsy	Libman-Sacks lesions
Klinkhoff et al. (6)	1985	47	21	Echo	Valvular thickening
Galve et al. (42)	1988	74	24	Echo	Valvular abnormality
Crozier et al. (7)	1990	50	60	Echo	Mitral or aortic regurgitation Consecutive patients
Khamashta et al. (43)	1990	132	23	Echo	Valvular abnormality Consecutive patients
Leung et al. (16)	1990	75	25	Echo	Left-sided insufficiency
Nihoyannopoulos et al. (8)	1990	93	28	Echo	Valvular abnormality
Cervera et al. (44)	1992	70	44	Echo	Valvular abnormality
Sturfelt et al. (9)	1992	75	27	Echo	Valvular abnormality
Gleason et al. (45)	1993	20	40	Echo	Valvular abnormality
Gabrielli et al. (46)	1996	46	39	Echo	Valvular abnormalities Consecutive patients
Roldan et al. (18)	1996	69	61	Transesophageal echo	Valvular abnormality 51% with valvular thickening 43% with vegetation
Jensen-Urstad et al. (47)	2002	52	27	Echo	Valvular abnormality
Leszczynski et al. (48)	2003	52	35	Echo	Valvular abnormality
Morelli et al. (49)	2003	71	43	Echo	Valvular abnormality

Valvular abnormalities are commonly associated with the antiphospholipid antibody syndrome (APS). This relationship was initially described in the late 1980s, when several published reports identified the presence of valvular heart disease in patients with known antiphospholipid antibodies (aPL) (50 ,51). During this time period, the association between anticardiolipin antibodies and the presence of CV abnormalities in SLE patients was also identified (52 ,53). An early case series of 11 patients detailed a syndrome consisting of aPL, recurrent thrombosis, valvulitis, and thrombocytopenia (54). These patients also had associated valvular insufficiency, with two having multiple valves affected. Although there has been speculation that aPL may be the primary etiology of valvular heart disease in SLE, data supporting this view has been inconsistent and suggests that other factors are involved in the pathogenesis of SLE-associated valvular lesions. Data supporting the role of aPL contributing to valvular disease was reported by Leszczynski et al., who evaluated 52 SLE patients and compared echocardiographic findings with those of 34 healthy controls (48). SLE patients more frequently had valvular abnormalities than healthy controls (35% vs. 6%). They also found that anticardiolipin antibodies were strongly associated with valvular heart disease, detected in 77% of the SLE patients with valvular disease. Another study of 93 SLE patients suggested that the influence of aPL on cardiac disease might go beyond valvular dysfunction. Fifty patients (54%) had elevated levels of anticardiolipin antibodies. Of these 50 patients, 40 (78%) had at least one cardiac abnormality including valvular disease (20), pericardial effusion (15), and myocardial dysfunction (5 ,8). However, data from Gleason et al. comparing primary APS patients with SLE patients (aPL negative) and healthy controls demonstrated no statistically significant difference in the prevalence of cardiac valvular disease among the patient groups (APS vs. SLE) (45). Gabrielli et al. compared the prevalence of valvular heart disease in a group of SLE patients with primary APS patients (46). Thirty-nine percent of SLE patients had valvular abnormalities, compared with no patients in the primary APS group. Among the patients with SLE there was no significant association between the presence of aPL and valvular disease. Although aPL are unlikely to be the sole pathogenic factor in valvular disease in SLE, the high prevalence of valvular

disease in both primary APS and in SLE patients with aPL implicates their role in valvulopathy.

Libman-Sacks lesions were initially reported most often affecting the tricuspid valve, but more recently have been noted to preferentially occur on the mitral valve (7 ,18 ,48 ,55). Similarly, valvular lesions in aPL-associated valvular disease more commonly occur on the valves of the left heart (16). Valvular lesions can affect valve leaflets, rings, and commissures; less often the chordae tendinea, papillary muscles, and endocardium. These lesions are visually indistinguishable from vegetations caused by infectious organisms, but have a more distinctive histopathologic appearance. In addition to proliferating and degenerating cells and fibrin, Libman-Sacks verrucae contain fine granular material composed of hematoxylin bodies and immunoglobulin deposits (56 ,57). Valvular disease associated with aPL is similar to Libman-Sacks lesions, both in gross morphology and in histopathologic appearance. A study comparing valvular specimens of primary APS patients with secondary APS patients demonstrated linear subendothelial deposition of immunoglobulins with complement components in both groups (58). These changes were not noted in any of the normal valve specimens. In both groups, anti-idiotypic antibody to anticardiolipin was detected in the same location with the same pattern of staining. aPL-associated valvulopathic changes probably represent part of the spectrum of Libman-Sacks endocarditis, but comparative systematic histopathologic studies to support this assertion are lacking.

The pathogenesis of Libman-Sacks endocarditis has not been clearly elucidated, however, several mechanisms have been considered. It has been assumed that the lesions are initially formed by fibrin-platelet thrombi that propagate and lead to structural deformation of the valve and associated structures, resulting in valvular dysfunction. However, this does not address what the inciting insult is to the valvular structure that leads to the development of these lesions. The presence of complement and immunoglobulin deposition in Libman-Sacks lesions has been postulated as evidence for a role of immunologic injury, perhaps related to immune complex formation associated with disease activity.

The pathogenic mechanisms of APS-associated valvular disease are not well understood. The presence of immunoglobulins and complement components in verrucous valvular lesions again implies an immune-mediated process (58). Furthermore, the presence of anticardiolipin antibodies in the immunoglobulin deposits suggests either a direct immunologic interaction with valvular tissue or secondary participation following a primary immunologic response elicited by another pathogenic source. Additionally, there are inflammatory changes noted in the valvular lesions associated with APS (59). These findings suggest that aPL contribute more than just a prothrombotic milieu.

Clinical Features and Diagnosis

The clinical presentation of Libman-Sacks endocarditis may be indistinguishable from that of infective endocarditis. Patients may manifest with fever, anemia, tachycardia, splinter hemorrhages, and other embolic phenomena. Murmurs are neither sensitive nor specific for Libman-Sacks endocarditis. Although more frequent in infective endocarditis, patients with Libman-Sacks may also develop hemodynamic changes from chordae tendinea rupture or papillary muscle or valve ring involvement. Echocardiography may reveal verrucous lesions, but they cannot be distinguished from infective vegetations or thrombotic lesions reliably on the basis of appearance. As in infective endocarditis, transesophageal echocardiography is more sensitive for detecting the presence of valvular vegetations in SLE and should be employed in SLE patients in whom valvular disease is suspect when transthoracic echocardiography is unrevealing. The presence of disease activity also is not specific for Libman-Sacks endocarditis, since disease activity and infection often occur simultaneously. Additionally, infectious and/or thrombotic valvular lesions can occur concurrently with Libman-Sacks lesions. The critical aspect in evaluation of valvular lesions in SLE is to identify other causes that would merit therapy other than treatment of SLE.

Although initially thought to have minimal clinical consequences, conflicting data exists regarding outcomes of Libman-Sacks endocarditis. One large retrospective analysis determined that only 3% to 4% of patients had clinically significant valvular dysfunction, with 1% to 2% requiring surgical intervention (43). A small prospective study had less optimistic findings; 18% of SLE patients were found to have clinically significant valvular dysfunction, with 8% requiring surgery (42). Roldan et al. detected valvular vegetations in 43% of patients, while valvular insufficiency and stenosis were noted in 25% and 4% respectively (18). In this study, valvular disease was not related to clinical disease features, age, disease duration, disease activity or severity, or disease therapy. Upon followup imaging, valvular vegetations were not static in most patients, with both the appearance of new vegetations and disappearance of prior vegetations noted. By a mean of 57 months of follow-up, 21% of patients developed complications of valvular disease including stroke, peripheral embolism, congestive heart failure, and infective endocarditis. Another study evaluating the consequences of left-sided heart valve abnormalities (thickening, regurgitation, stenosis, prolapse) followed 71 SLE patients over a mean period of 5.8 years (49). At baseline, 43% had evidence of left-sided valve involvement on transthoracic echocardiography. In the 40 patients who received followup echocardiographic studies, new valvular disease was present in 5 patients, worsening of valvular disease in 5 patients, and stable disease in 10 patients. Twenty-six percent suffered ischemic strokes during the study period. The odds ratio was 10.84 for the association of left-sided valvular disease with ischemic cerebrovascular events. Jensen-Urstad et al. demonstrated that valvular abnormalities might also be associated with the presence of CVD in SLE (47). In a study of 26 SLE women with CVD (cases), 26 SLE women without CVD (controls), and 26 healthy women, thirteen

SLE cases had valvular abnormalities as compared to one SLE control and one healthy control. Valvular abnormalities in the cases were associated with higher levels of serum triglycerides and homocysteine.

Valvular dysfunction requiring surgical repair or replacement has also been reported in patients with aPL-associated valvular disease (60 ,61).

Treatment

In patients in whom infection is suspected, antibiotic therapy directed at the most likely organism should be initiated immediately. If no infection is detected but is still considered likely, the most prudent course is to treat the patient with the appropriate full course of antibiotic therapy. There is no specific therapy directed at Libman-Sacks endocarditis. If disease activity is present by clinical and/or laboratory means, immunosuppressive therapy will be required for other disease manifestations. The impact of immunosuppressive therapy on Libman-Sacks endocarditis is not clear. There is some suggestion that the use of glucocorticoids has changed the natural history of valvular lesions in SLE. Bulkley and Roberts reported complete or partial healing of these lesions at autopsy, with resultant fibrous thickening and valvular calcification in patients from the poststeroid era (3). These changes were not noted in autopsies of SLE patients in the presteroid era. Valvular lesions leading to clinically significant valvular dysfunction need to be treated as those from any etiology. It should be noted that Libman-Sacks lesions might recur after surgical intervention (62).

Treatment of aPL-associated valvular disease in patients with primary or secondary APS is centered on the management of APS. The focus is on anticoagulation with agents such as warfarin or heparin, usually in the range described as high-intensity (INR 2.5 to 3.5 or equivalent). APS associated with SLE, or in catastrophic APS, therapy may also include immunosuppression. In patients with aPL antibodies and valvular disease without a prior history of thrombosis, the lack of evidence-based data makes management less straightforward. There is no evidence to support anticoagulation, the use of platelet inhibitors such as aspirin, or immunosuppressive therapy.

Prevention

Although no evidence points to proven means of preventing SLE- and aPL-associated valvular disease, it seems reasonable to focus efforts on controlling the underlying disease activity. No recommendations have been made by the American Heart Association (AHA) specifically regarding endocarditis prophylaxis in SLE, but based on their risk stratification many SLE patients with valvular disease would merit consideration. According to the 1997 guidelines, patients with prosthetic valves or the presence of valvular vegetations are considered high risk, while patients with mitral valve prolapse with mitral regurgitation or other acquired valvular dysfunction are considered moderate risk (63). By these criteria, a significant number of SLE patients would be considered moderate or high-risk, and antibiotic prophylaxis prior to undergoing bacteremia-inducing procedures would be indicated. A survey of dentists regarding endocarditis prophylaxis in SLE found that about 95% of those queried would not recommend prophylaxis in SLE, suggesting lack of awareness of the prevalence of valvular abnormalities in SLE (64). Hence, it falls upon the rheumatologist to ensure that SLE patients with valvular involvement receive appropriate prophylaxis.

Atherosclerotic Disease

Epidemiology and Pathogenesis

The description of a bimodal mortality pattern in SLE patients by Urowitz et al. in 1976 was an instrumental step toward identifying their increased risk of premature atheromatous CVD (65). Patients who succumbed to lupus early in the disease course were noted to die most often from complications of disease activity (e.g., organ failure) or therapy. Patients who died later often had quiescent disease, and died from CV events. These findings were substantiated by an autopsy series reported by Bulkey et al. in which the majority of a cohort of young women with a mean age of 35 years had significant obstructive atherosclerotic disease of at least one major coronary artery (3). As survival has improved from better means of disease detection and treatment, atherosclerotic CVD has emerged as a significant cause of morbidity and mortality in SLE. The prevalence of CV and cerebrovascular events in SLE ranges from 6% to 26% (9 ,10 ,18 ,49 ,65 ,66 ,67 ,68 ,69 ,70) (Table 34-5). Ischemic cerebrovascular events (stroke/transient ischemic attack) have been reported in 10% to 26%, (18 ,49 ,68 ,69 ,70), whereas MI and angina have been reported in 6% to 11% of SLE patients (9 ,10 ,65 ,66 ,67 ,68 ,69 ,70).

Not surprisingly, autopsy studies as well as the examination of surrogate markers of coronary atherosclerosis in SLE suggest that the prevalence of subclinical atherosclerosis is higher than overt events (3 ,71 ,72 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82) (Table 34-6). Autopsy studies reveal atherosclerotic disease of the coronary arteries in SLE in 22% to 54% of cases (3 ,71 ,72). Noninvasive studies including vascular ultrasound, electron beam tomography, and myocardial perfusion studies demonstrate atherosclerotic vascular disease in 17% to 40% of SLE patients (73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82). In a cohort of SLE women with a mean age of 44.9 years, 40% were found to have focal carotid plaque as measured by ultrasound (73). Another study of 75 SLE women with a mean age of 38.8 years demonstrated that 28% had coronary artery calcifications (77). Roman et al. found carotid plaque in 37% of SLE patients, compared with a prevalence of 15% in age, sex, and race-matched controls (78).

A striking feature of this comorbid condition of SLE is its predilection for premenopausal women. Ward evaluated rates of hospitalization for CV events in a cohort of SLE women compared to a control group (83). Younger SLE

women (age 18 to 44) were 2.27 times more likely to be hospitalized with MI and 3.8 times more for congestive heart failure than controls. In the middle-aged women (45 to 64 years), the frequency of hospitalization for heart failure was just 1.39 times higher, and the frequency for MI hospitalization did not differ significantly from that of controls. A study comparing SLE women with age-similar women from the Framingham offspring cohort demonstrated a 50-fold increased risk of MI in the SLE women between 35 and 44 years of age (84). In sharp contrast to women in the general population, where the risk of atherosclerotic CV events is highest after menopause, the mean age at the first event in SLE women is about 49 years (85).

Table 34-5: Cardiovascular Events in SLE

Author	Year	Patient (#)	Prevalence (%)	Comment
Urowitz et al. (65)	1976	81	11	MI
Petri et al. (66)	1992	229	8	Angina/MI
Sturfelt et al. (9)	1992	75	9	MI
Jouhikainen et al. (10)	1994	74	6	MI
Hearth-Holmes et al. (67)	1995	89	6	Angina/MI
Roldan et al. (18)	1996	58	12	Stroke
Rahman et al. (68)	2000	150	15	Angina/MI/Stroke/PVD 50% hypertensive
Morelli et al. (49)	2003	50	26	TIA/Stroke Consecutive patients; 23% with prior history
Bessant et al. (69)	2004	47	8/10	MI/Stroke
Toloza et al. (70)	2004	546	6	MI/Angina/Stroke/PVD

Table 34-6: Atherosclerotic Disease in SLE

Author	Year	Patient (#)	Prevalence (%)	Comment
Bulkley et al. (3)	1975	36	22	Autopsy (>50% coronary lesion)
Haider et al. (71)	1981	22	45	Autopsy (>75% coronary lesion)
Abu-Shakra et al. (72)	1995	40	54	Autopsy
Manzi et al. (73)	1999	175	40	Carotid ultrasound
Bruce et al. (74)	2000	130	40	Single photon emission computed tomography
				Dual isotope myocardial perfusion imaging
				Consecutive female patients
Asanuma et al. (75)	2003	65	31	Electron beam tomography (coronary artery calcification)
Doria et al. (76)	2003	78	17	Carotid ultrasound
Manger et al. (77)	2003	75	28	Electron beam tomography (coronary artery calcification)
Roman et al. (78)	2003	197	37	Carotid ultrasound
				Consecutive patients
Sella et al. (79)	2003	82	28	Myocardial perfusion scintigraphy
Selzer et al. (80)	2004	214	32	Carotid ultrasound
Wolak et al. (81)	2004	51	28	Carotid/femoral ultrasonic bx
				Consecutive patients
Jimenez et al. (82)	2005	70	28	Carotid ultrasound

Traditional risk factors for CVD occur frequently in SLE, both as a consequence of disease activity and treatment. The presence of subclinical inflammation in the general population has been demonstrated to correlate with the development of a number of traditional risk factors, including

insulin resistance, visceral obesity, and hypertension (86 ,87 ,88). It is possible that the sustained systemic inflammation and immune activation in SLE has similar influences on the development of CV risk factors. The Toronto Risk Factor Study compared 250 SLE patients with 250 controls and found that SLE patients had a higher number of CV risk factors per patient as well as a higher prevalence of diabetes, hypertension, and elevated levels of low-density lipoproteins, triglycerides, and homocysteine (89). Additionally, both Bruce et al. and Costenbader et al. have demonstrated that even when CV risk factors are identified in SLE patients, they often are not adequately treated (90 ,91). Although a large part of CVD risk in SLE is likely a result of a high prevalence of traditional CV risk factors, Esdaile et al. demonstrated that the presence of CV risk factors alone does not explain the increased incidence of CV events (92).

Glucocorticoid therapy has been implicated in atherosclerosis in both lupus and nonlupus patients, but it is unclear whether this reflects pro-atherogenic effects of the underlying disease process or adverse metabolic effects associated with steroid use (93 ,94 ,95).

Antiphospholipid antibodies have been considered as a contributory factor to SLE-associated atherosclerotic disease. In addition to their postulated role in arterial endothelial damage, they have been associated with renal arterial disease (both thrombotic and stenotic), which may result in hypertension from activation of the renal-angiotensinogen-aldosterone axis from renal hypoperfusion (96 ,97). Although cohort studies have failed to identify an association between the presence of antiphospholipid antibodies and surrogate markers of coronary atherosclerosis, there is a strong association between surrogate markers of CVD and CV events with hypertension with in SLE (68 ,98 ,99). The importance of this association becomes apparent when more closely evaluated.

Hypertension and Lupus

Hypertension occurs commonly in SLE, with its prevalence ranging from 22% to 48% (5 ,12 ,78 ,79 ,82 ,91). Several studies examining CVD risk factors in SLE document an increased risk of hypertension in SLE when compared to age- and sex-matched healthy controls, but this relationship has not been consistently demonstrated in other studies (75 ,78 ,81 ,82 ,83). As in the general population, hypertension in SLE patients is associated with surrogate markers of CVD including carotid plaque, carotid intimal-medial thickness (IMT), vascular stiffness, and abnormal myocardial perfusion scintigraphy (73 ,76 ,78 ,79 ,80). Hypertension in SLE has also been demonstrated to correlate with vascular events (MI, angina, stroke, and peripheral vascular disease) and with the presence of severe coronary artery narrowing on autopsy (66 ,68 ,71).

Hypertension in SLE may arise from a variety of etiologies, including essential onset, medication side effects (e.g., sodium and fluid retention or weight gain from glucocorticoids), increased arterial stiffness (from systemic inflammation or atherosclerotic disease), or renal disease. Fortunately, this risk factor is one that is readily amenable to therapy.

It has been assumed that there exists a “lupus factor” that explains the increased prevalence and incidence of CVD in SLE. However, serologic and clinical scales reflective of disease activity have not demonstrated a consistent association with surrogate markers of CVD or CV events. Despite these findings, disease activity could be an important pro-atherogenic factor. Patients with clinically apparent, severe disease activity generally receive the most aggressive and sustained therapies. This could lead to better control in patients with “worse” disease, while patients with low level, less severe manifestations continue to have “grumbling” disease, with low-grade but persistent systemic inflammation. The importance of low-grade inflammation has been demonstrated in the general population, in whom higher levels of C-reactive protein within the “normal” standard range predict future CV events (100 ,101). It could be that duration of systemic inflammation, rather than intensity, is more influential on atherogenesis. Additionally, it could be that the sicker patients, with more frequent visits and closer scrutiny, are more likely to have CV risk factors detected and treated.

In the general population, the recent identification of myeloperoxidase, a source of reactive oxidants, as a surrogate marker for coronary artery disease and independent predictor of CV events demonstrates the emerging utility of markers of subclinical inflammatory processes in CV detection and risk prediction (102 ,103). Additionally, in the general population, myeloperoxidase levels are associated with the presence of traditional CV risk factors (104). Oxidant stress is abundant in SLE, and is associated with disease activity, but its role in CVD risk prediction has not yet been evaluated (105 ,106).

Lastly, it may be that our current means of identifying traditional risk factors in SLE is not optimal. For example, the Quebec Cardiovascular Study recently revealed that lipoprotein subfractions, rather than the standard quantitative measures of serum lipoproteins, better predict the presence of and risk for CVD and related events (107). A pro-atherogenic subfraction distribution may be present even in the presence of a “normal” lipid profile. Furthermore, studies of lipoprotein subfractions in other inflammatory disorders suggest that the presence of systemic inflammation promotes a pro-atherogenic lipoprotein subfraction distribution (108 ,109). It is likely that lupus disease activity, which increases oxidant stress and may also influence production of pro-atherogenic lipoprotein subfractions, plays a significant role in the excess CVD risk seen in SLE.

Clinical Features and Diagnosis

Signs and symptoms of atherosclerotic CVD in SLE are similar to those reported in the general population. Symptoms of ischemic heart disease may include chest pressure or

pain (with exertion and/or rest pain), dyspnea, dizziness/lightheadedness, diaphoresis, nausea, “heartburn,” shoulder/jaw/arm pain, upper back pain, and fatigue. A unique caveat in SLE is the prevalence of atherosclerotic disease in young women, a group once thought to be at very low risk in general for CVD. Signs and symptoms that would prompt immediate CV evaluation in a middle-aged male may not provoke the same concern in many physicians when reported by a premenopausal female, and could be attributed to any number of SLE and non-SLE–related conditions.

Diagnosing atherosclerotic CVD in SLE utilizes the same diagnostic studies as for evaluation of CVD in the general population. Stress echocardiography, nuclear imaging modalities, and cardiac catheterization all are appropriate studies for the evaluation of coronary atherosclerotic disease in SLE. Limited exercise capacity in many SLE patients in addition to the poor sensitivity and specificity of isolated treadmill stress testing in women limits its usefulness as an investigative study. Carotid ultrasound, electron beam tomography, and MRI have been generally used as research tools for determining prevalence and association with risk factors of CVD in SLE. The utility of these imaging tools on an individual basis is less clear. The presence of carotid plaque or coronary calcification may prompt more aggressive management of traditional CV risk factors, although many experts believe that all lupus patients should be treated as coronary heart disease equivalents with rigorous CV risk factor management. The critical factor in detecting atherosclerotic vascular disease in SLE is maintaining an appropriately elevated index of suspicion of CVD, especially in young women. Any symptoms suspicious for ischemic heart disease, regardless of the patient's age, should prompt a thorough evaluation.

Treatment/Prevention

Hypertension is strongly associated with the development of surrogate markers of CAD and with CV events, and thus warrants aggressive management and diligent monitoring. The protective effects of antihypertensive therapies in the general population appear to come from both lowering of blood pressure and from beneficial metabolic effects (110 ,111 ,112). It is likely that the same effects are present in SLE patients receiving therapy. ACE inhibitors are efficacious in controlling blood pressure, but are also beneficial in maintaining renal function by decreasing proteinuria and possibly by their antioxidative effects. These agents should be used with caution in patients with known renal artery stenosis. Angiotensin-receptor blockers have been purported to have protective effects comparable to ACE inhibitors, and may be a reasonable substitute (113 ,114). Beta-blockers and aldosterone antagonism provide additional benefit for patients with compensated heart failure (115 ,116). The focus should be on obtaining and maintaining strict blood pressure control.

Dyslipidemia in SLE should be managed as in the general population. Diet modification and regular exercise may help improve lipids, but in many patients will prove insufficient to attain a desirable lipid profile. The use of beta-hydroxy-beta-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) in SLE is currently under study in two large, randomized placebo controlled trials. Interestingly, these agents are demonstrating anti-inflammatory and antioxidative effects in the general population, and in a small study of rheumatoid arthritis patients, were shown to have disease-modifying activity (117 ,118 ,119). Regular monitoring of hepatic function is mandatory with the use of these agents, and may be required more frequently when used with other hepatically metabolized drugs.

Obesity in SLE may arise from disease or treatment-associated physical disability, glucocorticoid use, poor dietary habits, or lack of exercise. Patients should be counseled at disease onset and periodically advised regarding proper nutrition and exercise habits, individually suited to their other comorbidities and physical capacities.

It has become clear that insulin resistance exists prior to the development of clinically detectable hyperglycemia. A high index of suspicion for this condition should be maintained, especially when other metabolic syndrome features are present. However, it has not yet been determined what measures are indicated to best manage this condition prior to glucose elevation other than other risk factor management, including dietary and exercise interventions. Steroid-induced hyperglycemia warrants treatment, rather than observation.

The major focus of CVD therapy in SLE should be on prevention. As the awareness of the presence of traditional risk factors in SLE has grown, the numbers of patients receiving screening, counseling, or therapy for these factors has increased, but remains suboptimal. Costenbader et al., examined a cohort of 110 SLE patients with mean disease duration of 15.3 years and found that only 58% of the modifiable risk factors had been addressed (91). Twenty-three percent had never had cholesterol screening, and only 21% with dyslipidemia had this risk factor treated by dietary or pharmacologic intervention. Even diabetes mellitus, which is globally recognized as a CVD risk equivalent, was not treated in 25% of patients in this cohort. A key facet in prevention of CVD and related events in SLE is patient education. Patients and their physicians need to be informed of the increased risk of CVD associated with SLE, and should be aware of CVD risk factors. This awareness may be helpful when providing counseling regarding modification of risk factors that require significant lifestyle changes, such as tobacco cessation, dietary habits, and body weight. The accelerated and premature atherosclerosis in SLE is reminiscent of that in diabetes mellitus, suggesting that we might be justified in considering SLE in the same manner-as a CVD equivalent. Risk factor screening should be initiated at the time of diagnosis, and be continued annually. Modifiable risk factors present, such as dyslipidemia, hypertension, hyperglycemia, hyperhomocysteinemia, and the lifestyle factors previously noted should be treated and monitored. The recent data regarding the contribution of inflammation to the atherogenesis implies that control of

disease will also be an essential component of CVD prevention. This is a straightforward undertaking in patients with clinically active and organ-threatening disease. Until we have a more sensitive means of detecting subclinical SLE activity and understand of how “grumbling” activity directly impacts CVD, it will be difficult to formulate a comprehensive approach to CVD prevention.

Cardiovascular manifestations occur frequently and can result in significant morbidity. An elevated index of suspicion for and familiarity of the differential diagnosis associated with each of these manifestations is essential for timely diagnosis and treatment. The increased prevalence of CV risk factors and the markedly increased risk of CVD in SLE mandate vigilant surveillance for modifiable risk factors and for their aggressive treatment and monitoring in all patients, regardless of age or sex. Although progress continues to advance in this area, there remains much to be understood about key mechanisms that result in the CV manifestations of SLE.

An approach directed at maintaining CV health in SLE must begin at disease onset and continue throughout the lifespan of the patient. At the time of diagnosis, patients should be screened for CV risk factors and treated appropriately (Table 34-7). Monitoring the response to therapy and adjusting therapy to attain treatment goals must be given equal priority. A low threshold of suspicion for CVD needs to be maintained throughout the patient's lifetime, regardless of the age at diagnosis. The prevalence of valvular heart disease in SLE dictates careful evaluation for this condition when cardiac abnormalities are detected by physical examination or by other means (e.g., imaging or functional studies). Additionally, in characterizing SLE as conferring the risk of a CVD equivalent, any signs or symptoms suggestive of ischemic heart disease must be promptly and thoroughly evaluated. The same concerns should be reflected in the preoperative evaluation of patients with SLE. Since many factors that are contributory to CVD and CV risk in SLE are initiated and/or intensified by disease activity, the control of disease activity has a central role in the prevention and treatment of the CV manifestations of SLE.

Table 34-7: Addressing CV Risk Factors in SLE

Factor	Intervention
Obesity	Nonpharmacologic interventions (weight loss, dietary, activity) Regular monitoring and feedback
Hypertension	Nonpharmacologic interventions Antihypertensive therapy Regular monitoring and medication adjustment
Dyslipidemia	Nonpharmacologic interventions Lipid-lowering therapy Regular monitoring and medication adjustment
Insulin resistance	Weight maintenance (loss if necessary) Regular exercise
Hyperglycemia/diabetes	Nonpharmacologic interventions Hypoglycemic therapy at onset of hyperglycemia Regular monitoring and medication adjustment
Hyperhomocysteinemia	Folic acid supplementation Monitoring and adjustment of dose
Tobacco use	Counseling Medical therapy
Inflammation	Aggressive control of disease activity Adequate control of above risk factors (they both result from and propagate inflammation)

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Chapter 35

Pulmonary Manifestations of Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) and the adverse effects of treatment may affect all regions of the thorax. Pulmonary involvement is common in SLE and may affect up to 60% of patients during the disease course and is part of the spectrum of presenting symptoms in 4% to 5% of patients (1). Furthermore, other complications of lupus such as cardiac failure or nephrotic syndrome may lead to lung involvement with the development of pleural effusions. Pulmonary involvement may increase the risk of mortality—in one study, lung involvement was one of several factors that was predictive for increased mortality (2).

The prevalence of pulmonary manifestations in SLE depends on the referral pattern to the unit where patients are studied, the population under scrutiny, and the methods used to detect pulmonary involvement. For example, the total cumulative prevalence of clinically apparent lung involvement was approximately 20%, with serositis seen in 45% of patients in a large cohort of patients (1). However, in a cross-sectional study of lung function, 90% of patients had subclinical abnormalities of pulmonary function tests (3).

The lungs may conveniently be considered in six main regions: the pleural space, the parenchyma, the pulmonary vasculature, the airways, the diaphragm, and the chest wall including the ribs and respiratory muscles. This chapter describes the clinical features, investigation, pathologic features, and management of the various pulmonary manifestations of SLE. Recent advances in diagnostic tools and therapy will also be addressed.

Historical Perspective

The contributions of Osler and Jadasson established that SLE was a distinct syndrome, and in 1904 Osler (4) described the occurrence of pneumonia in SLE. Further reports in the 1930s and 1940s described polyserositis, patchy lung consolidation, and other pulmonary changes including “atelectizing pneumonitis” in patients with SLE (5 ,6). Autopsy of these patients showed diffuse lung consolidation with hyaline alveolar thickening and marked obliterating cellular infiltration. Purnell et al. extended these observations and described a peculiar basophilic, mucinous edema of the alveolar walls and the peribronchial and perivascular tissues in association with interstitial pneumonitis and alveolar hemorrhage (7). These pathologic changes were distinct from the ordinary pyogenic and fibrinous bronchopneumonia that frequently complicates the terminal stages of SLE, but they were not pathognomonic of SLE (7). In atelectizing pneumonitis, the alveolar walls as well as the peribronchial and perivascular connective tissues appeared to be the primary sites of an inflammatory process that obliterated alveolar spaces.

In 1953 and 1954, two reports of radiographic studies in SLE emphasized the high frequency of pulmonary involvement. Israel (8) showed that non-specific pneumonia occurred frequently, not only during the terminal stages of SLE but throughout the course of the disease. Garland and Sisson (9) reported lung parenchymal changes in one third of their patients and observed both pleural and cardiac abnormalities to be prevalent. Comprehensive reviews of the pulmonary manifestations of SLE in adults (10 ,11 ,12) and in children (13) have appeared.

Pathology

A wide variety of lesions have been described in the lungs, but none are specific for SLE. For example, an autopsy study of 54 patients showed the following pathologic abnormalities in more than 50% of cases: bronchopneumonia, hemorrhage, pleural effusion, edema, interstitial pneumonia, and congestion (7). Fibrinoid necrosis and hematoxylin bodies occasionally were seen (14), whereas bronchiolar dilatation and foci of panacinar emphysema were often found (15). Fayemi (16) noted a high frequency of occlusive vascular changes of varying severity in the lungs of 8 of 20 patients with SLE; these changes affected arterioles, arteries, and veins. The acute lesions consisted of fibrinoid necrosis and vasculitis, and the chronic lesions included intimal fibrosis, medial hypertrophy, alteration of the elastic laminae, and periadventitial fibrosis.

Haupt et al. (17) examined pathologic changes in the lungs of 120 patients seen at autopsy and correlated these with their clinical features. In contrast to the high frequency of lung involvement in a clinical series (10), they showed that many of the pathologic lesions were not caused by SLE

itself but rather by secondary factors, such as congestive heart failure, infection, aspiration, and oxygen toxicity. Only 18% of the lung parenchymal lesions were found to be directly attributable to SLE.

Although informative, analyses of autopsy cases may not represent a cross-section of the general population of lupus patients and probably underestimate the prevalence of lung involvement. Certainly in the clinical setting pulmonary abnormalities can change rapidly and subside either spontaneously or with drug therapy, so anatomic lesions may not be evident later at autopsy.

The Clinical Assessment of Pulmonary Involvement

As with any other branch of medicine the clinical assessment of pulmonary disease in lupus patients is based on a careful history, thorough examination, and appropriate further investigations.

A useful screening question is: “Have you been short of breath or had chest pains?” The most common early symptom of serositis is pleuritic chest pains, which occur on both inspiration and expiration. There is nothing particular about the pain that differentiates lupus from other causes of pleurisy such as infection or pulmonary embolism. Resolution of pleuritic pain does not always imply improvement, because the pain at a particular site of the chest may disappear if a significant pleural effusion develops. Dyspnea may be the presenting symptom of many of the other complications of lupus to affect the lung. A sudden onset is more likely to be a result of pulmonary embolism, but the more usual presentation is an insidious onset of breathlessness. An estimate of the patient's walking distance is a useful guide to exercise tolerance and the extent of lung involvement. A careful history to exclude cardiac disease should be undertaken. Hemoptysis may be the presenting feature of infection, infarction, cardiac failure, or very rarely pulmonary hemorrhage.

The examination of the respiratory system should be part of a comprehensive clinical examination that includes blood pressure and urinalysis. For example, vasculitic lesions may indicate more widespread disease activity. Splinter hemorrhages raise the possibility of small vessel vasculitis or cardiac valvular disease in a breathless patient with SLE. Interestingly, although finger clubbing is common in patients with idiopathic fibrosing alveolitis, it is relatively unusual in patients with interstitial lung disease of lupus (12). The presence of central cyanosis should be noted and a careful search should be made for late inspiratory basal crepitations—a sensitive sign of interstitial lung disease that should prompt further investigation. Pleural rubs and effusions should be sought as indicated by a history of pleuritic pains, though other causes of pleural effusions should be considered such as infection, nephrotic syndrome, cardiac failure, and malignancy. A thorough cardiac assessment is essential, with particular attention to signs of cardiac valve disease, left or right ventricular failure, and pointers toward pulmonary hypertension.

Investigation of Pulmonary Involvement

Imaging

Chest Radiography in SLE

The chest radiograph is a simple and useful extension of the clinical examination of the patient with SLE. Previous films, when available, may give valuable information on disease progression or the appearance of new lesions.

Chest radiograph abnormalities are reasonably common in patients with SLE. For example, in a prospective study of 50 unselected patients, abnormalities were found in 38% (18), and in a study of 43 patients who were examined specifically for pulmonary dysfunction, 23% had abnormal chest radiographs (19). In contrast, none of 70 non-smoking patients with SLE who were free of respiratory complaints and enrolled in a study of pulmonary function had abnormal chest radiographs (20). Several factors may explain this difference in the prevalence of abnormalities, including patient selection, the many manifestations of the disease that may be transient, and the timing of the radiographic study.

Radiographic Findings

A wide variety of abnormalities may be seen in chest radiographs. Early studies documented radiographic changes in the pleura, lung parenchyma, and heart (21). However, none of these abnormalities are specific or diagnostic for SLE and may be seen in other conditions such as rheumatoid arthritis and systemic sclerosis.

Pleural changes as an isolated abnormality or in combination with cardiac or parenchymal lesions are the most common radiographic changes (21 ,22). The pleura may appear as a shaggy thickening of the pleural surface. Pleural effusions are usually small and, in 50% of patients, bilateral (12). With clearing of the effusion, residual pleural thickening may be seen. Parenchymal lesions are characterized by ill-defined focal patches, linear bands, infiltrates, and small nodules or plaques at the bases (22). Diffuse granular, reticular, or reticulonodular lesions throughout the lung fields, but especially at the bases, are found in a small number of patients with SLE and chronic interstitial fibrosis (10). Cardiomegaly, if present, is usually mild to moderate (21), and isolated cardiac enlargement is almost as frequent as isolated pleural disease. In patients with cardiomegaly, there are often other factors such as hypertension, pericarditis, valvular disease, myocardial involvement, and anemia to explain the cardiac enlargement (21). Elevated diaphragms are found in 5% to 18% of patients (18 ,19).

Early investigators often ascribed parenchymal changes in chest radiographs to primary lung involvement caused by SLE. The term lupus pneumonitis was used indiscriminately to encompass various types of pulmonary lesions and has been reported in 15% to 50% of patients (12 ,23). In 111 patients with SLE, Levin (23) found that radiographic parenchymal changes (infiltrates or small nodules) were mostly the result of secondary complications, such as infections, uremic pulmonary edema, and basilar atelectasis. Primary lupus pneumonitis was relatively rare, being found in only three (2.7%) patients, and the diagnosis therefore became one of exclusion.

High-Resolution Computed Tomography and Magnetic Resonance Imaging

High-resolution computed tomography (HRCT) is an essential tool in the evaluation of lung disease in SLE. Early inflammatory lesions may be visualized as alveolar or ground-glass shadowing, and later in the disease course fibrotic lesions may be visible as fixed reticular honeycombing. These appearances usually correlate with the clinical finding of late inspiratory crackles at the lung bases.

Two studies from the same group have demonstrated the usefulness of HRCT scans of the chest in determining the prevalence of pulmonary involvement (24 ,25). The most common computed tomography (CT) findings were interstitial lung disease (ILD), bronchiectasis, mediastinal or axillary lymphadenopathy, and pleuropericardial abnormalities. No correlation was found between disease activity, duration of disease, chest symptoms, drug therapy, smoking history, and the presence of abnormal HRCT findings. Interestingly, no correlation was found between pulmonary function abnormalities and the presence or grade of interstitial lung disease or bronchiectasis as determined by HRCT, and the prevalence of pleuropulmonary disease was lower than in previous studies. Thus, HRCT evidence of airways disease and interstitial lung disease was frequently present despite an absence of symptoms, a normal chest radiograph, and normal pulmonary function testing. Furthermore, the study by Sant et al. (25) showed that 72% of HRCT scans were abnormal when only 34% of plain chest radiographs were abnormal.When combined with contrast injections, CT pulmonary angiography is a highly accurate method of detecting pulmonary emboli where ventilation/perfusion (V/Q) scans give indeterminate results, especially if the clinical suspicion of pulmonary embolism is high. The use of D-dimer assays in this clinical situation may increase the predictive value of diagnosing pulmonary emboli considerably and is less invasive than the gold standard of pulmonary arterial angiography. However, if the V/Q scan is indeterminate, the CT angiogram is inconclusive, and the clinical suspicion of pulmonary embolism is high, then pulmonary arterial angiography should still be considered, especially if D-dimer assays are positive (26). It should be remembered however that elevated D-dimer levels may simply be a reflection of active lupus or infection as it is an acute phase reactant and negative D-dimers are more useful in excluding thrombosis.

The role of MRI in the evaluation of pulmonary disease in lupus remains the subject of continuing studies. Case reports suggest that MRI may be useful in the evaluation of pulmonary artery thrombosis and pulmonary hemorrhage in SLE (27 ,28). In other fields MRI has been useful in assessing respiratory diaphragmatic and chest wall dynamics in pulmonary emphysema (29) and chronic infiltrative lung diseases (30). The major limitation of MRI of the lung parenchyma is image degradation because of breathing, though breath-hold techniques are now possible with faster acquisition times and gadolinium enhancement (30).

Pulmonary Function Tests

Simple spirometry gives useful information on the pattern of pulmonary involvement. The ratio of forced expiratory volume at one second to forced vital capacity ratio (FEV1/FVC) distinguishes restrictive patterns, most often because of interstitial lung disease, from obstructive patterns as a result of lesions that may affect the larger airways as well as documenting lung volumes. The pulmonary transfer factor using a single breath carbon monoxide technique is particularly useful in the detection of early interstitial lung disease, documenting disease progression and monitoring responses to treatment.

Pulmonary Function Test Abnormalities

Pulmonary function test (PFT) abnormalities are exceedingly common in patients with SLE, even in those without respiratory complaints and with normal chest radiographic findings (18 ,19 ,31 ,32) (Table 35-1). In 1965, Huang et al. (32) studied 28 consecutive patients with SLE and found a high prevalence of physiologic abnormalities with a restrictive pattern. Reduction of the diffusing capacity was the most common finding with a mean value of 65% of the predicted value. Importantly, they observed a disparity between clinical and chest radiographic findings and PFT abnormalities.

In 1966, another study of PFTs in 20 patients with SLE and respiratory symptoms described three major abnormalities: (a) restrictive disease, (b) airway obstruction, and (c) pulmonary vascular obstruction (33). Twelve of the patients had evidence of restrictive disease, three had severe airway obstruction without pulmonary restriction, and five had pulmonary vascular obstruction. Three patients died, and the pathologic findings in the lungs correlated with the physiologic abnormalities observed during life.

Wohlgelernter et al. (3) found PFT abnormalities in 90% of their patients with SLE and a previous history of pleuritis and/or pneumonitis, and in 71% of their patients without

pulmonary complaints. The most common abnormalities were a decreased carbon monoxide diffusing capacity of the lungs for carbon monoxide (DLCO), lack of response to breathing helium, restrictive ventilatory defect, and arterial hypoxemia. None of their patients with chronic discoid lupus erythematosus had an abnormal PFT, which is a finding consistent with the limited disease process in this condition. Rolla et al. (34) suggested a relationship between disease activity and KCO, indicating that systemic inflammation correlated with alveolar inflammation. Likewise, disease severity was related to airway patency and airway reactivity indices, suggesting cumulative damage to the airways in these patients (34). A similar study in children confirmed that transfer factor measurements were related to disease activity, and decreases in disease activity with treatment resulted in better pulmonary function (35). An interesting study described elevated nitric oxide (NO) levels in the exhaled air of lupus patients and correlation with disease activity in addition to pulmonary function testing (36). It was not clear, though, whether the NO arose from lung or systemic inflammation.

Table 35-1: Respiratory Function Studies in Systemic Lupus Erythematosus (SLE)

Authors	Year	No. of Patients	Conclusions
Uncontrolled studies			
Huang and Lyons	1965	*n* = 20 with compared to *n* = 8 without lung disease	↓ DLCO in 57%, ↓ lung volumes; no correlations between PFT, CXR, or clinical findings
Gold and Jennings	1966	*n* = 20, 5 with lung	Restrictive pattern in 60%, obstructive symptoms in 10%. ↓ DLCO most common finding
Holgate et al.	1976	*n* = 30 SLE all with lung disease	All abnormal
Wohlgelernter et al.	1978	*n* = 10 (group 1) with and *n* = 14 (group 2) without lung symptoms or signs	↓ DLCO, arterial hypoxemia and/or restrictive pattern in 90% (group 1) and 71% (group 2); small airways disease in*n* = 10
Silberstein et al.	1980	*n* = 43	88% abnormal: ↓ DLCO (72%), ↓ lung volumes (44%), obstructive pattern (7%); no correlation between PFT and disease activity
Nakano et al.	2002	*n* = 110 SLE followed for up to 195 days	↓ Dlco in 47%. Restrictive defect in 8%. Correlation with Raynaud.
Zheng et al.	2002	*n* = 48 SLE	↓ Dlco in 42%. HRCT showing ILD in 5/14
Longitudinal studies			
Eichacker et al.	1988	*n* = 25 followed for 2-7 years	No changes in DLCO, forced vital capacity or lung volumes; reduction in small airway function unrelated to smoking
Cerveri et al.	1996	*n* = 15 children followed for 5 years	↓ TLCO 45% at baseline, 35% at follow-up; TLCO correlated with disease activity
Controlled studies			
Chick et al.	1976	*n* = 28 SLE and no lung symptoms, *n* = 28 controls	↓ DLCO, lung capacity and functional residual volume in SLE; smoking effects similar in both groups
Andonopoulos et al.	1988	*n* = 70 SLE nonsmokers, *n* = 70 control nonsmokers	Abnormal PFT in 63% SLE and 17% controls; isolated ↓ DLCO in 31% SLE and 0% in controls; small airways disease in 24% and 17%, respectively
Rolla et al.	1996	*n* = 24 SLE and *n* = 24 controls	↓ lung capacity, ↓ bronchial threshold to metacholine, increased AaO_2 gradient (n = 12); KCO correlated with disease activity

CXR, chest radiograph; DLCO, carbon monoxide diffusing capacity; KCO, pulmonary gas transfer; PFT, pulmonary function test; TCLO, total lung carbon monoxide diffusing capacity.

In a prospective study of 43 ambulatory, consecutive patients with SLE, Silberstein et al. (19) attempted to correlate PFT abnormalities with other measures of lupus activity. Pulmonary dysfunction was noted in 88% of patients. An impaired DLCO (72% of all patients), reduction in lung volume (49%), and hypoxia (44%) were the most common abnormalities that were found. No correlation was found between the type or severity of the abnormality and serum complement levels, anti-DNA antibody, lupus band test, or nephritis. Patients with SLE and abnormal PFT results did not differ from those with normal PFT results in regard to their clinical or immunologic features. In contrast, Holgate et al. (37) reported that patients with SLE and prominent pleuropulmonary disease had a lower prevalence of lupus nephritis, suggesting that this is partly a result of the low frequency of anti-DNA antibodies in this group of patients. These two studies are not comparable,

however, because the latter included only patients with SLE and pleuropulmonary disease.

Chick et al. (38) compared the PFT findings in 28 patients with SLE but without pulmonary involvement to those of healthy individuals matched for age, gender, and height. A restrictive pattern with reduced lung volume and vital capacity was seen in the SLE group. DLCO was reduced in patients with SLE in proportion to the reduction in lung volume, suggesting that this results from pleural thickening rather than from parenchymal disease. Cigarette smoking caused a reduction in flow at low lung volumes in both patients with SLE and normal controls.

Andonopoulos et al. (39) conducted the largest controlled study to date of PFTs in patients with SLE. They studied 70 lifelong nonsmoking SLE patients and an equal number of age- and sex-matched, nonsmoking healthy subjects. None of the patients had active pulmonary disease, and all had normal chest radiographs at the time of the study. An isolated reduction in the DLCO was the most prevalent abnormality found in the SLE patients. Isolated small airway disease, defined as less than 60% of the predicted value of the maximal flow at 25% of vital capacity, was common in both patients with SLE (24%) and controls (17%). Restrictive and obstructive patterns were uncommon in SLE, being seen in only 5.7% of patients. Overall, only 33% of the patients with SLE had normal PFT results, compared with 83% of the controls.

Eichacker et al. (40) evaluated the PFTs of 25 patients with SLE serially over a period of 2 to 7 years. Reductions in diffusing capacity, forced vital capacity, and total lung capacity did not change significantly with time. In contrast, small airway function decreased significantly with time and was unrelated to smoking history. The significance of this finding is not clear in view of the data found by Andonopoulos et al. (39) that the prevalence of small airway disease in SLE patients is the same as that in healthy controls.

More recently, Nakano retrospectively studied 110 patients with SLE who underwent HRCT and PFTs. Although only 5 had obvious pulmonary disease and 13% had clinical or radiological evidence of pulmonary fibrosis, 47% of patients had DLCO values <80% predicted and only 8% had restrictive defects (41). Furthermore, although reduced DLCO values were more common in patients with pulmonary fibrosis, reduced DLCO values were frequently observed even in patients with neither pulmonary fibrosis nor a restrictive pattern (39%; 34/88) (41).

Table 35-2: Comparison of Techniques in the Diagnosis of Pulmonary Embolism

	Sensitivity	Specificity	Advantages	Disadvantages
D-dimer	85%-100%	41%	High negative predictive value; cheap	Not compared to pulmonary angiography yet
Ventilation/perfusion				
High probability	41%	97%	Well validated, widely available	High proportion of nondiagnostic result
Highly/intermediate				
Probability	82%	52%		
Low probability	98%	10%		
Spiral CT	53%-100%	81%-97%	Well validated, widely available, detects other conditions	Cost, contrast load, subsegmental emboli may be missed
MR Angiography	50%-100%	91%-100%	Low contrast load, may be used to diagnose DVT	Cost, subsegmental emboli may be missed

CT, computed tomography; DVT, deep venous thrombosis; MR, magnetic resonance.

Another study by Zheng et al. assessed DLCO values in 93 patients with autoimmune connective tissue disorders including 48 with SLE. Of the SLE patients 7/48 (15%) had ILD by chest radiography. HRCT abnormalities of ILD were noted in 5/14 (36%) and abnormal DLCO values were present in 42% of the SLE patients (42).

Many of the studies of PFTs in patients with SLE that have been summarized here were uncontrolled, and did not consider the effects of cigarette smoking. In the absence of respiratory symptoms, isolated abnormalities in PFT such as a reduced DLCO do not require treatment but should be monitored, and further investigation with HRCT chest scanning should be considered.

Nuclear Medicine Imaging

Ventilation/Perfusion Scans

In any patient with SLE presenting with breathlessness and pleuritic pains, the possibility of pulmonary embolism should be considered. V/Q scans offer a simple and reasonably reliable screening test for pulmonary embolism that can be used with CT pulmonary angiography where the results are indeterminate. V/Q scans may also be abnormal in the absence of pulmonary embolism, and severe pulmonary hypertension may produce an appearance of hypoperfusion in the peripheral areas of the lung. The combined use of D-dimer assays, V/Q scanning, and, where appropriate, CT pulmonary angiography may raise the accuracy in diagnosing pulmonary embolism (26) (Table 35-2).

Gallium-67 (Ga-56) scans have been useful in the diagnosis and monitoring of patients with sarcoidosis, but there is little data on their use in the assessment of pulmonary disease in lupus. Witt et al. (43) studied lupus patients with interstitial lung disease and found an association between the presence of late inspiratory crackles clinically and increased uptake on Ga-67 scanning and abnormal bronchoalveolar lavage. However, abnormal Ga-67 scans were seen in only 37% of SLE patients compared to a prevalence of 74% with late inspiratory crackles and 95% of patients with abnormal CT chests, making the scans somewhat insensitive at picking up interstitial lung disease.

Diethylenetriamine Pentaacetic Acid Scans

An estimate of pulmonary epithelial permeability may be obtained by measuring the clearance of inhaled technetium- 99m-labeled diethylenetriamine pentaacetic acid (99mTc-DTPA) from the alveolar space to the blood. This technique has been used to diagnose early lung involvement in a variety of autoimmune connective tissue disorders even when HRCT scans are normal. In simple terms rapid clearance of the isotope implies active interstitial lung disease.

In the largest controlled study so far, Dalcin et al. (44) studied 46 SLE patients and 30 healthy controls. The SLE patients were divided into those with clinically active or inactive disease although no disease activity scores were given. Pulmonary clearance of 99mTc-DTPA was significantly faster in patients with active disease compared to either healthy controls or those with inactive disease. Neither carbon monoxide transfer factors nor imaging with HRCT was performed making it difficult to assess the place of 99mTc-DTPA clearance in the diagnosis of early lupus lung disease. Furthermore, although a correlation with cough and rapid 99mTc-DTPA clearance was found, there was no correlation with dyspnea or radiologic appearances (44). Although this does appear to be a very sensitive technique its use should be considered in the clinical context of the patient's symptoms and other investigations. It is also unclear whether asymptomatic patients with rapid isotope clearance will necessarily progress to overt lung disease or if treatment may influence outcome.

Another nuclear medicine technique being used in SLE is the use of technetium-99m hexamethylpropylene amine oxime (Tc-99m HMPAO) to assess pulmonary vascular endothelial damage. The degree of damage may be expressed as the lung/liver uptake ratio. There are two studies of this technique in SLE with conflicting results. Shih et al. measured lung uptake of Tc-99m HMPAO in 20 SLE patients and 25 controls without SLE, referred for brain imaging due to dementia or stroke (45). Of the 20 SLE patients, 10 had significant clinical pulmonary manifestations. The lung/liver ratio was significantly higher in the SLE patients. However, Chang et al., using Tc-99m HMPAO as well as quantitative Ga-67 citrate lung scans to evaluate the severity of inflammation in the SLE lungs found no correlations with clinical or radiologic features (46).

Arterial Blood Gases

Arterial blood gases give an accurate estimate of alveolar ventilation. It is essential in the assessment of pulmonary embolism, extensive pulmonary infiltration, severe pneumonia, pulmonary hemorrhage, and acute reversible hypoxemia where hypoxia (type I respiratory failure) may occur.

Bronchoscopy and Bronchoalveolar Lavage

These techniques may be useful when evaluating the etiology of interstitial pulmonary shadowing in patients with lupus. BAL fluid may be examined to exclude opportunistic infections especially in immunosuppressed patients. In patients with suspected interstitial lung disease where CT chest images show ground-glass shadowing with a honeycomb appearance, elevated cell counts in BAL fluid may suggest alveolar inflammation and direct treatment with immunosuppressive agents once infection has been excluded.

The technique of BAL facilitates the analysis of cellular and soluble components from the epithelial surface of the lower respiratory tract. BAL has yielded valuable information about immune responses and the pathogenesis of lung injury in the connective tissue diseases, especially systemic sclerosis and rheumatoid arthritis (RA). BAL findings may be diagnostic in some diseases, such as infections; in others, however, findings are nonspecific but may contribute to the management of these diseases. For example, an increased number of BAL eosinophils were associated with progressive lung disease in idiopathic interstitial pulmonary fibrosis, RA, and scleroderma (47).

In a multicenter study designed to standardize the test procedure, BAL was performed in 24 patients with diffuse ILD secondary to rheumatic disorders, including SLE (48). The total number of cells in the BAL fluid was increased with the percentage increase in neutrophils and decrease in macrophages. Total protein, immunoglobulin M (IgM), IgG, and IgA, but not albumin, increased in concentration in the BAL fluid. Walaert et al. (49) studied BAL fluid in 61 patients with collagen vascular disease without respiratory symptoms, and included in their study were 11 patients with SLE, all of whom had normal PFT results. An abnormal differential count of BAL fluid leukocytes was found in 48 of patients, including 3 with SLE. In contrast to patients with RA and systemic sclerosis, who had a predominant polymorphonuclear alveolitis, patients with SLE showed a lymphocytic predominance. An increased percentage of BAL fluid eosinophils was also found in two patients with SLE and ILD (47). Similarly, Witt et al. (43) found elevated lymphocyte and neutrophil counts in the BAL fluid from 19 lupus patients.

Alveolar macrophages in BAL fluid from 17 patients with inactive SLE were found to be normal in number, viability, and respiratory burst activity, but had severely impaired antibacterial function (49). This dysfunction, which was observed in both steroid-treated and untreated patients, may contribute to the increased frequency of pulmonary infections in this disease.

Walaert et al. (50) introduced the concept of subclinical alveolitis in SLE and in other systemic rheumatic diseases, which is characterized by the accumulation of inflammatory and immune cells in the BAL fluid of patients without respiratory complaints, with a normal chest radiograph, and with or without significant PFT abnormalities. The clinical significance of subclinical alveolitis, however, is not clear since it is not known how many of these patients will progress to develop overt interstitial lung disease.

Higher concentrations of soluble immune complexes have been observed in BAL fluid compared to the corresponding serum specimen from patients with ILD associated with rheumatic diseases, including SLE (51). Immune complexes were also seen within the cytoplasm of BAL neutrophils, indicating that locally formed immune complexes may induce an inflammatory response in the lungs of these patients.

Increased numbers of activated $CD8^+$ T lymphocytes and natural killer (NK) cells were found in the BAL fluid of lupus patients with abnormal pulmonary function tests and correlated with reduced carbon monoxide transfer factor and diffusing capacity values (52). In contrast, the number of $CD19^+$ B cells in the BAL fluid was lower than that seen in the fluid of healthy controls, despite the high percentage of these cells in SLE peripheral blood. These observations suggest a cell-mediated immune response in the lungs in SLE (52).

BAL, therefore, may be a potentially useful technique in the assessment and follow-up of patients with SLE and pulmonary involvement, especially in those with acute lupus pneumonitis and chronic diffuse ILD.

Clinical Manifestations of Pulmonary Involvement in Lupus

Respiratory tract involvement occurs in about half of lupus patients over their disease course (12). The following sections describe the main features of lung disease in SLE.

Pleurisy

Prevalence

Pleurisy is the most common manifestation of pulmonary involvement in SLE, and the pleura is involved more commonly in lupus than in any other connective tissue disease. Several early studies documented the prevalence of pleural involvement. For example, in Dubois and Tuffanelli's (53) 520 patients, the cumulative incidence of recurrent pleuritic pain was 45% and that of pleural effusions was 30%. McGehee et al. (54) reported pleurisy in 56% of their patients with recurrent pleuritic episodes in 13%, while 16% had associated pleural effusions. Several other studies have documented high prevalences of pleuritis, ranging from 41% to 56% (18 ,55), being found more commonly in blacks than whites (56). The largest study to date of 1,000 European lupus patients found a prevalence at disease onset of serositis (including both pleural and pericardial inflammation) of 17% with a cumulative incidence of 36%, with pleuritis occurring more commonly in men than in women (57). Lung involvement defined as acute or chronic lupus pneumonitis was much less common, with a prevalence at disease onset of 3% and cumulative incidence of 7%—almost certainly an underestimate. At follow-up, 10 years later, the prevalence of most manifestations had decreased and the prevalence of serositis had decreased significantly to 6.2% possibly reflecting the response to long term treatment and close medical supervision in a prospective study (58). The highest prevalence comes from a postmortem study: Ropes (59) described pleural changes in 93% of 58 patients with SLE at autopsy, with fluid in the pleural cavity in 33 cases.

Clinical Features

Pleurisy as the initial manifestation of SLE was noted by Dubois' (60) in 13 of 520 patients. Pleuritic symptoms may antedate other manifestations of lupus by months or even years, resulting in a delay in the diagnosis of SLE (61).

Pleuritic chest pain may be unilateral or bilateral, and is usually located at the costophrenic margins, either anteriorly or posteriorly. Attacks of pleuritic pain often last for several days, and, when associated with effusions, the pain may persist for weeks often accompanied by cough, dyspnea, or fever. The effusion generally occurs on the side of the chest pain (Fig. 35-1). Pleural effusions may also occur

in patients with SLE and nephrotic syndrome; infections, such as tuberculosis; or cardiac failure (23). Massive bilateral pleural effusion is a rare presenting feature of the disease (62). It is important to remember that the differential diagnosis of pleuritic pain in a patient with lupus may include infection and pulmonary embolism, especially in the presence of antiphospholipid antibodies.

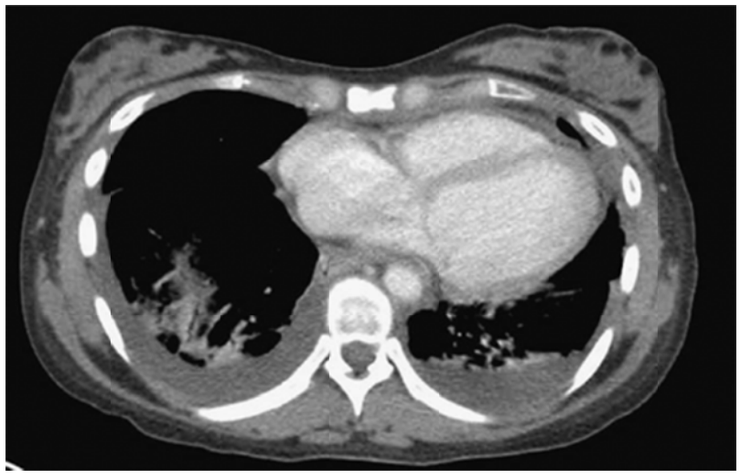

Figure 35-1. Computed tomography (CT) chest scan showing bilateral pleural effusions and right lower lobe consolidation in a woman presenting with pleuritic chest pain and breathlessness. There was rapid resolution with low-dose prednisolone.

Pleural Fluid

The volume of pleural effusions usually is small to moderate (400 to 1,000 mL) and may be unilateral or bilateral. Large pleural effusions are uncommon (10 ,63). Thoracocentesis is not always necessary in lupus patients unless the cause of the pleural effusion is uncertain and infection is suspected. The pleural fluid in SLE is usually exudative in character, although transudates have also been reported (10). The fluid can be yellow, amber, or slightly turbid in color. In a study of 14 patients with lupus pleuritis (63), the white cell count in the pleural fluids ranged from 325 to 14,950 cells/mL (mean, 4,895 cells/mL). Half the specimens showed a predominance of polymorphonuclear leukocytes, with cell counts ranging from 10% to 100% (mean, 57%). Kelley et al. (64) examined pleural effusions from 10 patients with SLE and found atypical cells resembling plasma cells. The presence of these cells with other inflammatory cells, fibrinoid debris, erythrocytes, and few mesothelial cells and in the absence of pathogenic organisms or malignant cells constituted a pattern that was characteristic of SLE in 4 of the 10 patients studied.

In most patients with lupus pleuritis, the pleural fluid glucose concentration is greater than 60 mg/dL, with a pleural fluid/serum glucose ratio of greater than 0.5. Good et al. (63) found the mean pleural fluid/serum glucose ratio to be 0.3 or lower. This contrasts with the finding of low glucose levels in the pleural fluid of patients with RA and pleurisy, in whom the glucose concentration is less than 30 mg/dL in 75% of patients (65). Low glucose concentrations, or a low pleural fluid/serum glucose ratio, may also occur in those with malignant effusions, empyema, or tuberculosis (65). The pH of SLE pleural fluid usually is greater than 7.35. A few patients have a pH of less than 7.3, which is associated with a low pleural fluid glucose level (63 ,65).

Classic LE cells have been documented in smears of pleural fluids from patients with SLE (63 ,66 ,67). It has been suggested that the presence of in vivo LE cells in the pleural fluid is highly characteristic of SLE (68). However, the LE cell test is now largely obsolete and has been replaced by searching for antinuclear antibodies (ANAs) in pleural fluid (12).

The presence of ANAs in the pleural fluid may be a useful diagnostic test for patients with undiagnosed pleural effusions. For instance, Leechawengwong et al. (69) tested pleural fluid from 100 consecutive patients with pleural effusion and found positive ANA in all 7 patients with SLE and in 1 patient with drug-induced LE, but not in patients with other diagnoses. Conversely, Small et al. (70) found that a positive ANA in the pleural fluid was not specific for SLE; it also was found in patients without SLE but with pleural effusions who tested positive for ANA in the blood. Khare et al. (71) found positive ANA in 8 of 74 nonlupus pleural effusions (10.8%), including those associated with malignancy.

Good et al. (63) measured the ANA titer in paired samples of pleural fluid and serum of patients with SLE. In lupus pleuritis, the pleural fluid/serum ANA ratio was greater than 1. In contrast, the ratio was less than 1 in patients with SLE who had pleural effusions from other causes, such as congestive heart failure. Moreover, none of 67 patients with pleural effusions of different causes had a positive ANA.

Pathogenesis

The pathogenesis of pleural effusion in SLE differs from that of RA. For example, immune complexes in RA are thought to be produced locally in the pleura, whereas immune complexes are derived from the circulation in SLE. Additionally, the concentration of soluble interleukin-2 (IL-2) receptor in the pleural fluid in RA is significantly higher than that in SLE. This suggests that a local T cell–mediated immune reaction may be a more important mechanism in rheumatoid pleurisy than in lupus (72).

In an autopsy study, 54 of 58 patients (93%) in Ropes' series showed pleural involvement (59). Fluid was found in the pleural space in 33 patients, and adhesions were seen in 63%. Microscopic changes of varying degrees were observed in 24%; these consisted of accumulations of lymphocytes and macrophages, pleural thickening, perivascular fibrinoid necrosis with neutrophilic and mononuclear infiltrates, fibrinous exudate, and rare hematoxylin bodies. Pleural biopsy in one patient with SLE and bilateral effusions revealed noncaseating pleural granulomas (73).

Several early studies demonstrated reduced levels of hemolytic complement, C1q, C4, and C3 in pleural fluid from lupus patients when compared to pleural effusions from patients with cancer, heart failure, and other conditions (74 ,75 ,76). These complement levels remained low even after adjustment for the total protein content of the pleural fluid (74). However, low pleural fluid complement levels are not specific for SLE, and may occur in patients with RA or empyema (76 ,77).

There is some evidence that low pleural fluid complement levels in SLE results from activation of the complement cascade by immune complexes. Thus, conversion products that are generated by activation of the complement cascade are present in SLE pleural fluid (77 ,78), and immune complexes abound in SLE pleural fluid (77 ,78 ,79). Furthermore, perivascular deposits of immunoglobulins and complement components in the parietal pleura have been found in patients with lupus pleuritis (77). The nature of the immune complexes in SLE pleural fluid is not clear, though they may well be DNA–anti-DNA complexes (80).

The immunologic abnormalities described in pleural fluid are not diagnostic of lupus pleuritis. Thus, the presence of immune complexes, complement activation products, and immune deposits in the parietal pleurae all have been reported in pleurisy associated with other rheumatic diseases, such as RA, as well as in nonrheumatic conditions, including cancer and empyema (70 ,77). This suggests that an immune-mediated mechanism(s) is a common pathway by which SLE and other diseases can cause pleurisy.

Treatment of Lupus Pleurisy

Analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) are useful treatments for mild lupus pleurisy. If the patient fails to improve or the symptoms are severe, systemic corticosteroids (10 to 20 mg of prednisone daily) are usually rapidly effective. Hydroxychloroquine may be added to provide longer-term benefit after tapering the prednisone dose. When present, the effusion begins to clear within days of beginning steroid therapy, though it may take several weeks for the radiographic changes to clear up completely. It is not necessary to consider chest tube drainage of these effusions. When clinical or radiographic changes are slow to improve, the addition of a steroid sparing agent such as azathioprine may be considered. In those very rare patients with chronic, unremitting lupus pleurisy that is refractory to medical therapy, pleurectomy (81), talc poudrage (82), and tetracycline pleurodesis (83 ,84) may be used. Intravenous immunoglobulin therapy was of limited value in a patient with lupus and refractory pleural effusion (85). Our own anecdotal experience of rituximab in a patient with lupus nephritis and refractory pleuritis was very successful both for the renal and pleural disease (unpublished data).

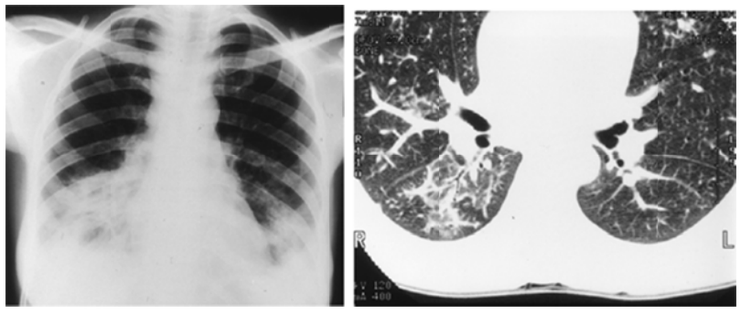

Figure 35-2. A, Chest radiograph in a patient with acute lupus pneumonitis. **B**, CT scan in this patient shows right-sided interstitial shadowing.

Acute Lupus Pneumonitis

Clinical Presentation

Acute lupus pneumonitis is an uncommon clinical manifestation of SLE. In Estes and Christian's (55) series of 150 patients, 48% had evidence of pulmonary involvement at some time during the course of their illness, but only 14 (9%) had acute lupus pneumonitis. Other studies found prevalences of between 1% and 7% in their series (18 ,21 ,23 ,57).

Patients with acute lupus pneumonitis usually present with fever, dyspnea, cough productive of scanty sputum, hemoptysis, tachypnea, and pleuritic chest pain (86). Physical findings commonly include basal crepitations, and, when severe, central cyanosis may be present. Chest radiographs demonstrate diffuse acinar infiltrates with a predilection for the bases in all patients and pleural effusion in 50% of patients (86) (Fig. 35-2). The vast majority of patients have some degree of arterial hypoxemia. The white cell count is usually normal, and anti-DNA antibodies are present in these patients with severe active lupus, often with other evidence of multisystem involvement. Multiple cultures and investigations for bacteria, fungi, and viruses are usually negative, and prompt diagnosis of lupus pneumonitis is essential, as the mortality rate may be as high as 50% (86).

Pathology

The histopathology of the lung in acute lupus pneumonitis has been examined in a few untreated patients; light microscopic changes are variable and nonspecific.

An open-lung biopsy obtained before therapy in one of Matthay et al.'s (86) patients showed a diffuse interstitial lymphocytic infiltrate with prominent lymphoid nodules and bronchiolitis. Despite therapy, the patient died, and at autopsy alveolar hyaline membranes and persistent cell infiltrates were found. Other findings at the autopsy of four patients included acute alveolitis, interstitial edema, hyaline membranes, and arteriolar thrombosis. Pertschuk et al. (87) reported eight patients with SLE who presented with a clinical diagnosis of acute lupus pneumonitis and 50% had changes of interstitial pneumonia. The pathologic picture was considered to be nonspecific and similar to that seen in those patients with oxygen toxicity, viral pneumonia, or uremia. The other four patients showed other pathologic changes: bronchiolitis, pulmonary infarction, focal atelectasis, and cytomegalovirus pneumonia, respectively. Vasculitis was not observed in any patient in either study (86 ,87). Widespread thrombosis was found in the lungs and other organs at autopsy of a patient with acute lupus pneumonitis and disseminated intravascular coagulation (88).

The wide variety of histologic changes in the lungs suggests that acute lupus pneumonitis may result from different pathologic processes (10). Many of the patients studied had received prior treatment, however, including oxygen, high-dose steroids, or cytotoxic agents, which can affect lung pathology either directly or indirectly. Thus, some of the pathologic abnormalities in the lungs may be secondary changes rather than resulting directly from SLE.

Granular deposits of IgG, C3, and DNA have been found in the alveolar septa of two patients with acute lupus pneumonitis (89). Electron microscopy revealed electron-dense deposits in the septal interstitium and in the walls of alveolar capillaries. Immunoglobulin eluted from the lung tissue had ANA activity, including IgG anti-DNA antibody. Another study described deposits of immunoglobulin within the nuclei of alveolar lining cells and pleural mesothelial cells, rather than in the septal interstitium, in acute lupus pneumonitis (87). The concomitant presence of C3 within the nuclei suggested that this was an in vivo rather than an artifactual phenomenon. These immunopathologic observations are consistent with the deposition of antigen-antibody complexes and may be important in the pathogenesis of the lung injury.

Parenchymal pulmonary infiltrates that develop acutely in a patient with SLE should not be considered as acute lupus pneumonitis until infectious processes such as viral pneumonia, tuberculosis, and other bacterial pneumonias and fungal and *Pneumocystis carinii* infections have been completely excluded (10). An elevated C-reactive protein (CRP) in such patients should encourage a vigorous search for lung sepsis.

Treatment and Prognosis

High-dose corticosteroids including bolus intravenous methylprednisolone together with supportive measures such as oxygen and mechanical ventilation where necessary are still the mainstay of drug therapy for acute lupus pneumonitis, although no controlled trials have established their efficacy (10 ,89). Cytotoxic agents such as azathioprine are added to the regimen in patients who fail to respond to steroid therapy or who relapse on steroid dose tapering (86). Intravenous pulse cyclophosphamide combined with systemic corticosteroids has also been used successfully (90).

Acute lupus pneumonitis carries a poor prognosis. Of 12 patients reported by Matthay et al. (86), the mortality was 50% during the acute episode—from respiratory failure, opportunistic infection, and thromboembolism. All six surviving patients remained relatively well after more than a year of follow-up, but three developed residual interstitial infiltrates with abnormal pulmonary function tests, indicating that the acute process can progress to chronic interstitial lung disease. Adult respiratory distress syndrome (ARDS) may occur with acute lupus pneumonitis, greatly increasing the risk of mortality (91 ,92).

Pulmonary Hemorrhage

Clinical Presentation and Diagnosis

Pulmonary hemorrhage is a rare, devastating, and frequently fatal manifestation of SLE with mortality rates of 70% to 90% (90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99). For example, Abud-Mendoza et al. (94) described 12 patients with massive pulmonary hemorrhage among 750 patients with SLE (1.6%) of whom 11 died. A further study found 34 patients in a cohort of 630 patients, all of whom had severe respiratory failure (97). A higher prevalence of 3.7% of all inpatient admissions for SLE had pulmonary hemorrhage reported by Zamora et al (93). Autopsy studies found pulmonary hemorrhage to be the cause of death in 11% to 14% lupus fatalities (94 ,98). Several children with SLE and lung hemorrhage also have been reported (100 ,101 ,102 ,103). Single-case reports also describe pulmonary hemorrhage in pregnancy (104) and the antiphospholipid syndrome (105).

The clinical presentation is similar to that of acute lupus pneumonitis, with a sudden onset of fever, dyspnea, cough, blood-stained sputum, and occasionally frank hemoptysis. The clinical course is rapidly progressive over hours or days, with increasing tachypnea, arterial hypoxemia, tachycardia, acute respiratory distress, and frank hemoptysis. The hemoglobin and hematocrit drop suddenly, and chest radiographs show bilateral pulmonary infiltrates, with a predominantly alveolar pattern. The infiltrates are coarsely nodular, fluffy, or homogeneous in pattern, often extending to the bases but occasionally unilateral in distribution. Single breath diffusing capacity for carbon monoxide may well be raised because of the presence of blood in the alveolar spaces, though patients are often too ill to undergo this investigation (106). Mechanical ventilation is frequently required for marked arterial hypoxia (93), and patients usually have clinical and laboratory evidence of

multisystem involvement, including positive anti-DNA antibodies and hypocomplementemia. The mortality in these patients is 62%, rising to 78% to 100% if there is concomitant infection (93).

Frank hemoptysis does not always occur, even with massive intra-alveolar hemorrhage, so the clinical diagnosis is often delayed (94 ,97). For example, in a series of 140 patients with SLE, 3 developed pulmonary hemorrhage, and in 2 of these patients the diagnosis was made only at autopsy (99). In the absence of hemoptysis, a rapidly falling hematocrit and diffuse lung infiltrates in a patient with SLE should alert the clinician to the possibility of lung hemorrhage (94). Bronchoalveolar lavages are universally hemorrhagic, and transbronchial lung biopsies may be helpful (93). Although open lung biopsy is sometimes advocated, these patients are usually very unwell and are poor operative risks. MRI and CT have been reported to be helpful in the diagnosis of diffuse alveolar hemorrhage in SLE (107 ,108).

Although pulmonary hemorrhage is very rarely the presenting feature of SLE, the majority of cases arise in patients with established lupus. The median disease duration of such patients ranges from 31 months to 3.2 years (93 ,98). Multisystem disease is often present in patients with pulmonary hemorrhage and renal disease is the most common occurrence. In one study, 94% of patients had both pulmonary hemorrhage and lupus nephritis (93), justifying the inclusion of SLE in the list of diseases associated with the pulmonary-renal vasculitic syndromes (109).

Pathology

The histopathology of the lung is that of diffuse, intra-alveolar hemorrhage with intact erythrocytes and hemosiderin-laden macrophages in the alveoli (93 ,98 ,110). Other microscopic findings include thickening of the alveolar septa, hyaline membrane formation, and fibrin deposits within the alveolar cavities. Evidence of vasculitis usually is not seen. A distinctive microangiitis that is characterized by acute inflammation and necrosis of alveolar capillaries, arterioles, and small muscular arteries also has been described in four patients (111), while in another study pulmonary capillaritis was seen in 80% of lung tissue specimens (93).

Electron microscopic studies in a small number of cases have shown type II alveolar-lining cell hyperplasia and electron-dense deposits in the alveolar septa within the basement membrane of alveolar capillaries and in the walls of small arteries (93 ,110 ,112 ,113 ,114). Direct immunofluorescence studies have demonstrated the presence of granular deposits of immunoglobulin, principally IgG, and complement components in the alveolar septa and in the walls of small blood vessels (110 ,112 ,113 ,115). Other investigators, however, have failed to find immune deposits in the alveolar septa in patients with SLE and pulmonary hemorrhage (110 ,116).

Pathogenesis

The pathogenesis of pulmonary hemorrhage in SLE is not known. Deposition of immune complexes in the alveolar septa and in blood vessels, with activation of the complement system, has been proposed as being the major mechanism, which is analogous to the lung changes that are seen in experimental models of chronic serum sickness. Brentjens et al. (117) described changes of interstitial pneumonitis with proliferation of septal cells, thickening of the alveolar septa, accumulation of leukocytes in capillaries, and alveolar hemorrhages in rabbits that were hyperimmunized with foreign serum protein. Conversely, Eagen et al. (110) identified several factors that potentially contributed to the pathogenesis of lung hemorrhage in their patients with SLE, including bleeding diathesis, oxygen toxicity, infection, uremia, and shock lung. Desnoyers et al. (116) described a patient with SLE and cutaneous vasculitis, nephritis, and pulmonary hemorrhage but without the complicating factors described earlier that could cause nonspecific alveolar damage. No immune deposits were found in the alveolar septa, although they were present in the renal glomeruli. These authors suggested that pulmonary hemorrhage could occur in SLE by mechanism(s) other than immune complex deposition, such as vascular injury with disruption of the alveolar capillary membrane. Unlike Goodpasture syndrome, in which pulmonary hemorrhage is characteristic, there is no association with smoking (93).

Treatment and Prognosis

The prognosis of massive pulmonary hemorrhage in patients with SLE is grave, despite treatment with high-dose systemic corticosteroids combined with a cytotoxic agent (10 ,86 ,94 ,110). Other investigators have reported a better prognosis, however. Six of eight patients (75%) with SLE and pulmonary alveolar hemorrhage who were reported by Schwab et al. (118) recovered. In another large study the overall survival rate was only 38% (97). The mortality rate of pediatric lupus cases with lung hemorrhage has been reported to be 50% (102).

The poor prognosis in these patients suggests that it may be advisable to employ a regimen of high-dose corticosteroids, cyclophosphamide, and perhaps plasmapheresis (119 ,120 ,121).

Erickson et al. (120) reported dramatic improvement in three patients who underwent intensive plasmapheresis combined with steroids and cyclophosphamide. A review of reported cases of pulmonary hemorrhage in SLE showed that the survival rate of those patients who received corticosteroids with or without cytotoxic agents was 43% (23 of 53 patients). In contrast, 7 of 11 patients (64%) who also underwent plasmapheresis survived (120). Complications (especially a high rate of serious infections) have been reported in patients receiving plasmapheresis combined with steroids and cyclophosphamide (122). Pagnoux et al.

wrote a useful review on the indications for plasmapheresis in severe lupus including pulmonary hemorrhage (123).

Carette et al. (124) reviewed more than 400 SLE patients who were followed over 10 years, of whom 8 became acutely ill with diffuse lung infiltrates. Pulmonary hemorrhage was eventually diagnosed in 6 patients, of whom 4 had other complicating factors, including uremia, coagulopathy, and infection. Based on their experience, a practical management approach was suggested. Four major conditions should be considered in the differential diagnosis: (a) congestive heart failure, (b) noncardiogenic pulmonary edema, (c) infection, and (d) pulmonary hemorrhage. A careful clinical history should be taken, and a thorough physical examination performed. Evidence of lupus activity in other organ systems should be documented. If the diagnosis of congestive heart failure is unclear on clinical grounds, an echocardiogram or Swan-Ganz catheter should be considered. Infection and factors that are associated with noncardiogenic pulmonary edema, including uremia, pancreatitis, and side effects from drugs, should be excluded. If a definite diagnosis is not reached at this point, broad-spectrum antibiotics and high-dose corticosteroids should be considered while bronchoalveolar lavage, transbronchial lung biopsy, and brushings are obtained. If pathogenic organisms are not found after appropriate stains and cultures, antibiotics should be discontinued. Systemic corticosteroids should be continued until clinical improvement occurs, but if the patient fails to respond, an open-lung biopsy sample should be considered, bearing in mind the operative risks that this procedure entails.

Chronic Diffuse Interstitial Lung Disease

Clinical Presentation

Diffuse ILD is a well-recognized pulmonary manifestation of systemic rheumatic diseases, particularly systemic sclerosis, dermatomyositis, and RA. Somewhat surprisingly, it is much less common in SLE than in other connective tissue diseases. ILD in SLE was described in detail in 1973, by Eisenberg et al. (125). The prevalence of symptomatic ILD in SLE has been calculated to be approximately 3% (126 ,127). In an early prospective study of 150 patients, 9 developed radiographic changes of pulmonary fibrosis, but it is unclear whether these patients were symptomatic or how severe the pulmonary functional abnormalities were (55). A retrospective study of 63 SLE patients noted 16 patients (25%) with ILD (127). The high prevalence was partly due to the inclusion of severely ill, hospitalized patients in their series.

The initial presentation of diffuse ILD in SLE can be one of two types. The more common is an insidious onset of a chronic nonproductive cough, dyspnea on exertion, and a history of recurrent pleuritic chest pain. Less commonly, ILD may develop in a patient following acute lupus pneumonitis: two studies suggest that between 43% and 50% of patients with acute lupus pneumonitis may go on to develop chronic diffuse ILD (86 ,127).

The presentation of ILD in SLE resembles that of lung disease in systemic sclerosis and RA. The most common clinical manifestations are persistent dyspnea on exertion, pleuritic chest pains, and nonproductive cough. ILD can occur at any time during the course of SLE, but in most patients it develops in those with long-standing disease. Multisystem involvement is relatively common, and patients usually test positive for both ANAs and anti-DNA antibodies.

In the study by Eisenberg et al. (125), the mean age of their 18 patients with SLE and ILD was 45.7 years, with a mean disease duration of 10.3 years. Pulmonary manifestations were present for a mean of 6 years. Initially, 7 patients presented with pulmonary symptoms developing over weeks to months with dyspnea on exertion, and 3 with dyspnea at rest. Twelve complained of cough with scanty sputum, and a similar number had pleuritic chest pain. All patients had poor diaphragmatic movement, with diminished resonance to percussion over the lung bases. Cyanosis and clubbing were present in 1 patient, and 12 had basilar rales. All 18 had persistent, diffuse interstitial infiltrates on chest radiography that could not be attributed to other complication. Markedly elevated diaphragms were seen in 8 patients, and diaphragmatic excursion was decreased in 6 as evaluated by chest radiography on deep inspiration and expiration. A pleural reaction was present in 9 patients, and plate-like atelectasis in 6, which was usually seen just above the diaphragm and persisted for several months to years. The diffusing capacity of the lung, as measured by the carbon monoxide method, was decreased below the predicted normal values in all but 1 patient.

Laboratory Evaluation

To investigate the possibility of pulmonary infarction, Eisenberg et al. (125), obtained ventilation-perfusion lung scans in 13 patients. Of these, 8 had matched ventilation and perfusion defects occurring at the same site. In seven of 13 patients, ventilation-perfusion defects were noted in areas other than those occupied by long line shadows, effusions, or atelectasis.

Spirometry and lung-volume measurements were consistent with a restrictive defect or loss of lung volume without obstruction to air flow. Diffuse pulmonary interstitial infiltration typically results in this type of ventilatory defect. Infiltrated lung areas are less elastic and change their volume less with each breath. Underventilation results in under oxygenation of the blood perfusing these regions, and if this ventilation-perfusion mismatching is extensive, significant arterial hypoxemia may occur. Obstructive defects were not seen (125).

The arterial blood oxygen tension (PaO_2) was diminished in all patients, and the alveolar-arterial difference for oxygen (PaO_2–PaO_2) increased significantly. The PaO_2 was lowest and the PAO_2–to-PaO_2 difference greatest in patients

with the most extensive pulmonary involvement, as noted in their radiographs. The oxyhemoglobin saturation, however, was above 94% in all but four patients, and ventilation-perfusion mismatching was responsible for these changes. Thickening and fibrosis of the interstitial wall and microinfarcts in the lung alter the elastic properties of the ventilatory lung units, leading to underventilation relative to perfusion. Lung biopsy in four patients showed nonspecific interstitial fibrosis, with chronic inflammation in two (125).

Boulware and Hedgpeth (127) found precipitating anti-Ro/SSA antibodies in 81% of patients with SLE and ILD compared to 38% in their general SLE population. In contrast, the frequency of other specific types of ANA, such as anti-U1 ribonucleoprotein (RNP), and anti-La/SSB, was not significantly increased. This possible association with anti-Ro/SSA has also been found by Mochizuki et al. (128) but not by Weinrib et al. (126), and the discrepancy may result from differences in patient selection.

There is evidence that chronic ILD occurs in a subset of lupus patients with features of scleroderma, including Raynaud phenomenon, sclerodactyly, and nailfold capillary abnormalities (129). Additionally, there is an increased frequency of anti-U1RNP and anti-U1RNA antibodies in these patients. However, in this study, 7 of the 19 patients with SLE and chronic ILD had concomitant connective tissue disease, including RA, scleroderma, and mixed connective-tissue disease (MCTD) (129).

High-Resolution CT Scans

HRCT scans of the lungs are essential in the diagnosis, assessment of disease activity in the lung parenchyma, and provision of prognostic information in patients suspected of having ILD associated with connective tissue diseases (130 ,131 ,132 ,133) (Fig. 35-3).

Johkoh et al. (134) compared HRCT lung scans and pulmonary function tests in idiopathic pulmonary fibrosis and ILD associated with connective tissue disease including SLE. The extent of morphologic changes correlated with the severity of impairment of the DLCO in both patient groups. Steroid-responsive patients showed a ground-glass and alveolar consolidation and no honeycomb lesions on the HRCT scans. In contrast, patients who failed to respond to therapy had severe honeycomb lesions on the HRCT scans. A further study of 15 HRCT scans in ten lupus patients with respiratory symptoms found 14 of the 15 scans to be abnormal (135). The main finding was that of chronic lower zone interstitial lung disease with honeycombing, architectural distortion, parenchymal bands, and pleural changes. Pleural thickening was seen in 87% of scans, and all patients had abnormal lung function tests, especially reduced carbon monoxide diffusion capacity.

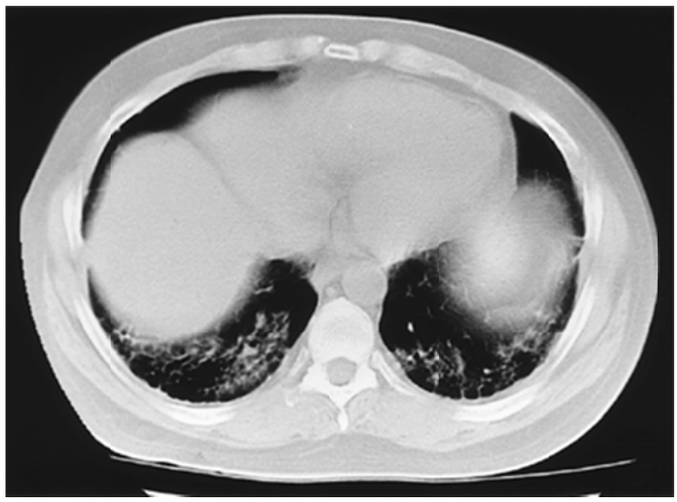

Figure 35-3. High-resolution CT chest scan showing bilateral basal interstitial fibrosis with honeycombing.

Pathogenesis of Chronic ILD

Studies in idiopathic pulmonary fibrosis and systemic sclerosis suggest a key role for cytokines and other mediators that are secreted by alveolar macrophages and inflammatory cells as well as resident structural cells of the lung. IL-1, tumor necrosis factor (TNF), endothelin, and especially growth and differentiating cytokines (transforming growth factor, platelet-derived growth factor [PDGF], and granulocyte-macrophage colony-stimulating factor [GM-CSF]) are believed to be important in the alteration of cell phenotypes in the lung, accumulation of inflammatory cells from the circulation, and increased collagen secretion by stimulated fibroblasts (136 ,137).

Chronic ILD is a predominantly T-lymphocyte–mediated immunologic reaction to some unknown antigen, in contrast to acute lupus pneumonitis, which primarily is an immune-complex–mediated condition (138). This study found increased numbers of activated $CD8^+$ T cells, $CD56^+/CD16^+/CD3^-$ NK cells, and other markers of T cell activation in the BAL fluid of patients with SLE (138). These cellular abnormalities were associated with upregulated local production of oxygen radicals and with impaired pulmonary diffusing capacity. Additionally, cells in the BAL fluid of patients with SLE and ILD spontaneously secrete increased amounts of PDGF and TNF, which can induce proliferation of fibroblasts (139).

Treatment and Prognosis

The clinical course of chronic ILD is variable in individual patients, but as a group, most patients follow a slow course, tending to improve or stabilize with time. In one study of 14 patients, all received high-dose prednisone (60 mg daily) for at least 4 weeks early in the course of the pulmonary disease (126). The DLCO and inspiratory vital capacity (IVC) either improved or remained unchanged in most patients, and respiratory symptoms improved in all patients. Two died of progressive pulmonary fibrosis, however, and another succumbed to bacterial pneumonia (126). A study comparing patients with ILD associated

with connective tissue diseases, including SLE, and those with idiopathic pulmonary fibrosis showed that lung function deteriorated significantly over 2 years in the idiopathic group, but remained essentially unchanged in the connective tissue diseases group (140).

Patients with SLE and symptomatic, chronic, progressive ILD should undergo assessment of the extent and activity of the lung disease with pulmonary function tests, gallium lung scan, BAL, and/or transbronchial biopsy. An open-lung biopsy is usually not indicated. HRCT scans of the lungs give useful information on the extent and activity of disease, and repeat HRCT may document changes, especially if the changes are predominantly of ground-glass alveolar shadowing. Serial pulmonary function tests are useful in monitoring the disease and the response to therapy (10).

In the presence of an active inflammatory process in the lung, and once infection has been excluded, high-dose prednisone (40 to 60 mg daily) for at least 6 to 8 weeks should be considered. The dose is tapered depending on the clinical and laboratory responses of the patient. Immunosuppressive agents such as azathioprine and intravenous cyclophosphamide may be needed in patients who fail to respond satisfactorily to steroids.

Corticosteroid therapy of ILD in lupus and other connective tissue diseases results in a decrease in total cell count, immune complexes, and immunoglobulin levels in BAL fluid (141). The efficacy of systemic corticosteroids and immunosuppressive agents in the treatment of ILD and SLE has not been validated by well-designed, controlled trials.

Pulmonary Embolism

Pleuritic chest pain and dyspnea are relatively common, and most patients with SLE presenting with these symptoms usually have pleurisy or pneumonitis. However, pulmonary embolism (PE) should always be considered especially if antiphospholipid antibodies are present or there is a previous history of PE. The predictive value of V/Q scans, D-dimer levels, and CT pulmonary angiography has been discussed above (Table 35-2), and it should be remembered that abnormal perfusion scans may occur in patients with active lung disease in the absence of PE. Nevertheless, if PE is suspected clinically in a patient with a low probability V/Q scan, angiography should be performed.

Peripheral deep venous thrombosis (DVT) is common in patients with SLE and predisposes to PE (13 ,53 ,55 ,142). Perhaps the most widely recognized risk factor for venous thromboembolism in SLE is the presence of circulating lupus anticoagulants and other antiphospholipid antibodies (143 ,144 ,145). In the European study of 1,000 patients, antiphospholipid antibodies including the lupus anticoagulant were significantly associated with thrombosis, fetal losses, thrombocytopenia, and livedo reticularis (57). Patients who develop DVT and/or PE in the context of antiphospholipid antibodies will usually require lifelong anticoagulation (146).

The catastrophic antiphospholipid syndrome is an accelerated form of the antiphospholipid syndrome characterized by the rapid onset of widespread thrombosis and multiorgan failure with a high mortality. Recently classification criteria have been validated for use in research studies (147). The lungs are very commonly involved and the usual presentation is acute respiratory distress syndrome. Thus, Bucciarelli et al. reported pulmonary involvement in 150 out of 220 (68%) patients with catastrophic APS and 47 (21%) patients were diagnosed as having acute respiratory distress syndrome. Prognosis was poor and 19 (40%) of these patients died. Seven of 10 patients had a thrombotic microangiopathy on histology (148). (See Chapter 65 for a further discussion of thrombosis and antiphospholipid antibodies.)

Reversible Hypoxemia

A relatively rare syndrome of acute reversible hypoxemia in acutely ill patients with SLE but without evidence of parenchymal lung involvement may occur (149 ,150 ,151). The first description was by Abramson et al. (149); among 22 inpatients with acute disease exacerbation, 6 (27%) had this syndrome. Although some patients had mild pleuropulmonary symptoms, chest radiographs and lung scans were normal. The patients had hypoxemia and hypocapnia with a wide alveolar-arterial (A-a) gradient, which reversed with corticosteroid therapy. The pathogenesis of the syndrome is unclear, but a correlation between hypoxemia and the level of complement split products was noted, and others have suggested a relationship with disease activity (150 ,151). Complement activation may lead to diffuse pulmonary injury with the aggregation of neutrophils in the lungs similar to that seen in cardiopulmonary bypass, hemodialysis with cuprophane membranes, and ARDS.

Pulmonary Hypertension

Clinical Presentation

Severe, symptomatic pulmonary hypertension (PH) is a relatively rare manifestation of lung involvement in SLE, but mild subclinical cases are surprisingly common with prevalences of between 5% and 14% (reviewed in (12)).

Perez and Kramer (152) were the first to report four patients with severe PH in a group of 43 patients with SLE over a period of 2 years. Other studies, however, found rather lower prevalences (153). Of great interest is a 5-year follow-up study of a cohort of lupus patients that showed that the prevalence of PH rose from 14% to 43%, with the mean pulmonary artery pressures rising from 23.4 mm Hg to 27.5 mm Hg (154).

The clinical diagnosis of PH is difficult to make in early and mild cases, and only the severe cases, with right ventricular hypertrophy and/or congestive heart failure, have been reported in the literature. Pulmonary artery pressure at rest and during exercise is significantly higher in unselected

patients with SLE as compared to normal subjects, probably secondary to increased pulmonary vascular resistance in lupus patients (155). A study of the prevalence and severity of PH in a group of 36 patients with SLE and healthy controls was undertaken by Simonson et al. (156) using two-dimensional and Doppler echocardiographic data to calculate pulmonary artery systolic pressure. Five patients (14%) and none of the controls had PH, as defined by a pulmonary artery pressure of greater than 30 mm Hg. This study suggests that PH is common in SLE, but usually mild in degree. In another study, Shen et al investigated 84 Chinese patients with SLE and 99 healthy controls to investigate the prevalence and the mechanism of pulmonary hypertension with 12 patients undergoing right heart catheter studies as well as echocardiography (157). The lupus patients had significantly higher systolic pulmonary artery pressures, mean pulmonary artery pressures and total pulmonary resistance compared to controls. Nine of the 84 patients had pulmonary hypertension (11%). Pulmonary hypertensive patients had higher endothelin levels than nonpulmonary hypertensive patients, were more likely to have active disease and presented Raynaud phenomenon and positive rheumatoid factors. Endothelin levels correlated with echocardiographic pulmonary pressure (157).

The symptoms of PH in SLE are in general similar to those of patients with idiopathic or primary PH (153). In most of the reported cases, the symptoms of PH occurred within a few years of onset of the multisystem disease, with a mean duration of approximately 2.3 years (153). The most common complaints are dyspnea on exertion, chest pain, and chronic nonproductive cough. Chronic fatigue, weakness, palpitations, edema, and/or ascites may also occur. Symptoms usually develop insidiously and progress gradually. The physical findings may include a loud second pulmonary heart sound, systolic murmur, and right ventricular lift. Chest radiography findings include cardiomegaly with a prominent pulmonary artery and clear lung fields. Electrocardiography may show changes of right ventricular hypertrophy. Although PFTs may show restrictive abnormalities, these are mild in degree and disproportionate to the severity of the PH. Pulmonary angiograms in severe cases demonstrate symmetric dilatation of the central pulmonary artery trunk, with pruning of the peripheral blood vessels. Cardiac catheterization is the definitive investigation and demonstrates the characteristic elevation of the pulmonary artery pressure and normal wedge pressure without evidence of intracardiac or extracardiac shunting.

Pathology

The histopathology of the lung in patients with SLE and PH is that of plexiform lesions similar to those seen in primary PH (153). Medial hypertrophy and intimal fibrosis of the branches of the pulmonary artery may be seen. Thrombosis and vasculitis have also been reported in a few patients (153). Deposits of IgG, IgM, and C3 in the walls of the pulmonary blood vessels have been found (153). These immunoglobulin deposits, when eluted with acidic buffer, showed ANA activity, including anti-DNA antibody and rheumatoid factor activity (153). The putative antigen DNA also was found in the walls of blood vessels, indicating the deposition of immune complexes.

Pathogenesis

The pathogenesis of PH is not well understood, and it is likely that multiple etiologic factors are involved (158). For example, although immune deposits have been found in the large pulmonary vessels, it is not clear whether this is important in the pathogenesis or merely represents a secondary phenomenon. Vasculitis affecting the pulmonary artery is rarely seen and is unlikely to be a major cause of PH (159 ,160 ,161). Occasionally, PH can develop as a complication of diffuse pulmonary fibrosis.

Asherson et al. (162) reported a high frequency of anticardiolipin antibodies in patients with SLE and PH, indicating the possible causative role of recurrent thromboembolic phenomena, but others have not observed this association (159). Additionally, other features of the antiphospholipid syndrome are uncommon in patients with SLE and PH (162), and thrombosis is not commonly seen in autopsied cases (153). Thrombosis of the pulmonary vessels has been confirmed in a small number of patients with antiphospholipid antibody syndrome with PH (163 ,164).

Miyata et al. (165) reported that patients with SLE or MCTD and anticardiolipin antibodies have higher mean pulmonary artery pressures than those without anticardiolipin antibodies. The high frequency of Raynaud phenomenon in the PH group (up to 75%, compared to 25% to 40% of other lupus patients) suggests that PH may be a complication of pulmonary arterial vasospasm (156 ,162). Another intriguing study suggested a significant correlation between nailfold capillary density and pulmonary gas transfer (KCO) in patients with SLE. It may be that in SLE, poor gas transfer may be dependent on alveolar capillary loss and that nailfold capillary density may be a good indicator of alveolar capillary density (166). This group also assessed muscle biopsy specimens in relation to pulmonary involvement in lupus but found no strong correlation (167).

Some studies point to the possible role of endothelial dysfunction and abnormal vascular response in PH. One study showed an increase in the release of thromboxane A2, a potent pulmonary vascular vasoconstrictor and procoagulant, and a decrease in the release of prostacyclin in patients with both primary and secondary PH, including SLE (168). Endothelin levels are elevated in lupus patients with PH and in Shen et al.'s study correlated with echocardiographic pulmonary pressure (157). Antiendothelial cell antibodies, which are associated with lupus nephritis (169) have also been described in patients with SLE and PH (170). More recently the potential role of brain natriuretic peptide (BNP) levels in the assessment of functional status and right heart performance in primary pulmonary hypertension has been highlighted (171) and this may be a useful test in screening for early pulmonary hypertension in lupus.

Primary PH in children is associated with human leukocyte antigen (HLA)-DR3, DRw2, and DQw2, whereas PH in systemic sclerosis is associated with DRw52, suggesting a role for genetic factors (172 ,173). This is supported by a report of fatal PH in identical twins with lupus (174).

Prognosis and Treatment

The overall outcome of severe PH in SLE is poor. Of the patients reported in the literature, cardiac failure or sudden death presumably because of an arrhythmia were the most common modes of death. Although the presence of Raynaud phenomenon is associated with a poorer outcome, the most accurate predictors are pulmonary artery pressure, right atrial pressure, and cardiac index (reviewed in (12)).

A trial of various vasodilator agents is certainly worth trying in these patients. For example, nifedipine was useful in patients with PH associated with MCTD and scleroderma (175), although some cardiologists feel that this is inappropriate, particularly in scleroderma patients with severe PH. However, the effect on exercise tolerance and survival was not examined in this study. In primary PH, high doses of nifedipine and other calcium channel blockers have been shown to improve hemodynamic abnormalities and to prolong survival (176).

Other strategies include anticoagulants, systemic corticosteroids, or cytotoxic agents (reviewed in (12)). Some patients have experienced symptomatic improvement, although hemodynamic abnormalities remained unchanged with drug therapy (153 ,177 ,178). Improvements in cardiac hemodynamics have been reported in a few patients (179) and intermittent intravenous infusions of cyclophosphamide have resulted in partial improvement in hemodynamics in a patient with lupus and PH (180). Sequential administration of cyclophosphamide and cyclosporin A improved the hemodynamic and clinical course of a patient with MCTD and PH (181).

Short-term infusion of prostacyclin and prostaglandin E1 has been tried successfully in the treatment of a patient with lupus and PH (182). Chronic prostaglandin infusion either intravenously and more recently by inhalation and other treatment modalities, including nitrous oxide, have been used in primary PH and may be applicable to SLE, but the evidence in support of these therapies in SLE is anecdotal at present.

Until recently, despite combinations of these drugs, most of the patients reported by Asherson et al. (162) died within 5 years after the diagnosis of PH. Two of their patients underwent heart-lung transplantation, with satisfactory results in one but poor results in the other because of chronic rejection (183). However, in the last 2 years, specific endothelin receptor antagonists against the ETA and or ETB receptors have been subjected to clinical trials. Bosentan has been shown to improve exercise capacity, hemodynamics, echocardiographic and Doppler variables and time to clinical worsening in patients with primary and secondary (to connective tissue diseases) PH (184). Other agents such as sitaxsentan and sildenafil and combinations of endothelin receptor antagonists and prostacyclins such as epoprostenol are undergoing clinical trials with very encouraging results.

Shrinking Lung Syndrome

In 1965, Hoffbrand and Beck (185) described a group of patients with SLE with breathlessness and reduced chest expansion, but no cyanosis, clubbing, or abnormal auscultatory findings. Many of the patients had a previous history of pleurisy. Chest radiography revealed clear lung fields but with an elevated diaphragm, which moved sluggishly and not paradoxically (Fig. 35-4). The vital capacity was extremely reduced. The authors coined the term shrinking lung syndrome and suggested that the main pathologic lesion is that of alveolar atelectasis secondary to deficiency of the surface tension reducing film that lines the normal alveoli. Gibson et al. (186) measured diaphragmatic function in these patients by determining the transdiaphragmatic pressure using a double-balloon technique, and they found it to be grossly abnormal. This finding led them to suggest that diaphragmatic dysfunction, rather than parenchymal or pleural disease, accounts for the unexplained dyspnea in these patients. Martens et al. (187) concluded that the restrictive ventilatory defect in these patients results primarily from the weakness of expiratory and inspiratory muscles. Diaphragmatic dysfunction correlated with the degree of dyspnea but not with overall disease activity, proximal muscle weakness, or serologic markers. Contrary to these reports, however, Laroche et al. (188), using a wide range of tests for determining respiratory muscle strength, found no evidence of isolated weakness of the diaphragm in 12 patients with SLE and this syndrome. The discrepancy between their results and those of previous studies is not entirely clear, but it may result partly

from patient selection and differences in the methods that were used to assess diaphragmatic function.

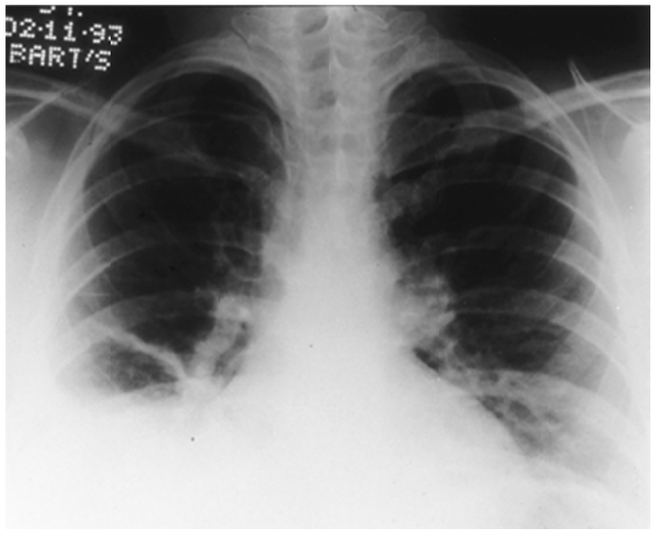

Figure 35-4. Chest radiograph showing raised right hemidiaphragm and a linear atelectasis in the right midzone.

The pathogenesis of the diaphragmatic weakness in patients with SLE is not well understood. Wilcox et al. (189) found no evidence of phrenic nerve neuropathy as the cause of this weakness. Diffuse fibrosis of the diaphragm without evidence of acute inflammatory infiltrates was observed in one patient examined at autopsy (190), supporting an extrapulmonary, restrictive cause for this unusual syndrome. An electromyographic study of the diaphragm and external intercostal muscles demonstrated that fatigue of the respiratory muscles occurs at lower loads in patients with SLE as compared to those in healthy controls (191). Whether myopathy is an isolated process affecting primarily the diaphragm and other respiratory muscles or is part of a generalized muscle disease in SLE is not entirely clear. When the diagnosis of shrinking lung syndrome is suspected, measurements of transdiaphragmatic pressures and elastic recoil of the respiratory system should be considered.

The clinical course of the syndrome is a chronic, low-grade, restrictive defect. Follow-up of some patients over a period of several years has shown that the volume restriction is not progressive (186 ,187). In symptomatic patients, prednisone therapy (30 to 60 mg daily for several weeks) is clinically beneficial and tends to stabilize the PFT abnormalities (191 ,192 ,193 ,194 ,195 ,196 ,197). Agonist agents and theophylline and immunosuppression with methotrexate may also be useful in the treatment of this syndrome (194 ,195 ,196 ,197).

Airway Obstruction

Severe airway obstruction has been reported in a small number of patients with SLE (198 ,199). Lung biopsy in one patient showed obliterative bronchiolitis and an acute inflammatory process that affected small bronchi and bronchioles, resulting in necrosis and eventual endobronchiolar proliferation of epithelial cells and peribronchial infiltration by lymphocytes. Dense plugs composed of alveolar debris and fibrin strands within the bronchioles caused partial or complete obstruction. Bronchiolitis obliterans with organizing pneumonia has been described in SLE (200).

Evidence of airway obstruction has been described in several controlled studies of PFT in patients with SLE (3 ,201 ,202 ,203 ,204). Some series did not take into consideration the effect of cigarette smoking, but in those that did it was evident that airway obstruction occurred even in nonsmoking patients with SLE. The frequency of airway obstruction is variable, primarily because of differences in the criteria that are used to define the abnormality and in the selection of patients. In the only controlled study of lifelong nonsmoking patients with SLE, Andonopoulos et al. (39) observed a high prevalence (24%) of isolated small airway disease in patients with SLE. The clinical significance of this observation remains unclear, however, because a similarly high frequency (17%) of small airway disease was found in their healthy, nonsmoking age- and sex-matched controls.

Very occasionally the upper airways have been affected in lupus. Case reports have described hypopharyngeal ulceration, laryngeal inflammation, vocal cord paralysis, epiglottitis, and subglottic stenosis (reviewed in (12)).

Fatigue, Physical Fitness, and Aerobic Capacity in SLE

Fatigue is a major clinical problem in patients with SLE and is often their most persistent symptom. It is multifactorial in origin and difficult to treat. A number of studies have addressed whether poor physical fitness and aerobic capacity may be contributory factors. Two studies assessed physical deconditioning in SLE (205 ,206). Robb-Nicholson et al. showed that compared to published age- and sex-matched norms, patients with SLE performed at 45% of their expected maximal aerobic capacity (VO_2max) and fatigue, measured using a visual analog scale, was inversely correlated with the duration of the exercise test, although not with VO_2max itself (205). A study by Daltroy et al. measured exercise duration during a graded exercise test in 34 patients with SLE and concluded that the patients were deconditioned compared to published age- and sex-matched norms (206). However, both studies were limited by the small number of patients involved, the use of published normative data, and the insensitivity of the fatigue measure. Tench et al. studied 93 patients with mild stable SLE and 41 healthy, but sedentary female controls and found that the SLE patients had significantly shorter test duration, lower VO_2peak, lower maximum minute ventilation, and lower respiratory exchange ratio, and terminated the exercise treadmill test at a significantly lower maximum heart rate (207). Significantly more of the SLE patients (33%) had a peak respiratory exchange ratio less than 1.1 compared to 12% of the controls. The maximum voluntary quadriceps contraction was also significantly reduced in the SLE patients compared to controls. Furthermore, resting lung function measures were significantly reduced in the lupus patients. There were 15/93 (16%) SLE patients who had FEV_1 less than 75% of predicted and 18/93 (19%) SLE patients who had FVC less than 75% of predicted. One control patient had FEV_1 and FVC less than 75% of predicted. The SLE patients had significantly lower submaximal oxygen uptake compared to controls, but there were no significant differences in submaximal perceived exertion or heart rate between the groups (207). A smaller study of 18 women with SLE and 16 controls described very similar findings with significant functional aerobic impairment (208).

In order to address this a randomized trial of an exercise programme was conducted by the same authors (209). Although fatigue scores and exercise duration improved significantly aerobic capacity did not change in these patients who had no clinical evidence of lung disease.

Infections and the Lung in SLE

Pulmonary infections are common in patients with lupus, especially in those taking corticosteroids and immunosuppressive therapies. Organisms include viruses, bacteria, mycobacteria, parasites, and opportunistic fungal infections. For a more thorough review of infectious complications in SLE, see Chapter 43 .

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Chapter 36

Clinical Aspects of Vasculitis and Selected Cutaneovascular Manifestations of Systemic Lupus Erythematosus

Daniel J. Wallace

Inflammation of the blood vessels is a common feature of SLE. Its manifestations in various organ systems are reviewed in their appropriate chapters. This section overviews clinical vasculitis with emphasis on its cutaneovascular features and subsets.

Vasculitis

Defined as inflammation of a blood vessel, vasculitis associated with lupus is defined using clinical, histopathologic, and arteriographic criteria. In SLE, vasculitis is cutaneous or visceral, with the former making up the overwhelming majority of cases. The clinical signs of vasculitis vary from organ to organ and are covered individually in chapters in this book. A literature review by Calamia and Balabanova estimates that vasculitis complicates lupus in only 4% of cases (1). The prevalence of vasculitis varies widely and depends the nature of referral patterns of the reporting center. Lupus usually involves the small- and medium-sized vessels with intimal thickening, medial necrosis, and adventitial fibrosis, but cutaneous vasculitis can include capillaritis. The pathophysiologic mechanisms of inflammation resulting in vasculitis can be found in the basic science chapters of this monograph.

Alarcon-Segovia et al. followed 667 lupus patients in Mexico City. Of 540 who were evaluable, 194 had vasculitis (2). Five percent had vasculitis at 1 year, but 41% at 10 years. As noted above, most of the cases consisted of cutaneous vasculitis (160, or about 80%). Visceral lesions were found in 24 and both in 10. Those with vasculitis tended to be younger and male. The cutaneous lesions were (from most to least) punctuate lesions, palpable purpura, papules, urticaria, ulcers, erythematous plaques, erythema with necrosis, and panniculitis. The visceral lesions included mononeuritis multiplex, digital necrosis, large artery vasculitis (3), mesenteric arteritis, and coronary arteritis. The definitive diagnosis was made by biopsy in 46 and imaging studies in 5. Visceral disease was associated with a poor prognosis. Ramos-Casals et al. reported on 630 patients in their Barcelona cohort seen between 1980 and 2002 (4). Sixty-two, or 10% had vasculitis with 22% being male and a mean age of 32.8. 94% had cutaneous vasculitis; 18% systemic vasculitis (with obvious overlap). The presentation was similar to the Mexican group, except that 27% with vasculitis had cryoglobulins.

Clinical Aspects of Cryoglobulinemia in SLE

The prevalence of cryoglobulinemia varies depending upon referral patterns and the vigor and rigor for which it is defined and looked for, ranging from 16% to 83% (5 ,6 ,7 ,8). The lower figure is probably more accurate if one examines published reports since 1990, because hepatitis C could be differentiated from clinical SLE (5 ,7). Chapter 28 reviews the basic science aspects of this protein.

Cryoglobulinemia is associated with cutaneous vasculitis, rheumatoid factor, hypocomplementemia, antiphospholipid antibodies, and concurrent hepatitis C infection. Two thirds have cryocrits less than 1%, and only hepatitis C is associated with higher values. Hepatitis C accounts for approximately 20% of SLE associated cryoglobulinemia, which responds to antiviral regimens and interferon-α. Anti-inflammatory therapies used to manage active lupus ameliorate cryoglobulinemia with the suggestion that apheresis might be helpful in cases where the cryocrit is critically elevated (9).

Raynaud Phenomenon

Paroxysmal vasospasm of the fingers, or Raynaud phenomenon, is a frequent abnormality in SLE. Raynaud phenomenon represents an abnormally regulated neuro-endothelial

control of vascular tone as a form of dysautonomia (10 ,11) (Fig. 36-1 .) Here again, as with alopecia and fractured frontal hair, incidence varies with the observer's specific questioning of the patient. Raynaud phenomenon was present at some time in 10% to 57% of the patients listed in Table 32-1 . Because a history of Raynaud phenomenon may be vague, nonspecific, and difficult to document, it was deleted from the 1982 revised American College of Rheumatology (ACR) criteria for SLE. Its prevalence takes the middle range between the 95% found in scleroderma and the 3% seen with rheumatoid arthritis (12). Cholesterol emboli, cryoglobulinemia, digital infarcts from the lupus anticoagulant, and reflex sympathetic dystrophy can mimic Raynaud phenomenon and must be excluded. The activity of Raynaud phenomenon usually is independent of SLE disease activity and the presence of antiphospholipid antibodies (13).

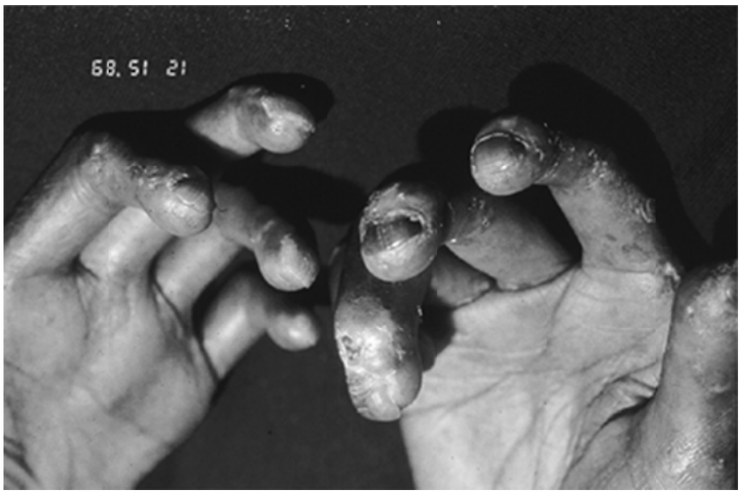

Figure 36-1. Severe Raynaud's phenomenon with digital gangrene.

Raynaud phenomenon is nonspecific and may be present years before the development of other changes caused by SLE, scleroderma, or dermatomyositis. De Takats and Fowler (14) reviewed 66 patients with Raynaud phenomenon with follow-up for 1 to 25 years, and they observed that 32 subsequently developed scleroderma, 2 developed SLE, and 1 developed dermatomyositis. Three later reports repeated their survey and found that 0 of 96, 0 of 85, and 0 of 87 patients with Raynaud phenomenon carried a diagnosis of SLE, with a mean 5-, 6-, and 9-years of follow-up, respectively (15 ,16 ,17). Studies of patients referred to rheumatologists because of Raynaud phenomenon revealed that 5% to 9% had SLE (1 ,18 ,19 ,20). Raynaud phenomenon was the first manifestation of SLE in two of Dubois' patients (Table 32-2).

Dimant et al. (21) compared 91 of their 276 patients with Raynaud phenomenon to those without it. Those in the Raynaud phenomenon group had significantly more arthritis, malar rash, and photosensitivity and less renal disease, lower steroid requirements, and fewer deaths than those in the unaffected group. The Raynaud phenomenon that is associated with SLE is similar to that seen in Raynaud disease and is less severe than that in scleroderma. The vasospasm rarely leads to permanent damage; small ulcers on the fingers can occur following prolonged and frequent attacks. Raynaud phenomenon usually operates independent of disease activity and is not steroid responsive. It may be associated with antibodies to hn-RNP protein A1 (22), migraine, and chest pain (23). Arteriograms of 10 patients with SLE and Raynaud phenomenon showed severe vasospasm and severe digital artery involvement that did not correlate with disease activity (24). Evaluation of two studies (25 ,26) leads to the conclusion that cold-pressor testing improved or did not change the diffusing capacities in patients with SLE and Raynaud phenomenon and worsened it in those with primary Raynaud phenomenon. Renal Raynaud phenomenon after cold exposure with 99m-technetium scanning (27), colonic cyanosis (28), Raynaud phenomenon of the tongue (29), and pulmonary hypertension have been associated with Raynaud phenomenon in SLE (30). Single-photon emission computed tomography (SPECT) brain-imaging correlates with hypoperfusion associated with Raynaud phenomenon in SLE (31), as are elevated homocysteine levels (32), and lupus pernio (chilblains) (33).

The treatment of Raynaud phenomenon is not different in those with concurrent SLE. Avoidance of cold or inciting drugs (e.g., beta blockers, ergot alkaloids), along with wearing gloves, biofeedback (34), and use of vasodilators (e.g., nifedipine, nicardipine, nitroglycerin paste, nitroprusside) (35 ,36 ,37) may be advised. Steroids have no effect upon Raynaud phenomenon. Pentoxifylline (38) may be useful. Prostaglandin E1 infusions or sildenafil (39 ,40 ,41 ,42 ,43 ,44) improve digital ulcers and early gangrene in severe cases.

Nailfold Microcapillaroscopy

In 1935, Baehr et al. (45) noted that the involved skin about the nailbed, when viewed under the capillary microscope, contained many more patent and dilated capillaries than normal. In 1968, Buchanan and Humpston (46) observed hemorrhage in 62% of 29 patients and abnormal capillary loops in 93%. These findings were confirmed by others (47 ,48 ,49).

Maricq and LeRoy (50) as well as Kenik et al. (51) have studied these phenomena extensively in connective tissue diseases. Capillary loops in SLE (independent of coexistent Raynaud phenomenon) appear meandering and tortuous, with most having some disorganization and glomerulization (52 ,53) (Fig. 36-2). They claimed that a trained, blinded observer could identify SLE 75% of the time. SLE patients have less frequent microvascular abnormalities (54). Three independent studies have given photomicrographs of patients with scleroderma, rheumatoid arthritis (RA), SLE, and mixed connective tissue diseases to blinded observers. Lefford and Edwards (55) could not find any relation between capillary morphology and those clinical diagnoses. Granier et al. (56) noted that

64% of patients with mixed connective tissue disease (MCTD) had a scleroderma pattern and only 23% an SLE pattern, and McGill and Gow (57) found an 89% specificity and 80% sensitivity in selecting the correct diagnosis. Increased nailfold capillary density may indicate pulmonary capillary loss in SLE (58) and correlate with anticardiolipin antibody along with the presence of microhemorrhages (59 ,60). SLE patterns are similar to those seen in primary Sjögren syndrome (61).

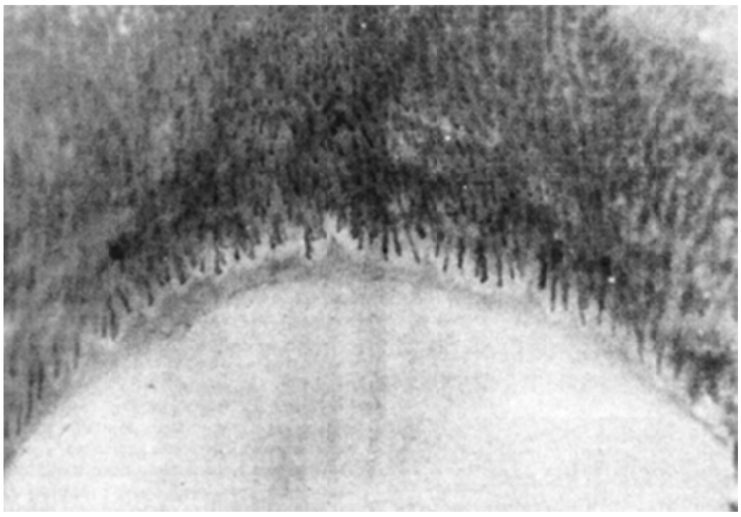

Figure 36-2. Nailfold microcapillaroscopy in systemic lupus erythematosus. (Courtesy of Dr. J. Kenik.)

The presence of anti RNP or Raynaud phenomenon in SLE is associated with greater intercapillary distance, capillary width, and more scleroderma-associated features (62). In one report, 8.5% of 47 patients had a scleroderma capillary pattern (63). Patients with primary antiphospholipid syndrome tend to have swollen capillary diameters and lower flow rates (64).

Complications of Cutaneous Vasculitis: Ulceration and Gangrene

A small percentage of these patients with active vasculitis may manifest necrotic ulcerations, digital and peripheral gangrene, and/or cutaneous infarctions. They frequently have a high-titer antinuclear antibody (ANA), elevated levels of serum anti-DNA and IgG, and reduced serum complement levels (65). Direct immunofluorescence has demonstrated IgG, complement, and fibrinogen, with fibrin in the vessel walls surrounding the involved tissue (66 ,67 ,68). Approximately 10% of patients with peripheral vascular disease have a positive antinuclear antibody or antiphospholipid antibodies. Few of these have an inflammatory process (69).

Vasculitic leg ulcers were found in 29 of Dubois' 520 patients in 1963 (70) and in 3 of Brogadir and Mejers' patients (71), and 6.2% of Petri's Hopkins Cohort (72). Dermal vasculitis was present in 18 to 70 patients in the series listed in Table 32-1 . Of Dubois' 520 patients, 7 developed peripheral gangrene, as did three of Estes and Christian's 150 patients (73).

Ulcerations and gangrene can occur as a result of active vasculitis, the antiphospholipid syndrome, or both (74 ,75 ,76 ,77 ,78) (Fig. 34-3). Asherson et al. (75) followed six patients with gangrene of the extremities. Three had the antiphospholipid syndrome with no vasculitis, two had a classic immune-complex–mediated vasculitis without evidence of a lupus anticoagulant, and one had both. Similar findings were reported by Alarcon-Segovia et al. (74) and by Lockshin et al. (79 ,80). Additionally, the lupus anticoagulant rarely is associated with a syndrome of cutaneous necrosis, not unlike what has been reported in protein C deficiency states (81 ,82). Cutaneous necrosis, antiphospholipid antibodies, livedo reticularis, and central nervous system findings have been termed Sneddon's syndrome (see Chapter 57). The differential diagnosis of cutaneous ulcerations requires ruling out ischemia from degenerative arterial disease, venous stasis, cryoglobulinemia, hyperviscosity syndrome, cholesterol

emboli, and other hypercoagulable states. Thrombosis is reviewed in Chapter 65 .

The optimal treatment of peripheral vasculitis includes systemic corticosteroids, cyclophosphamide, and if necessary, plasmapheresis (79 ,83 ,84). If the antiphospholipid syndrome is present, anticoagulation is advised (79 ,80). Short-term treatment with the prostaglandin E-derivative alprostadil, or sildenafil is the most effective method of managing critical peripheral ischemia of the hands, feet, and legs (39 ,40 ,41 ,85 ,86). Cutaneous vasculitis may respond to colchicine (see Chapter 56).

Livedo Reticularis

Livedo reticularis occurs because of disordered autonomically mediated blood flow through subpapillary and dermal blood vessels. It can be brought on by cold, connective-tissue diseases, fibromyalgia-cold agglutinins, and cryoglobulinemia. In SLE, it presents as a reticulated poikiloderma, most often on the arms and legs. Usually painless, livedo rarely can appear as a cutaneous vasculitis known as livedoid vasculitis (Fig. 36-3). This reddish-purplish mottling on the skin blanches on pressure, is independent of temperature changes, and represents a vasospastic phenomenon of dermal-ascending arterioles (87 ,88 ,89 ,90 ,91). Livedo racemosa is a subset of livedo reticularis with irregular, ill-circumscribed and broken segments and is associated with Sneddon syndrome (92).

Livedo reticularis has been shown in case-controlled studies to be unequivocally associated with cutaneous necrosis, central nervous disease, pregnancy-related morbidity, and the antiphospholipid syndrome (92 ,93 ,94 ,95 ,96 ,97 ,98). Livedo was observed in 11 of 66 patients with SLE in one report (99), and anticardiolipin antibodies were found in 81% of patients with SLE and livedo (see Chapters 29 , 30 , and 31). Unless livedoid vasculitis is present, no treatment is indicated.

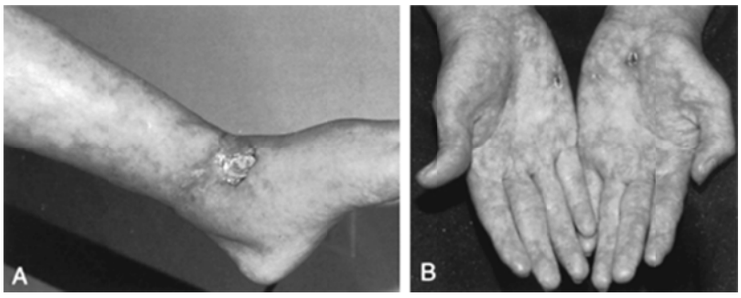

Figure 36-3. A, Livedo reticularis and ulceration of the leg of systemic lupus erythematosus patient with high-titer IgG anticardiolipin antibodies. **B**, Similar changes on the hands on this same patient.

Antiphospholipid Antibodies and the Skin

See Chapter 65 .

Purpura

Ecchymoses and petechiae may be noted depending on the platelet count and whether the patient has received steroid therapy. Cutaneous hemorrhages were seen in 9% to 21% of patients in various series (73). The most common cause of hemorrhagic lesions was steroid therapy, although salicylates and nonsteroidal anti-inflammatory drugs (NSAIDs) also could induce them. NSAIDs can induce ecchymoses through their antiplatelet actions, and long-term steroid administration is associated with skin atrophy. Patients with untreated SLE occasionally report that they bruise easily, and many are thrombocytopenic. Occasionally, petechiae may occur because of active cutaneous vasculitis. Purpuric leg lesions should be differentiated from pigmentary changes resulting from long-term antimalarial therapy. Thrombotic thrombocytopenic purpura, idiopathic thrombocytopenic purpura, cryoglobulinemia, and other dysproteinemias may be seen in SLE and need to be ruled out.

Erythromelalgia

Erythromelalgia consists of burning distress of the extremities that is accompanied by increased redness and skin temperature, initiated by an increase in environmental skin temperature, and diminished by measures that cool the skin (100 ,101 ,102). Mostly seen in myelodysplastic disorders with thrombocytosis, erythromelalgia can occur in patients with SLE and normal platelet counts (87 ,102 ,103 ,104 ,105 ,106). It usually is treated with antiplatelet agents (e.g., low-dose aspirin, dipyridamole) or corticosteroids (107). Clonazepam (105), propanolol with methysergide, or cyclosporin may be useful (108 ,109).

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Chapter 37

The Nervous System

Sterling G. West

Neuropsychiatric manifestations of systemic lupus erythematosus (NP-SLE) are frequent, vary from mild to severe, and often are difficult to diagnose and distinguish from those of other diseases. Any location within the nervous system may be affected, with symptoms and signs ranging from mild cognitive dysfunction to seizures, strokes, and coma. At the initial development of neurologic manifestations, many patients have other medical conditions or are receiving medications, which can affect the central or peripheral nervous systems. The challenge to the clinician is to determine the exact cause of the nervous system dysfunction so that appropriate therapy can be instituted. This chapter describes the classification, etiopathogenesis, clinical signs and symptoms, laboratory and radiographic findings, differential diagnosis, and treatment of lupus involving the nervous system.

Historical Considerations

Neurologic involvement in SLE was first noted by Hebra and Kaposi (1) in 1875, who described stupor and coma as terminal manifestations of the disease. Osler (2 ,3 ,4), in several papers on the systemic effects of the erythema group of skin diseases, discussed associated cerebral changes and reported a patient who “imagines all sorts of things.” In 1904, Baum (5) related active delirium, aphasia, and hemiparesis to probable disseminated lupus erythematosus. During the next 40 years, the psychiatric and neurologic correlates of SLE were recognized, but seldom discussed.

The first modern study of NP-SLE was conducted by Daly in 1945 (6). He correlated clinical symptoms with abnormal spinal fluid findings and with the pathologic finding of vasculitis. In 1948, Sedgwick and Von Hagen (7) discussed five cases in detail. In 1953, Dubois (8) described clinical neurologic subsets among 62 cases and, in 1954, Lewis et al. were the first to focus on the importance of electroencephalographic findings and psychometric testing in patients with NP-SLE.

From the 1950s through the 1970s, hundreds of reports delineated the various manifestations of neurologic involvement in SLE. Over the last three decades, however, appreciation of the clinical significance of antineuronal, antiribosomal P, and antiphospholipid antibodies, as well as advances in brain imaging, have again altered our concept of NP-SLE.

Classification and Clinical Presentation

The incidence of neuropsychiatric manifestations in SLE ranges from 14% to 75% depending on the ascertainment methodology. A recent prospective study of 1000 SLE patients followed for 10 years (1990–2000) found that 19.4% developed NP-SLE (9). However, it often is impossible to compare past studies of NP-SLE, because no standardized definition or classification system was used. Some reports included patients with minimal, nonspecific symptoms, while others restricted themselves to those with objective neurologic findings. In 1999, an international, multidisciplinary committee developed case definitions, including diagnostic criteria and important exclusions, for 19 neuropsychiatric lupus syndromes (10) (Table 37-1). This American College of Rheumatology (ACR) nomenclature should help clinicians classify NP-SLE, as well as help investigators in future studies. The complete case definitions are available on the ACR world wide web site: http://www.rheumatology.org/ar/ar.html.

SLE patients with NP-SLE can present with a myriad of diffuse and/or focal symptoms and signs involving the brain, spinal cord, or peripheral nervous system. The central nervous system (CNS) manifestations include headache, confusion, altered consciousness, seizures, focal or generalized neurologic deficits, ataxia, chorea and other movement disorders, papilledema, psychosis, and severe depression. Peripheral nervous system manifestations include cranial nerve dysfunction, acute weakness, paresthesias, and abnormalities of autonomic function. The pathologic abnormalities observed in the nervous system also are diverse (Table 37-2). Oftentimes, there is no clear-cut clinicopathologic relationship between CNS signs and localized CNS lesions. This section discusses the prevalence of NP-SLE as described in some of the principal surveys from the last 50 years (Table 37-3).

Table 37-1: Neuropsychiatric Syndromes of SLE

Central nervous system
Acute confusional state
Cognitive dysfunction
Psychosis
Mood disorder
Anxiety disorder
Headache (including migraine and benign intracranial hypertension)
Cerebrovascular disease
Myelopathy
Movement disorder
Demyelinating syndrome
Seizure disorders
Aseptic meningitis
Cranial neuropathy
Peripheral nervous system
Polyneuropathy
Plexopathy
Mononeuropathy, single/multiplex
Acute inflammatory demyelinating polyradiculopathy
(Guillain-Barré syndrome)
Autonomic disorder
Myasthenia gravis

Adapted from ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology Nomenclature and Case Definitions for Neuropsychiatric Lupus Syndromes. *Arthritis Rheum* 1999;42:599–608.

Important Surveys: 1950–1980

Of Dubois' 520 patients (11) who were followed between 1950 and 1963, 25% had some type of central or peripheral nervous system involvement. In a 1956 review of the cliniconeurologic changes that were observed in 24 of 100 patients with SLE followed at the Mayo Clinic, Clark and Bailey (12) reported changes that varied from convulsive seizures to monoplegia, and they revealed an entire gamut of neurologic findings. Signs and symptoms of neurologic disease alone were noted in 11 patients, and neurologic and psychiatric disease together were present in 13.

Table 37-2: Pathologic Classification of Central Nervous System Changes Observed in SLE

Vasculopathy
Hyalinization
Perivascular inflammation without infection
Endothelial proliferation without infection
Thrombosis
Vasculitis
Infarction
Microinfarcts
Large infarcts
Hemorrhage
Subarachnoid
Microhemorrhages
Subdural
Intracerebral
Infection
Meningitis
Perivascular inflammation with infection
Septic hemorrhages
Focal cerebritis
Vasculitis with infection

Adapted from Ellis SG, Verity MA. Central nervous system involvement in systemic lupus erythematosus: a review of neuropathologic findings in 57 cases, 1955–1977. *Semin Arthritis Rheum* 1979;8:212–221.

Table 37-3: Neurologic Manifestations of SLE in Selected Large Series (%)

Parameter	Clark & Bailey 1956	Ester & Christian 1971	Ropes* 1976	Gibson & Myers 1976	Feinglass 1976	Lee 1977	Hochberg** 1985	Wallace 1990	Sanna 2003
No. of cases	100	150	150	81	140	110	150	464	323
CNS manifestations	24	59	—	51	52	40	55	50	57
Seizures	14	26	11	20	17	8	13	6	8
Cranial neuropathy	—	7	—	4	16	3	—	—	—
Vasculopathy	8	8	15	10	16	3	—	11	18
Peripheral neuropathy	3	7	5	2	15	8	21	5	—
Psychosis	17	16	28	27	14	16	16	5	

*Ropes MW. *Systemic Lupus Erythematosus*. Cambridge, MA: Harvard University Press, 1976.

**Hochberg MC, Boyd RE, Ahearn JM, et al. Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic in 150 patients with emphasis on demographic subsets. *Medicine* 1985:285–295.

In 1966, O'Connor and Musher (13) reviewed 150 patients with SLE who were followed at Columbia Presbyterian Medical Center. Of these, 4 patients presented with NP-SLE. CNS disturbances were more frequent among patients who were

followed through terminal illness. Sixty-seven percent of the 150 patients had psychiatric disorders, and 43% had neurologic disorders. Neurologic signs were seen at one time or another in 46 of the 150 patients.

In 1971, Estes and Christian (14) observed neuropsychiatric manifestations of SLE in 59% of 150 patients who were followed mostly in the 1960s. Disorders of mental function were found in 42% and grand mal seizures in 26%. The neurologic manifestations were cranial nerve involvement (7 patients), oculomotor signs (6 patients), and optic atrophy and blindness (3 patients). Intention tremor was observed in 8 patients and was associated with cogwheel rigidity in 2. Hemiparesis occurred in 8 patients, 6 of whom had chronic renal disease with hypertension and/or uremia. Peripheral neuropathy with predominantly sensory deficits developed in 10 patients.

In 1975, Sergent et al. (15) reported on 52 episodes in 28 patients with SLE: 10 had seizures, 9 encephalopathy, 4 aseptic meningitis, 7 focal neurologic deficits, 15 psychiatric abnormalities, and 1 chorea. They found no evidence that large doses of steroids were helpful in treating these patients. Also in 1975, Klippel and Zvaifler (16) compiled a literature review of NP-SLE in 995 reported patients with SLE (one half of these were Dubois' 520 patients). Overall, neuropsychiatric abnormalities were found in 33%, seizures in 16%, neuropathy in 10%, psychopathology in 18%, and myelopathy and chorea in 4% each.

In 1976, a review of 140 patients with SLE at the Johns Hopkins Hospital revealed neuropsychiatric changes in 52% (17), and 63% of these had episodes in the first year of disease. Only two instances of documented steroid-induced psychosis occurred. Of these 140 patients, 84% improved with treatment of SLE. A striking, positive correlation was noted among CNS involvement, vasculitis, and thrombocytopenia. Five- and 10-year survival rates for those in the CNS group were 94% and 82%, respectively. Urowitz et al. at the University of Toronto published two large SLE series (18 ,19) that emphasized CNS findings; those with CNS disease had lower serum complement levels and more disease manifestations than those without CNS pathology. Grigor et al. (20) found neuropsychiatric symptoms in 50% of 50 patients. No correlations could be found with any other SLE manifestations, including vasculitis and thrombocytopenia.

Gibson and Myers (21) noted episodes of NP-SLE in 41 of 80 patients (51%) who were followed before 1976. More episodes were noted in blacks, and an increased incidence of renal failure as well as a poorer survival were seen in this group as opposed to the non-CNS group. In contrast to most of the preceding reports, Seibold et al. (22) calculated that CNS involvement occurred at a mean of 4.3 years after the onset of disease in 26 patients.

Important Surveys: 1980–2000

The 1980s saw a continuing decline in the reported frequency of classic NP-SLE. Pistiner et al. (23) observed 49 cases of cerebritis and, comparing patients who were evaluated from 1950 to 1963 with those between 1980 and 1989, this represented a decrease from 26% to 11%. Using the ACR definition for CNS-lupus as a criterion (i.e., seizures or psychosis), 30 patients (6%) had seizures and 24 (5%) psychotic episodes. The prevalence of significant CNS abnormalities was 27% in two European multicenter studies (24 ,25) surveying 1704 patients with lupus; 12% had these abnormalities at presentation. West et al. (26) noted CNS lupus in 50 of 184 patients (28%) in Colorado, and others (27 ,28 ,29) noted CNS disease in 48 of 266 patients (18%) in Saskatchewan, in 35 of 222 (16%) in Australia, in 22 of 53 (42%) in Italy, and in 171 of 1203 (14%) in China (30).

The recognition of antineuronal, antiribosomal P, and antiphospholipid antibodies in the 1980s has affirmed the value of clinically classifying CNS episodes into “diffuse” and “focal” presentations. Several studies showed that serum and cerebrospinal fluid (CSF) antineuronal antibodies are found more commonly in patients with diffuse, global dysfunction such as acute confusion, altered level of consciousness, seizures, cognitive dysfunction, and psychiatric abnormalities (31 ,32 ,33 ,34 ,35). Bonfa and Elkon were first to associate antiribosomal P antibodies with psychosis and severe depression (36). Some investigators confirmed this association, whereas others did not (37). Antiphospholipid antibodies were associated with thromboembolic episodes leading to focal manifestations, including strokes, transverse myelitis, and chorea (38).

West et al. reported in a 10-year prospective study of 184 SLE patients that certain combinations of tests were most useful in establishing a diagnosis of severe NP-SLE, depending on whether the patient had a diffuse or focal presentation. Of these SLE patients, 52 developed NP-SLE and 14 had CNS dysfunction because of a nonlupus cause. Cases were divided into diffuse, focal, and complex (both diffuse and focal symptoms) presentations. SLE patients with diffuse presentations were more likely to have elevated CSF antineuronal antibodies, abnormal CSF IgG indices with oligoclonal bands, or serum antiribosomal P antibodies. Patients with focal symptoms had evidence of vasculitis or high-titer antiphospholipid antibodies with abnormal brain MRI showing multiple lesions (26). More recently, Isshi and Hirohata reported that serum antiribosomal P and CSF antineuronal antibodies were detected more frequently in lupus psychosis patients than in lupus patients with other symptoms of NP-SLE or lupus patients without NP-SLE (39).

Clinical surveys confirmed that NP-SLE can occur early in the course of SLE (40 ,41) and usually is associated with lupus that is clinically and serologically active (42). Two studies pointed out that secondary causes such as infection always need to be ruled out in SLE patients presenting with mental confusion or alterations in consciousness. Infections were found in 14 of 91 SLE patients with CNS dysfunction by Futrell et al. (43) and in 28% of 36 acute CNS presentations by Wong et al. (44).

Studies by the Denburgs and others (33 ,34 ,35 ,45) established that mild cognitive dysfunction is common in SLE patients and should be considered a subset of NP-SLE. Symptoms of cognitive dysfunction were often present in

the absence of objective neurologic, clinical, laboratory, or neuroimaging abnormalities, but could be demonstrated on neuropsychologic testing. Some of these patients can progress to more severe neuropsychiatric disease.

Two Canadian studies that followed 900 patients with lupus for 15 to 20 years reported that most CNS events were self-limited, reversible, and not associated with a poor outcome (46 ,47). However, patients with focal findings had a worse prognosis than patients with diffuse presentations (46).

Important Surveys: Since 2000

Since publication of the ACR nomenclature and case definitions for Neuropsychiatric Lupus syndromes (see Table 4-2), several investigators have used these criteria in their surveys (48). In a cross-sectional population-based study from Finland, Ainala et al. found that 42 of 46 (91%) SLE patients had NP-SLE using the ACR nomenclature (49). Many of these were mild syndromes including cognitive dysfunction, headaches, and mood disorders. When these SLE patients were compared to well-matched controls, 56% of the controls fulfilled at least one of the ACR criteria (50). The criteria, therefore, had a high sensitivity (91%) but low specificity (46%). If the ACR criteria were revised to exclude syndromes without objective findings such as anxiety, headaches, mild depression, subjective cognitive complaints, and polyneuropathy symptoms with a negative electromyogram/nerve conduction velocities, the specificity improved to 93%. Other studies have also found a high prevalence of NP-SLE using the ACR nomenclature including investigators from San Antonio (80% of 128 SLE patients), Italy (72% of 61pts), Britain (57% of 323 patients), Canada (37% of 111 patients), Brazil, and Thailand (48 ,49 ,50 ,51 ,52 ,53 ,54).Overall the ACR nomenclature was felt to be useful for major NP-SLE syndromes, although problematic when applied to mild and subjective syndromes such as headaches, cognitive dysfunction, and minor psychiatric symptoms, which are common in patients without SLE. Notably, these mild syndromes are more common than major presentations of NP-SLE (55).

Summary

SLE can involve the nervous system in many ways. Until recently, the presence of specific neuropathology causing seizures, stroke, and paresis was considered to constitute NP-SLE and was found in approximately 25% of patients. Over the last three decades, the incidence of these features has decreased as early intervention and the diagnosis and treatment of active SLE became more common. Over the last decade, however, the recognition of lupus headache and cognitive dysfunction as distinct syndromes, in the absence of specific neuropathology, has resulted in a net increase in the incidence of what many rheumatologists call “CNS lupus.” NP-SLE is now regarded as being present in many patients with SLE at some point during the course of their disease. Specific neuropathology is seen in a minority and usually is short-term, but can lead to chronic neurologic deficits.

Etiopathogenesis

The Clinicopathologic Studies

Several autopsy series have reported detailed analyses of the neuropathology of patients with NP-SLE. Many of these studies are hampered by the inclusion of patients with secondary causes of CNS dysfunction, as well as patients with prolonged intervals between NP-SLE manifestations and death. Despite these limitations, these studies provide important insights into the pathogenesis of NP-SLE. Johnson and Richardson (56) reviewed the brain sections of 24 patients observed at Massachusetts General Hospital in the 1960s. Neurologic and psychiatric manifestations were found in 18 patients (75%). In 9 of these 18, neurologic involvement occurred during the last 6 weeks of life. Death was attributable to CNS disease in 6 patients (intracerebral hemorrhage in 4, status epilepticus in 2). Seizures were present in 54%, cranial nerve disorders in 42%, hemiparesis in 12%, paresis in 4%, peripheral neuropathy in 8%, and mental disorders in 33%. Significant, gross brain abnormalities were found in 10 of the 24 patients, including large intracerebral hemorrhages (3 patients), multiple pontine hemorrhages (1 patient), fresh hemorrhages (2 patients), small areas of old infarction (4 patients), and a small, subapical hemorrhage (1 patient). Microscopic lesions were more common than macroscopic lesions; microinfarcts with increased pericapillary microglia were found in 20 of the 24 patients. Johnson and Richardson concluded that the nervous system involvement of SLE was caused primarily by vascular disease affecting small vessels and producing micro-infarcts with hemorrhages. True vasculitis was rare; inflammatory cells within the vessel wall were found in only 3 of the 24 patients. In contrast, perivascular inflammation was more common. Destructive lesions in the wall of small vessels, which were described as fibrinoid degeneration, were found in 5 patients.

Funata (57) performed detailed neuropathologic evaluations on 26 patients with SLE. Twelve died as a result of uremia, and one half of these 12 had perivascular inflammation in the brain. Thrombi associated with endothelial swelling and proliferation and fibrinoid degeneration were noted in five patients.

Ellis and Verity (58) reviewed 57 autopsied SLE cases at UCLA Medical Center. Vasculopathy was observed in 65%, infarction in 44%, hemorrhage in 42%, and infection in 28%. In the vasculopathy group, hyalinization (54% of patients), perivascular inflammation (28%), endothelial proliferation (21%), thrombosis (7%), and vasculitis (8%) were found. The infarctions consisted mostly of microinfarcts. Hemorrhages included subarachnoid (30%), intracerebral (10%), and subdural (4%) hemorrhages, as well as microhemorrhages (19%). In those with infections, meningitis (18%), perivascular inflammation (14%), septic

hemorrhages (5%), and focal cerebritis (3%) were seen. Many patients in this group had received combined corticosteroids and azathioprine therapy. All of the studies discussed so far have antedated the availability of anticardiolipin antibody testing. They certainly may reflect the most severe end of the NP-SLE spectrum.

In 1988, Devinsky et al. reported autopsy results of 50 SLE patients (59). Neuropsychiatric manifestations had occurred in 74% of the patients. Half of the patients had microscopic lesions, with embolic brain infarcts (10 patients) and intracerebral infection (8 patients) being most common. A cardiac source was found in 9 of the 10 patients with embolic brain infarcts. A true vasculitis was not seen. Interestingly, 14 patients had clinical evidence of thrombotic thrombocytopenic purpura during the terminal phase of their illness. Unfortunately, the antiphospholipid antibody status of these patients was not reported, although Greisman et al. (60) have indicated in a subsequent report that many of them had antiphospholipid antibodies. Importantly, a correlation between the patients' neuropsychiatric manifestations and pathologic brain lesions could only be made in half of the patients. Recently, Hanly et al. associated brain microinfarcts with seizures in 10 patients, but not with antiphospholipid, antiribosomal P, or antineuronal antibodies (61).

These autopsy series agree on several important points. First, there is no distinct typical or pathognomonic lesion that NP-SLE causes in the brain that is diagnostically specific, like the “wire loop” lesion of the kidney or the “onionskin” lesion of the spleen. Notably, vasculitis is unusual at autopsy. The most common finding is a small vessel, bland, noninflammatory, proliferative vasculopathy characterized by hyalinization. These degenerative and proliferative changes in the small cerebral vessels are not distinct from the vascular changes seen in hypertensive encephalopathy and thrombotic thrombocytopenia purpura. The neuropathologic lesions of SLE, however, are characterized as more focal or more scattered and by the fact that they vary in age from region to region, rather than appearing to have occurred simultaneously. Finally, clinical manifestations may not be readily explained by pathologic findings. Some NP-SLE patients, particularly those with diffuse manifestations, may have normal or relatively unremarkable brain pathology.

Table 37-4: Pathogenic Mechanisms Causing Neuropsychiatric Symptoms in SLE

Primary	Secondary
Vascular occlusion/hemorrhage	Infection
Immune complex-mediated vasculitis	Medications
Immune complex-mediated anaphylatoxin	TTP
release causing leukoagglutination	Hypertension
Antiphospholipid antibody-associated	Uremia
hypercoagulability/thrombosis	Electrolyte imbalances
Emboli from cardiac source	Fever
Cryoglobulinemia/hyperviscosity	Thyroid disease
Autoantibody-mediated	Atherosclerotic strokes
Antineuronal antibodies	Subdural hematoma
Choroid plexus dysfunction	Berry aneurysm
Cytokine effects	Cerebral lymphoma
Other mechanisms	Fibromyalgia
Abnormal HPA axis response	Reactive depression
Abnormal noradrenergic response	Sleep apnea
Nitric oxide/oxidative stress	Other primary neurologic or psychiatric disease
Excitatory amino acid toxicity	

HPA, hypothalamic-pituitary-adrenal; TTP, thrombotic thrombocytopenic purpura

Adapted from West SG. Neuropsychiatric lupus. *Rheum Dis Clin North Am* 1994;20:129–158.

Pathogenesis

The pathogenesis of NP-SLE is unknown. However, it is unlikely that a single pathogenetic mechanism is responsible for the myriad of neuropsychiatric manifestations seen in NP-SLE (Table 37-4). Diffuse cerebral manifestations that are often transient, reversible on therapy, and not consistently associated with abnormal brain pathology, most likely have a different pathogenesis from the focal symptoms, which are usually acute in onset, permanent even with therapy, and frequently associated with pathologic lesions at autopsy.

Vascular Occlusion

All clinicopathologic studies have reported that multiple vascular occlusions resulting in large and small infarcts and hemorrhages are commonly observed in the brains of

patients with NP-SLE at autopsy (56 ,57 ,58 ,59 ,60 ,61). These studies report that less than 15% of these arterial occlusions can be attributed to immune-mediated vasculitis. This may be because the tight junctions between endothelial cells and the blood-brain barrier (BBB) hinder deposition of immune complexes in the cerebral blood vessels. However, there have been several case reports of angiographically and/or histologically proven large and small vessel vasculitis occurring in SLE patients presenting with acute symptoms of NP-SLE (62 ,63 ,64 ,65 ,66). Rarely, an isolated phlebitis and venulitis can be observed. Cerebral vasculitis is a dramatic process that presents with fever, confusion, and headache, followed within hours to days by seizures, psychosis, and encephalomyelitis. If untreated, it can lead to coma and death. Cerebral changes usually are generalized. Laboratory investigation often reveals active, multisystem SLE with elevated serologies and decreased serum complement components. The CSF usually demonstrates pleocytosis, an elevated protein level, increased IgG, and an elevated Q-albumin, indicating disruption of the BBB. Magnetic resonance imaging (MRI) of the brain frequently reveals multiple ischemic gray and white matter lesions, and PET scan reveals hypoperfusion.

The most common lesion leading to vascular occlusion is a noninflammatory vasculopathy with marked endothelial proliferation, obliterative intimal fibrosis, thrombosis, and occasionally perivascular lymphocytes. Brain microinfarcts and microhemorrhages frequently occur in association with this microangiopathy. The etiology of this bland vasculopathy is unclear. Some investigators have suggested these lesions are scarring from healed or treated vasculitis or represent a unique response of cerebral arterioles to immune reactants, which can occasionally be demonstrated in the blood vessel walls. Indeed, several animal models have shown that chronic, low levels of circulating immune complexes are associated with an endothelial and intimal proliferative lesion and thrombosis in blood vessel walls, rather than an inflammatory vasculopathy (67 ,68). However, others have pointed out that this bland vasculopathy is similar to lesions observed in patients with antiphospholipid antibodies (60 ,69) and in patients with thrombotic thrombocytopenic purpura (70). Antiphospholipid antibodies have been shown to activate endothelial cells, possibly leading to upregulation of adhesion molecules and release of coagulation proteins, which can contribute to the development of vasoocclusive thrombosis (71). Platelet deposition in this thrombus could result in release of platelet-derived growth factors, resulting in endothelial cell proliferation and intimal fibrosis, commonly observed in this bland vasculopathy. Immunohistochemical staining with monoclonal antibodies have demonstrated CD 31 (endothelial cells), factor VIII antigen, and CD 61 (platelet membrane glycoprotein IIIa) in these thickened vessels, indicating their local incorporation in the thrombus (72 ,73). Finally, although uncommon, vasculitis has been reported to occur in patients with antiphospholipid antibodies (74). Perhaps antiphospholipid antibody activation of endothelial cells results in influx of leukocytes into the blood vessel walls via the upregulated adhesion molecules.

Clinically, Hughes et al. in the 1980s were first to correlate antiphospholipid antibodies with focal manifestations and seizures in NP-SLE patients (75 ,76). Subsequently, Hughes et al. observed neuropsychiatric manifestations in 96 of 340 SLE patients (28%) with 55% of patients with NP-SLE having antiphospholipid antibodies, compared to 20% of patients without NP-SLE ($p < 0.001$) (77). The most common clinical manifestations were transient ischemic attacks and strokes. Chorea, dementia, migraine headaches, transverse myelitis, seizures, cerebral venous thrombosis (78), and anterior spinal artery syndrome have also been associated with antiphospholipid antibodies (79 ,80). Patients with antiphospholipid antibodies in NP-SLE frequently have livedo reticularis (81), although other clinical and laboratory manifestations of active lupus may or may not be present. The CSF usually demonstrates elevated protein, but commonly a low white blood cell count. Antiphospholipid antibodies are not generally present in the CSF (38). Brain MRI shows focal or generalized ischemic white matter lesions (82 ,83).

Other causes of vascular occlusion have also been described. Activation of inflammatory cells by complement-mediated anaphylatoxins (C3a, C5a), resulting in leukothrombi causing vessel occlusion, has been observed in the brains of some patients dying of acute cerebral lupus (84). These leukothrombi have been shown to cause infarction, in the absence of vasculitis, in the brain as well as other organs of SLE patients. Another cause of cerebrovascular occlusion is emboli, usually from a cardiac source. Emboli from Libman-Sacks vegetations can cause acute vascular occlusion resulting in strokes. A recent autopsy series has suggested that a cardiac source of emboli in NP-SLE patients may be more common than previously reported (59). Antiphospholipid antibodies have been associated with cerebral artery embolization with fragments from Libman-Sacks-like endocarditis and abnormal heart valves (85 ,86 ,87 ,88). Whether these antibodies can directly cause valvular damage, or only contribute to thrombus formation (which can subsequently embolize) on already damaged valves, is presently unclear. Finally, although not truly vascular occlusion, sludging as a result of cryoglobulinemia and hyperviscosity has rarely been reported in patients presenting with mental clouding, dizziness, and confusion (89 ,90 ,91 ,92).

Vascular Hemorrhage

Although microhemorrhages are common, larger subarachnoid, intracerebral, and subdural hemorrhages occur in 0.4% to 7.0% of NP-SLE patients. One survey of 500 patients with SLE revealed evidence for cerebrovascular disease in 15, being occlusive in 11 and hemorrhagic in 4 (93). Several cases of subarachnoid hemorrhage caused by a Berry aneurysm have been described in SLE. It is not

known if these aneurysms are more common in SLE patients than in the general population (94 ,95 ,96). A small subset of SLE patients with antiphospholipid antibody have an increased bleeding tendency with neurologic consequences, but this group usually has an associated thrombocytopenia or hypoprothrombinemia. Other causes of cerebral hemorrhage, such as hypertension or thrombotic thrombocytopenic purpura, must be excluded.

Antineuronal Antibodies

Antineuronal antibodies injected into the ventricles of experimental animals cause a variety of neurologic symptoms, such as convulsions and impaired memory, suggesting that brain-reactive antibodies may cause certain NP-SLE manifestations (97 ,98 ,99). Several investigators have reported that up to 75% of SLE patients have elevated levels of serum antineuronal or antilymphocyte antibodies that cross-react with brain tissue (100). Some of these studies indicate that NP-SLE patients, particularly those with diffuse manifestations, have these antibodies more commonly than SLE patients without CNS dysfunction and in close temporal relationship with clinical events (100 ,101).

A variety of epitope specificities have been demonstrated as the targets for some of these antineuronal antibodies. Several studies have demonstrated neural tissue-specific autoantibodies including those directed against a 50-kD antigen in the plasma membrane of brain synaptic terminals (102), neurofilaments (103), glial fibrillary acidic protein (104), and microtubule-associated protein 2 (105). Interestingly, antineurofilament antibodies have been associated more closely with diffuse NP-SLE manifestations (103), while the others have been associated with various neuropsychiatric manifestations.

Other antineuronal antibodies are cross-reactive with nonneural tissue antigens. Early studies showed some of these antibodies are directed against gangliosides, which are a family of acid glycolipids located on many tissues including neuronal and myelin membranes (106). Additionally, lymphocytotoxic antibodies that are cross-reactive with brain tissue have been demonstrated in patients with seizures and diffuse encephalopathy (107). Antiribosomal P antibodies have been shown to react with an homologous 38-kd protein on the surface of human neuroblastoma cells (108). Antiphospholipid antibodies have been shown to permeabilize and depolarize brain synaptoneurosomes (109). This suggests antiphospholipid antibodies have the ability to disrupt neuronal function by direct action on nerve terminals, possibly explaining the nonthrombotic CNS manifestations observed in some lupus patients with antiphospholipid antibodies. Furthermore, anti-β_2 glycoprotein-1 antibodies have been reported to bind to astrocytes and neurons, both in culture and histologic sections, in 11 of 20 SLE patients with these antibodies (110). Recently, DeGiorgio et al. demonstrated that a subset of anti-double-stranded (ds)DNA antibodies cross-react with a pentapeptide consensus sequence that is present in the extracellular, ligand binding domain of the N-methyl-D-aspartate (NMDA) receptor, NR2, in the CNS (111). The NR2 receptors bind the principle excitatory amino acid of the brain, glutamate. Interestingly, the NR2 glutamate receptors are located throughout the forebrain and are in highest concentration in the hippocampus, which is responsible for learning and memory. These cross-reactive antibodies have been demonstrated to cause apoptotic death of neurons by leaking from the serum into the CSF through a breakdown in the BBB. This caused memory impairment in the animal model (112). Studies in human SLE are ongoing.

Serum antineuronal antibodies may be useful markers for NP-SLE, but can only be considered pathogenic if they are demonstrated in the CSF and have the ability to bind directly to brain tissue. Investigators have found CSF IgG antineuronal antibodies in up to 90% of NP-SLE patients compared with less than 10% of SLE patients without CNS manifestations (26 ,31 ,32 ,39 ,114). Furthermore, the antineuronal antibody activity was concentrated to a greater extent in the CSF of these patients relative to a paired serum sample. Similar to serum antineuronal antibodies, the presence and amount of these CSF antibodies correlated best with diffuse manifestations, such as acute confusion, psychosis, generalized seizures, and cognitive dysfunction (26 ,31 ,39 ,113).

Several theories have been proposed to explain how these antineuronal antibodies are generated and get into the CSF. As previously mentioned, some of the serum antineuronal antibodies are cross-reactive with both neural and nonneural epitopes. Other serum antineuronal antibodies may have been generated by neuronal antigens released from the brain. Notably, as shown experimentally, the brain does have lymphatic drainage and antigens in the brain may cause a systemic immune response (115). Although normally the BBB would prevent these antineuronal antibodies from coming into direct contact with cortical tissues, this barrier could be breached at sites of brain microinfarction caused by vascular occlusions, allowing influx of antineuronal antibodies from the serum. Additionally, ischemia without infarction and increased serotonin (from platelet thrombi) can facilitate immunoglobulin transport from the serum to cerebrospinal fluid. Finally, immune-mediated injury and/or endothelial activation leading to cytokine production could result in local breakdown of the BBB allowing serum antineuronal antibodies to access to the CNS (116).

An alternative explanation is that antineuronal antibodies do not come from the serum, but are derived from local production within the CNS. Recent studies have demonstrated that activated T cells, B cells, and monocytes/macrophages can get through the BBB (117). Neuronal antigens, which are normally in an immunologically privileged site protected by the BBB, may stimulate these immunocompetent cells within the CNS. Interleukin-6, which has been found to be elevated in the CSF of patients

with NP-SLE, may facilitate the differentiation of B cells into plasma cells capable of intrathecal synthesis of antineuronal antibodies. The demonstration of an elevated IgG index, oligoclonal bands, a normal Q-albumin ratio (suggesting an intact BBB), and antineuronal antibodies in the CSF in some NP-SLE patients, who lack serum antineuronal antibodies, supports this hypothesis (22 ,26 ,39 ,118 ,119 ,120 ,121 ,122).

Antineuronal antibodies are hypothesized to be able to bind to neuronal membranes and interfere with cell function without causing nerve cell death or inflammation. Binding to neuronal ion channels could lead to recurrent depolarization, increasing susceptibility of the neuron to excitatory amino acid or oxidative, stress-mediated nerve cell injury. Alternatively, antineuronal antibodies could interfere with receptor ligand binding, resulting in neuronal dysfunction. Other immunopathogenic mechanisms are also possible. Each of these mechanisms would explain the reversibility of symptoms and the poor correlation of CNS pathologic changes with clinical manifestations seen in NP-SLE patients with antineuronal antibodies.

Choroid Plexus Dysfunction

The choroid plexus differs from the BBB in that it has a fenestrated capillary bed and glial cells, which have receptors for immune complexes (123 ,124). The choroid plexus is important in the production of CSF and provides a transport-mediated pathway for the influx of certain hormones, vitamins, and other molecules into the CSF. Postmortem examination has demonstrated IgG and complement deposits in the choroid plexus of some SLE patients (125 ,126). This deposition could alter the secretory or transport properties of the choroid plexus or result in cytokine release into the CNS, resulting in neuronal dysfunction. However, the significance of choroidal immune reactant deposition remains unclear, since these deposits are seen in SLE patients with and without neurologic symptoms (126).

Cytokine Effects

Patients with active SLE can have elevated serum levels of interleukin (IL)-2, IL-10, and interferon-α (IFN-α) and IFN-γ (see Chapter 10). Cytokines in the systemic circulation may affect the hypothalamus, which lacks the BBB, and/or the cerebral microvasculature, resulting in upregulation of adhesion molecules (84). These effects, as well as others, can facilitate the recruitment of activated lymphocytes (T cells more than B cells), monocytes, and macrophages to pass through the BBB into the CNS (117). Patients with active NP-SLE can have IL-1, IL-2, IL-6, IL-8, IL-10, TNF-α and IFN-α elevated within their CSF (127 ,128 ,129 ,130 ,131). These cytokines can be produced intrathecally by neurons and glial cells or by infiltrating immunocompetent cells from within the CNS (132). IL-1 is important in neuronal injury and repair, causes release of neurotransmitters from brain structures, and through prostaglandin E causes corticotropin-releasing hormone (CRH) release from the hypothalamus (133). Injection of IL-1 into the CSF of experimental animals causes behavioral disturbances, anorexia, drowsiness, slow wave sleep, and coma with neuronal destruction (134). IL-6 in the CNS can stimulate B cell differentiation into plasma cells with resultant intrathecal synthesis of immunoglobulin. IL-6 is also important in neuronal differentiation, neuronal repair, and hypothalamic CRH release. IL-8 is a chemokine that, after alteration of the permeability of the BBB, attracts B and T cells to the site of the site of inflammation. Thus, the contribution of these and other cytokine effects on the brain in NP-SLE needs to be considered and further investigated.

Neuroendocrine-Immune Systems

There is a complex intercommunication between the nervous, endocrine, and immune systems (135). Most important are the connections between the limbic system (hippocampus, amygdala) of the CNS with the hypothalamic-pituitary-adrenal (HPA) axis and the central noradrenergic and peripheral autonomic nervous systems. The function of these interconnected systems is to restore the body to basal state after being exposed to stress of physical, psychologic, or inflammatory stimuli (136). The degree an individual responds to stress depends on several physical, genetic, and environmental factors, such as age, gender, and reproductive status. Several investigators have postulated that an abnormal stress response partially explains the predilection for SLE to occur in young females in their reproductive years (see Chapter 17).

The systemic immune activation occurring in SLE could result in a chronic stress response, resulting in persistent activation of the HPA axis and noradrenergic systems. Chronically elevated glucocorticoid levels can result in hippocampal atrophy, resulting in abnormalities in spatial and declarative memory (137). Furthermore, excessive cortisol decreases the synaptic uptake of glutamate, resulting in increased glutamate levels, which could cause excitatory amino acid (EAA)–mediated neuronal injury (138). To what extent this plays a role in neuropsychiatric manifestations in SLE patients is presently unclear and is the subject of ongoing research (139).

CNS Tissue Injury

Oxidative stress, EAA toxicity, and matrix metalloproteinase (MMP) injury are the three major mechanisms responsible for nerve cell injury and death within the CNS. Oxidative stress causing nerve cell injury and apoptosis involves the production of nitric oxide (NO). NO is produced by the interaction of the enzyme, nitric oxide synthetase (NOS), and L-arginine (140). There are three major forms of NOS. Two of the isoforms are constitutively expressed (cNOS) (eg., neuronal cNOS and endothelial cNOS). One of the isoforms is inducible (iNOS) and

expressed by many cells, including those that can constitutively express cNOS. Constitutively-expressed nitric oxide synthetase produces small amounts of NO, which plays a protective role in the nervous system and microvasculature. This protection is caused by the capacity of NO to inhibit platelet and neutrophil adhesion to endothelial cells, as well as to inhibit leukocyte superoxide anion production. In contrast to the cNOS isoforms, iNOS is expressed after exposure to various stimuli, including inflammatory cytokines such as IL-1. The inducible form of NOS binds to calmodulin and generates large and sustained amounts of NO. NO is labile and, in the presence of oxygen, is rapidly metabolized to nitrate and nitrite. NO can also react with superoxide anions, resulting in the production of toxic hydroxyl radicals, which leads to nerve cell death. NO also can promote oxidative injury via formation of peroxynitrous acid. Additionally, NO can directly nitrosylate the NMDA receptor, causing inactivation of membrane ion channels and nerve dysfunction (141). Increased expression of iNOS by endothelial cells has been found in patients with active SLE (142). This upregulation of vascular iNOS in SLE patients may be the result of immune complexes, complement components, cytokines, and/or antiphospholipid antibodies. This could result in excessive NO production leading to nerve cell injury as well as other toxicities.

EAA toxicity is a second mechanism for nerve cell injury and death (143). Glutamate is the most important EAA neurotransmitter in the brain and is important in many normal neurologic functions. However, overproduction of glutamate, causing overstimulation of glutamate receptors, can result in excessive influx of positively charged ions and water, resulting in neuronal cell swelling, injury, or death. Some neurons, such as those in the hippocampus, are more vulnerable to glutamate than are other neurons. This is most likely a result of variation in repertoire and density of glutamate receptors in different areas of the brain. Furthermore, stresses such as ischemia, recurrent depolarization (seizures), and cytokines within the CNS can make individual neurons more sensitive to EAA toxicity, even when exposed to only minor insults. The role EAA toxicity has in NP-SLE is unclear and an area of future research.

Another mechanism of nerve cell injury is by MMP, which can degrade extracellular matrix components. MMP-9 is a gelatinase that is secreted by a variety of cells including macrophages, T cells, and endothelial cells. Consequently, MMP-9 could contribute to neuronal and BBB damage. Recently, levels of MMP-9 were found to be elevated in patients with NP-SLE compared to controls (129 ,144). Trysberg et al. further demonstrated elevated levels of free, enzymztically active MMP-9 levels in the CSF of NP-SLE patients, which correlated with elevated intrathecal levels of IL-6 and IL-8, suggesting these cytokines may be involved in the MMP-9 activation pathway (145). This same group has shown elevated levels of soluble molecules indicating neuronal and astrocytic damage such as neurofilaments and astroglial fibrillary acidic protein in the CSF of NP-SLE patients compared to controls. This was particularly apparent in NP-SLE patients with abnormal brain MRIs (144). Interestingly, patients with NP-SLE can make autoantibodies against some of these neuronal and astroglial self-antigens (103 ,104).

Summary

Although our understanding of the pathogenetic mechanisms involved in NP-SLE has advanced over the past three decades, it is still incomplete. Clearly, the cerebral vasculature plays an important role. If the vascular endothelium allows the deposition of immune complexes, then an inflammatory vasculitis can result. Alternatively, simultaneous activation of endothelial cells and neutrophil adhesion molecules by cytokines (IL-1) and biologically active complement split products (C5a desarg) can cause a local Shwartzman phenomenon, resulting in leukothrombosis and vasoocclusive plugs. Additionally, antiphospholipid antibodies are prothrombotic and may result in acute vasoocclusive thromboemboli or chronically cause endothelial cell proliferation and intimal fibrosis, commonly observed in the bland vasculopathy seen in NP-SLE patients. Emboli from cardiac valves can also occur. Each of these mechanisms can lead to vascular occlusion (less commonly hemorrhage) leading to focal neurologic manifestations and seizures.

Antineuronal antibodies with specificity against lymphocytic, neuronal membrane, and neuronal intracellular antigens have been demonstrated to occur with increased frequency in the serum of NP-SLE patients. Some of these may breach the BBB and gain entrance into the CSF. Alternatively, activated lymphocytes within the CNS may make antineuronal antibodies, which can bind to neuronal cells or receptors, resulting in neuronal dysfunction without nerve cell death or inflammation. These antineuronal antibodies could lead to diffuse manifestations such as acute confusion, coma, and seizures.

Cytokine effects and abnormalities in the neuroendocrine- immune system undoubtedly play a role in some of the neuropsychiatric manifestations observed in NP-SLE patients. Cytokines such as IL-1 can upregulate endothelial adhesion molecules, contributing to the development of vasculitis or leukothrombi. Cytokines within the CSF have been shown to cause cognitive and behavioral disturbances commonly observed in SLE patients. Additionally, cytokines such as IL-1 can upregulate iNOS, resulting in excessive NO production and nerve cell injury. IL-8 may facilitate the influx of mononuclear cells into the CNS, whereas IL-6 facilitates the growth of activated B cells into autoantibody producing plasma cells. Intrathecal cytokines may also contribute to activation of MMP-9, which can injure the BBB (allowing influx of cells/autoantibodies) as well as neurons and astrocytes causing neuropsychiatric manifestations. Additionally, inflammatory cytokines within the CNS

can sensitize neurons to be more prone to injury from EAA toxicity.

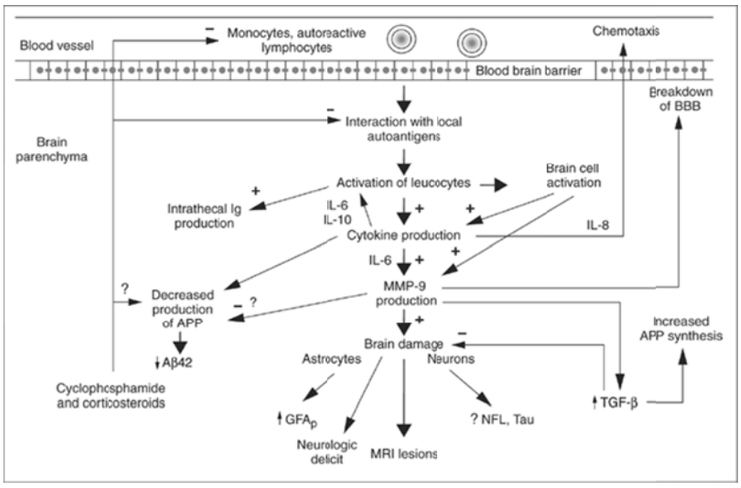

Figure 37-1. Hypothetical mechanisms of inflammation and tissue destruction in neuropsychiatric systemic lupus erythematosus. APP, amyloid precursor protein; BBB, blood-brain barrier; GFAp, astroglial fibrillary acidic protein; Ig, immunoglobulin; IL, interleukin; MMP, matrix metalloproteinase; TGF, transforming growth factor; Aß42, ß-amyloid protein. (From

Trysberg E, Tarkowski A. Cerebral inflammation and degeneration in SLE. *Rheumatol* 2004;16:531.

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Finally, the chronic immune stimulation may stress normal homeostasis, resulting in abnormalities in the neuroendocrine-immune system. Persistent activation of the HPA axis may result in chronically elevated glucocorticoid levels, leading to hippocampal atrophy. Likewise, chronic activation of the sympathetic and autonomic nervous systems may cause vasoconstriction and alteration in cerebral blood flow. Both of these mechanisms could lead to the mild cognitive dysfunction, memory problems, and abnormalities in neuro-imaging observed in many SLE patients.

In any single patient with NP-SLE it is likely that a combination of these mechanisms are contributing to their clinical manifestations (Figure 37-1). Some investigators have attempted to relate certain neuropsychiatric manifestations with specific pathophysiologic mecahanisms (146).

Clinical Manifestations

NP-SLE can involve the CNS, the peripheral/autonomic nervous system, and/or myoneural junction. An SLE patient can present with diffuse, focal, or a combination of symptoms (Table 37-1) The chief manifestations are symptoms of global dysfunction without any focal abnormalities, whereas focal symptoms are manifestations that can be attributed to a specific brain area. Clinical signs and symptoms range from mild and transient dysfunction to severe presentations, resulting in permanent neurologic sequelae and/or death. This diversity of manifestations and severity results from the several different immunopathogenic mechanisms that can affect various areas of an anatomically and physiologically complex nervous system. The clinician must always be aware that neurologic abnormalities in SLE patients may not be NP-SLE, but secondary to infection, electrolyte abnormalities, or numerous other causes (Table 37-4) (147 ,148).

Many of the cognitive, level of consciousness, behavioral, and personality abnormalities observed in SLE patients are difficult to classify. In the past, these have been termed acute or chronic organic brain syndrome, which has now been abandoned. This has been replaced by the term encephalopathy, which is diffuse cerebral dysfunction associated with a disturbance in consciousness, cognition, mood, affect, and behavior. The ad hoc ACR committee on neuropsychiatric lupus nomenclature felt that encephalopathy was too generalized and decided to separate it into its more specific presentations (10). This committee's

recommended classification has been used for the discussion of clinical manifestations.

Acute Confusional State

Acute confusional state is defined as disturbance of consciousness or level of arousal characterized by reduced ability to focus, maintain, or shift attention to external stimuli, and accompanied by disturbances of cognition, mood, affect, and/or behavior (10). This has been termed delirium in the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM IV)* and *International Classification of Diseases, 9th Edition (ICD-9)* diagnostic classifications. Disorganized thinking, loss of orientation, agitation, and delusions can be present. Symptoms may fluctuate or progress. An ominous sign is progression to a reduced level of consciousness, such as stupor or coma. Acute confusional state is one of the most common presentations observed in up to 30% of NP-SLE patients (26). Vasculitis, leukothrombosis, and autoantibodies have all been described as causes of acute confusion. Notably, this is also a common presentation of SLE patients with neuropsychiatric disturbances caused by cerebral infections, medications, TTP, and metabolic disturbances, which must always be excluded.

Cognitive Dysfunction

Cognitive dysfunction can range from mild cognitive impairment to dementia, where there are abnormalities on neuropsychological testing in multiple domains of attention, reasoning, memory, language, visual-spatial processing, psychomotor speed, and executive function (10). The recommended ACR neuropsychological battery of tests has recently been validated (149). Mild cognitive dysfunction (“lupus fog”) was first noted in SLE patients without a history of NP-SLE in the 1970s. The Denburgs, as well as others, have extended this observation. A recent review of 14 cross-sectional studies of cognitive function in SLE patients without overt neuropsychological symptoms revealed subclinical cognitive impairment in 11–54% (150). This dysfunction includes various deficits, as there is no specific SLE pattern of abnormalities (151). In the majority of patients, these abnormalities are subclinical and do not significantly impact their quality of life. In a 5-year prospective study, Hanly et al. reported that only 20% of patients, who had mild cognitive impairment on neuropsychological tests, went on to develop clinically overt NP-SLE, while 19% resolved their cognitive dysfunction on follow-up testing without any therapy (152). Those patients most likely to develop NP-SLE were the ones who showed cognitive decline on serial testing. Other investigators have confirmed that cognitive performance remains stable over time in the majority of patients with mild deficits on testing and does not predict the subsequent development in NP-SLE (153 ,154).

The pathogenesis of cognitive dysfunction in SLE is unclear. Most studies have demonstrated an association between cognitive impairment and active NP-SLE, but have not shown an association with active SLE, corticosteroid use, or psychological distress (150). However, Hay et al. reported that many SLE patients with mild cognitive abnormalities have psychiatric problems, which could cause the cognitive dysfunction (155). An association has been reported by some investigators between cognitive abnormalities and elevated serum anti-DNA antibodies, serum antilymphocytotoxic/antineuronal antibodies, serum antiphospholipid antibodies, and CSF IgG antineuronal antibodies, suggesting this dysfunction is a result of autoantibodies or cytokines (33 ,34 ,35 ,155 ,156 ,157 ,158 ,159). The strongest agreement is the association between cognitive dysfunction, cognitive decline, and antiphospholipid antibodies (150 ,160 ,161). Notably, antiribosomal P antibodies are not associated (162 ,163), and some investigators have not confirmed the association between antilymphocytotoxic/antineuronal antibodies and mild cognitive impairment (163). Recently, Omdal et al. have found a significant association between antibodies against the NMDA receptor, NR2, and a decrease in short-time memory and learning (164).

Many SLE patients (up to 88%) with a previous history of NP-SLE have significant cognitive dysfunction on neuropsychological testing (165). Some patients can progress to dementia with global cognitive dysfunction marked by impairment in short and long-term memory and disturbances in judgment, abstract thinking, and other higher cortical functions. The degree of cognitive impairment may be severe, interfering with the patient's ability to live independently. Dementia can be a result of active NP-SLE, the result of scarring from previously active NP-SLE, or from multiple infarctions because of antiphospholipid antibodies (160 ,161).

Most studies of cognitive impairment have used adult SLE patients as study subjects. However, Papero et al. (166) have reported cognitive impairment associated with antineuronal antibodies in children with SLE. Wycokoff et al. reported a study of 8 children with SLE demonstrated mean intellectual scores were in the low-to-average range and visual memory was depressed. Academic achievement was globally depressed and reading comprehension averaged 5 years below grade placement (167). Recently, Sibbitt et al. reported that isolated cognitive dysfunction was present in only 6% of pediatric outpatient SLE patients who did not have other associated NP-SLE syndromes (168).

Psychosis

Psychosis is defined as a severe disturbance in the perception of reality, characterized by delusions and/or hallucinations (usually auditory). Psychosis occurs in 8% of SLE patients with the initial episode occurring within the first year after diagnosis of SLE in the majority (60%). The sudden onset of psychosis in an SLE patient without a prior

psychiatric history or precipitating cause is usually indicative of NP-SLE. Some investigators have reported an association between antiribosomal P antibodies and psychosis (169 ,170). Titers of these antibodies reportedly rise with exacerbation of psychosis and decrease in response to corticosteroid therapy. Other studies have not found a correlation between these antibodies and psychosis (37 ,171 ,172) (see Chapter 38).

Mood and Anxiety Disorders

Severe affective disorders such as major depression and anxiety/panic disorders can be NP-SLE manifestations. Some previous studies have included these manifestations under the category of “lupus psychosis.” Whether these psychiatric problems are a direct manifestation of NP-SLE, or a reaction to a chronic illness or other psychosocial factors, is unclear. However, antiribosomal P antibodies, cytokine effects, and alterations of the HPA and other neuroendocrine axes have each been associated with severe depression (136 ,170), suggesting that mood disorders can be a manifestation of NP-SLE.

Previous studies have reported a high prevalence of nonpsychotic psychopathology in SLE patients (173). However, a recent review noted that previous studies were too methodologically limited to permit drawing confident conclusions about the prevalence and etiology of psychiatric abnormalities in SLE patients (174). An attempt was made to overcome some of these limitations in a population-based study reporting the prevalence of lifetime psychiatric disorders among all SLE patients in Iceland (175). Approximately 50% of SLE patients received one or more psychiatric diagnoses over the course of their disease. This was in agreement with a 46.5% prevalence of nonpsychotic, psychiatric disturbances in a cross-sectional study of 42 SLE patients, compared with 15.6% of healthy controls (176). Another study of 80 consecutively treated SLE patients found that psychiatric dysfunction was more closely associated with psychosocial factors than with measures of disease activity (177). In a study of 52 SLE patients without NP-SLE, Kozora et al. found greater psychological distress and depressive symptoms related to poor coping styles compared to rheumatoid arthritis patients or normal controls (178). These studies found depression, anxiety, phobias, and difficulty coping to be the most common psychiatric abnormalities. Determining if these abnormalities are a result of NP-SLE or emotional stress can be a challenge to the clinician. However, clearly, patients with good disease-coping strategies and better social support have significantly less depression and a better functional outcome, when followed over time (179) (see Chapter 38).

Headache

Headaches are common in SLE patients, occurring in up to 57% of patients (45 ,180 ,181). Because of the high prevalence of headache in the general population (40%), the association between headache and SLE is controversial (182). In 1978, Atkinson and Appenzeller described a headache syndrome that was distinct for SLE and independent of hypertension and other secondary causes of headache (183). Brandt and Lessel studied, in detail, 11 SLE patients that had “migrainous phenomenon.” These patients' headaches were associated with disease activity, abnormal CSF findings, abnormal electroencephelograms (EEGs), and usually responded to corticosteroid treatment (184). Most investigators believe that headache, as a manifestation of NP-SLE, is characterized by an acute presentation during a lupus flare, frequent association with other neurologic complications and abnormal laboratory tests, and resolves with corticosteroid therapy, as the lupus disease activity improves.

Many SLE patients have headaches, which are not related to the disease activity or other manifestations of lupus. Earlier studies suggested that SLE patients had an increased prevalence of headaches, but did not use standardized criteria for definition of headache. A recent pooled analysis of all studies using International Headache Society criteria for diagnosis found that the prevalence of migraine and tension headaches was not different from healthy controls (182). In this study, migraine headaches occur in 31.7% and tension headaches in 23.5% of SLE patients. Studies suggest that migraine headache in the general population has a strong genetic component and is a result of an abnormality of the trigeminovascular system (185). Previous studies have suggested that migraine headache in SLE patients was associated with Raynaud's phenomenon, antiphospholipid antibodies, and/or thrombotic events (186 ,187 ,188). However, three controlled studies of over 275 patients have failed to confirm these observations (180 ,189 ,190).

Benign intracranial hypertension (pseudotumor cerebri) can occasionally occur in NP-SLE patients. Patients present with refractory headaches, papilledema, and no focal neurologic symptoms. Lumbar puncture reveals increased intracranial pressure (greater than 200 mm H_2O), normal protein, and no white blood cells in the CSF. There have been less than 30 reported cases (191). Although it can occur in adults, most patients are young, adolescent females with severe SLE. Several patients had rapid corticosteroid withdrawal and half had dural venous sinus thrombosis as a potential cause of their pseudotumor cerebri. Up to 60% had clinical or laboratory evidence of hypercoagulability manifested by history of thromboembolic episodes, nephrotic syndrome, or elevated antiphospholipid antibodies (191). Therapy included multiple lumbar punctures and/or corticosteroids in all patients. Some patients also received acetazolamide, anticoagulants (two patients), or intravenous gammaglobulin (one patient).

Secondary causes must be ruled out in all patients before ascribing a severe headache to NP-SLE. The most common or important ones include: hypertension, infection, nonsteroidal anti-inflammatory medications, antimalarial

therapy, sleep apnea, subdural hematoma, and intracranial hemorrhage.

Cerebrovascular Disease

Cerebrovascular accidents occur in 5% to 18% of SLE patients (38 ,40 ,192 ,193). Stroke syndromes secondary to NP-SLE can affect any area of the brain (194 ,195). Patients can present acutely with transient ischemic attacks, hemiplegia, aphasia, cortical blindness (196), or other deficits of cerebral function. Strokes usually occur within the first 5 years of the onset of SLE; and between 13% and 64% of patients who have had a stroke will have a recurrent stroke resulting in significant morbidity and a 28% to 40% mortality rate (40 ,197).

The etiology of strokes in SLE can be from vasculitis, a noninflammatory vasculopathy, thrombosis associated with a coagulopathy, leukothrombosis, emboli from cardiac valvular lesions (198 ,199), and intraparenchymal or subarachnoid hemorrhage (see Pathogenesis). Strokes can be from large or small vessel disease. Large vessel strokes because of SLE can be from vasculitis, thrombosis from a coagulopathy, and cardiogenic emboli. Small vessel strokes and TIAs can be from vasculitis, noninflammatory vasculopathy, leukothrombosis, emboli, and antiphospholipid antibody-associated thrombosis.Patients with stroke because of antiphospholipid antibodies frequently have evidence of livedo reticularis, which has been called Sneddon's syndrome (81). Hemorrhagic strokes from intraparenchymal or subarachnoid bleeding also can occur. In any SLE patient with stroke, hypertension and accelerated atherosclerosis must also be considered. There is abundant evidence that accelerated atherosclerosis occurring in SLE patients can lead to early cerebrovascular disease (200).

Several investigators have identified risk factors for strokes in SLE patients. Futrell et al. reported that 94% of their 18 SLE patients with strokes had at least one of the following risk factors: age older than 60 years, previous stroke or TIA, antiphospholipid antibodies, or cardiac valvular disease (40). The five SLE patients with both cardiac valvular disease and coagulopathy had a 100% risk of stroke, compared to the 77 SLE patients who did not have these risk factors and did not subsequently develop a stroke. Levine and Welch reported that over 50% of patients with antiphospholipid antibodies and strokes had hypertension, hyperlipidemia, cigarette smoking, or diabetes mellitus, each of which can contribute to increased stroke risk (201). Each of these factors (particularly smoking) has been confirmed in recent studies (202 ,203). Clinical experience suggests that the use of the specific cyclooxygenase-2 (COX-2) inhibitors in SLE patients with these risk factors may contribute to the risk of subsequent clotting especially in patients with antiphospholipid antibodies. Each of these studies point out that control of hypertension, elevated cholesterol, and blood glucose levels, as well as smoking cessation, must be part of the treatment plan to prevent stroke or recurrence of stroke.

The diagnosis of cerebrovascular disease is made clinically and supported by neuro-imaging studies. A computed tomography (CT) scan of the brain is capable of detecting cerebral hemorrhage and large infarcts, making it a useful study in screening SLE patients with acute neurologic deterioration. Cranial magnetic resonance imaging (MRI) with contrast is superior to CT scan in detecting smaller and frequently transient lesions. MR imaging typically shows hyperintense gray and white matter lesions on T2-weighted images, which account for the patient's clinical symptoms. Additional lesions in clinically silent areas are also frequently observed. Magnetic resonance angiography (MRA), carotid Doppler ultrasound, and echocardiogram are noninvasive procedures, which can be useful in detecting large vessel vasculitis, thrombosis, or sources of emboli, leading to vascular occlusion and stroke. Angiograms are more likely to show abnormalities in patients with larger infarcts. CSF examination may show pleocytosis and high protein in patients with cerebral vasculitis or blood in patients with subarachnoid hemorrhage. Otherwise, the CSF examination is usually normal or demonstrates nonspecific abnormalities, such as a few cells and/or high protein.

Treatment of strokes in SLE patients is based on the suspected pathogenesis. Patients with suspected vasculitis are treated with corticosteroids and cytotoxic drugs, whereas those with a coagulopathy or cardiac emboli are treated with anticoagulation. Treatment of patients with strokes because of a noninflammatory vasculopathy is difficult since the pathogenesis of these vascular lesions is unclear. Although not proven to reduce stroke in SLE patients, most clinicians put these patients on aspirin or other platelet inhibitors and aggressively treat stroke risk factors. The value of corticosteroids in these patients is uncertain and could potentially contribute to stroke risk by increasing hypertension, cholesterol, and blood glucose. Patients, however, are often given corticosteroids to control other accompanying lupus manifestations.

Myelopathy

Spinal cord myelopathy is an infrequent, but devastating, manifestation of NP-SLE. Patients present with progressive or sudden weakness or paralysis (paraplegia or quadriplegia), bilateral sensory deficits, and impaired sphincter control. It occurs in less than 1% of patients and can be the initial presentation of SLE (204 ,205). Dubois (11) found lupus myelopathy in 2 of his 520 patients between 1950 and 1963, and Pistiner found myelopathy in 2 of his 464 SLE cohort followed between 1980 and 1989 (23). Alarcon-Segovia et al. in Mexico reported 4 cases of myelopathy among 500 SLE patients (206). Spinal cord myelopathy can also occur in pediatric and neonatal lupus (207).

There have been several reviews of the reported cases of lupus myelopathy (204 ,205 ,208 ,209). These reports agree that most patients (80%) are young females between ages 20 to 40 years old. Of the first 28 patients, myelopathy was the initial manifestation in 3, and 11 had no prior diagnosis

of SLE. Two were quadriplegic, 7 paraparetic, and 19 paraplegic (205). Weakness, discrete sensory level, and impaired sphincter control were abnormal in all patients, whereas hyperreflexia was seen in less than 25% at initial presentation. These reviews report CSF abnormalities in the majority of patients, including elevated protein (greater than 80%), pleocytosis (50% to 70%), and decreased glucose levels less than 30 mg% (50%). The cerebrospinal fluid, however, can be normal and may contain elevated levels of myelin basic protein and/or oligoclonal bands. Of the 9 patients who underwent myelograms, 8 had normal studies. There have been several additional case reports and case series, which have reinforced these clinical findings.

The diagnosis of myelopathy is made clinically. MR imaging of the spinal cord can help confirm the diagnosis and exclude other causes of spinal cord compression, which may benefit from surgery. MR imaging in lupus myelopathy typically shows edema with abnormalities of T2-weighted images, which may be accompanied by spinal cord enlargement in 75% of patients (210 ,211). Any level of the spinal cord can be involved (212). Notably, some patients (30% to 40%) may have a normal MRI, especially if the examination is delayed (greater than 5 days) or the patient has received treatment (211). The differential diagnosis includes: compressive myelopathy (tumor, abscess, hematoma (213)), epidural lipomatosis (214), vertebral compression fracture (215), anterior spinal artery syndrome (216), infection (herpes zoster, tuberculosis, polyoma JC virus) (217 ,218 ,219), and Guillain-Barré syndrome (220). The etiology of lupus myelopathy is multifactorial. Small series have reported neuropathologic changes to include: arteritis (Fig. 37-2), perivascular lymphocytic infiltrates and myelitis, thrombosis of small and big arteries and veins, ischemic necrosis of the cord, microhemorrhages, spinal cord subdural hematomas, and myelomalacia of the cord (221). Although vasculitis during an acute exacerbation of SLE has been reported, many patients do not have active SLE at the time of their presentation. Lavalle and others have reported that SLE patients with myelopathy frequently have antiphospholipid antibodies (76 ,206 ,222) while other investigators have not (209). Whether or not the recently described antineuromyelitis optica antibodies plays any role in the development of lupus myelopathy is unknown (223).

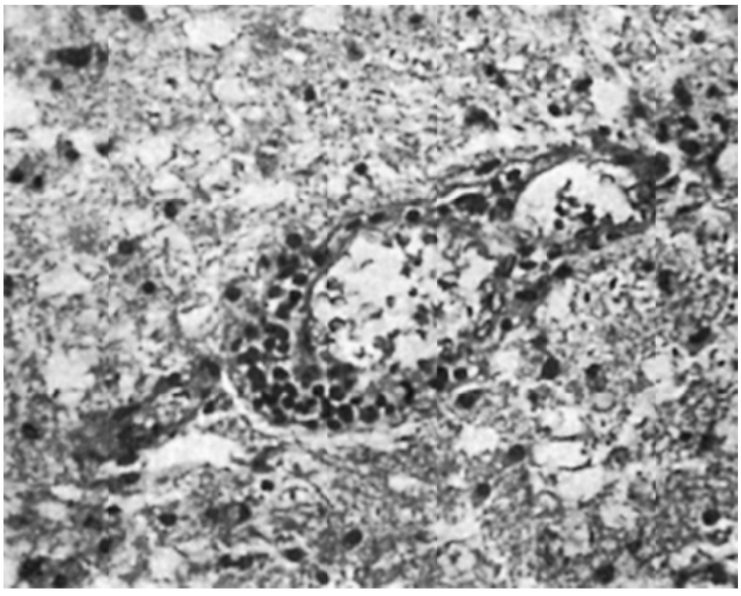

Figure 37-2. Acute arteritis and edema of surrounding tissue in the spinal cord of a 22-year-old patient with a paraplegia caused by lupus vasculitis (H&E; 750).

In their 1989 review, Propper and Bucknall emphasized the poor prognosis of the reported cases of lupus myelopathy (206). Of 26 patients, 7 had a full recovery, 9 a static or slowly-deteriorating course, and 10 died. Of 8 patients who promptly received high-dose corticosteroids, 5 recovered, compared with only 2 of 18 who did not receive steroids. More recent reports have emphasized that the use of pulsed methylprednisolone and cyclophosphamide may improve the prognosis of patients with lupus myelopathy (204 ,222 ,224 ,225 ,226). This therapy must be used early, since 50% of patients will reach their peak severity of myelopathy symptoms within 3 to 5 days of onset. Early use of aggressive therapy has resulted in reversal of symptoms and stabilization in the majority of patients with 50% having a complete recovery and 29% a partial recovery (205). In patients with significant titers of antiphospholipid antibodies, anticoagulation should probably be used, although studies are limited (204). Recurrences of myelopathy, particularly within the first year, are common. Rehabilitation measures to prevent pressure sores; preserve range of motion, strength, and mobility; and institute appropriate bladder management should also be initiated early.

Movement Disorders

Chorea, hemiballismus, cerebellar ataxia, and Parkinsonian- like rigidity/tremor are rare manifestations. Chorea is the most common, occurring in 1% to 4% of patients with SLE. Chorea is characterized by rapid, brief, involuntary, and irregular movements. It may be generalized or limited to the extremities, trunk, or face. Choreoathetosis is diagnosed when chorea is accompanied by slow, writhing movements of the affected extremity. Chorea occurs most commonly in young females, children, and during pregnancy (chorea gravidarum) or the postpartum period (227 ,228 ,229 ,230 ,231). It may be the initial presentation of SLE or precede other manifestations of SLE by as long as 7 years (228). Chorea usually occurs early in the course of SLE, tends to be bilateral, rarely is recurrent, and frequently is associated with other NP-SLE symptoms such as strokes. The cerebrospinal fluid examination is frequently unremarkable (227). The symptoms of chorea usually last for several weeks, but rarely can last for up to 3 years (229).

Infarction of the subthalamic nucleus can result in hemiballismus (232). It rarely has been reported in SLE. Ballismus may be steroid responsive (233) or related to antiphospholipid antibodies (234). Cerebellar ataxia is reported in less than 1% of patients with SLE (235 ,236).

Patients have an inability to stop or end purposeful movements. The abnormalities may involve the trunk or extremities. The etiology is uncertain, but some cases may be a result of cerebellar/brainstem infarction (195 ,237), antiphospholipid antibodies, or associated with Purkinje cell antibodies (238). In patients with cerebellar atrophy associated with antibodies against Purkinje cells, a paraneoplastic syndrome must be ruled out before attributing it to NP-SLE (239).

Tremor of all types has been reported in up to 5% of SLE patients during the course of their disease (240). However, Parkinsonian-like symptoms as a result of alterations of the substantia nigra are an extremely rare manifestation of NP-SLE. Miyoshi et al. (241) presented a case with a literature review of three previous reports. Recently, two adolescent females with severe, extrapyramidal Parkinsonism complicating SLE were reported (242). Patients presented with behavioral alterations (irritability or apathy), rigidity and progressive bradykinesia, and/or akinetic mutism. The EEG showed mild abnormalities, but single-photon emission computed tomography (SPECT) cerebral scanning detected decreased regional cerebral blood flow at the basal ganglia. Treatment with dopamine-agonist drugs led to complete recovery within 3 months along with normalization of the EEG and SPECT scans.

The etiology of chorea is unknown. Autopsy studies have not shown vasculitis (243). Multiple infarction of the basal ganglia (caudate, putamen, globus pallidus, or subthalamic nucleus) has been reported, which may lead to defective control of the thalamus, leading to abnormal movements. Recently, chorea has been associated with antiphospholipid antibodies, which can cause brain infarctions. In 1986, Hadron et al. reanalyzed the previously reported cases of chorea and determined that at least 20 of the patients had spontaneous abortions, false-positive serologic test results for syphilis, or evidence for the lupus anticoagulant (230). Asherson et al. observed chorea in 12 of 500 SLE patients (231). Of these 12 patients, 9 had antiphospholipid antibodies and 7 also had TIAs or cerebral infarcts. Others have confirmed this association (244 ,245); and case reports have claimed an association of chorea with antiphospholipid antibodies, SLE, and oral contraceptive use (246 ,247). Not all patients with chorea have antiphospholipid antibodies or abnormal brain MRIs. Furthermore, the association of chorea with antiphospholipid antibodies does not mean this manifestation is always caused by basal ganglia infarction. Notably, a study of 4 patients with SLE and chorea failed to find basal ganglia hypometabolism, which should be found if the striatum had been infarcted (248). This suggests that, in some patients, other mechanisms such as antineuronal antibodies may be an alternative pathogenetic process. It has been postulated that antiphospholipid antibodies can directly interact with neuronal structures in the basal ganglia.

There is a long differential diagnosis of illnesses rarely associated with chorea. Sydenham's chorea, secondary to rheumatic fever, is the most common and can be ruled out by obtaining antistreptococcal antibodies. However, the onset of chorea in a young woman with a positive ANA should strongly suggest SLE. The recommended treatment of chorea has been corticosteroids and neuroleptic drugs such as haloperidol. Some patients recover spontaneously, whereas others fail to respond to immunosuppressive therapy. Asherson et al. has recommended aspirin or anticoagulation in patients with chorea and antiphospholipid antibodies (231). More studies are needed, however.

Demyelinating Syndrome

A multiple sclerosis-like syndrome, sometimes called lupoid sclerosis, has been described in SLE patients (249 ,250 ,251 ,252 ,253 ,254 ,255). Interestingly, both multiple sclerosis and NP-SLE can share many features including: clinical presentation, Lhermitte's sign (256), positive antinuclear antibody, abnormal CSF with elevated IgG index and oligoclonal bands, and abnormal brain MRIs. Whether both diseases can coexist in one patient, or lupoid sclerosis is just an unusual presentation of NP-SLE, is unclear. Recently, antiphospholipid antibodies have been demonstrated in a number of patients with multiple sclerosis-like illnesses, suggesting these antibodies may be pathogenic in lupoid sclerosis (254 ,255 ,256 ,257).

Patients with definite multiple sclerosis have an acute, relapsing, demyelinating encephalomyelitis with discrete neurologic events distributed in place and time (258). Patients characteristically develop, at different time points, the following: limb weakness with sensory loss, transverse myelitis, optic neuritis, cranial nerve palsies often leading to diplopia, sphincter dysfunction, and/or brainstem or cerebellar abnormalities. Cognitive dysfunction and mood alterations can also be seen. CSF analysis shows elevated IgG index, multiple oligoclonal bands, and myelin basic protein (259). Brain MR imaging (260) shows discreet, low-density lesions often in the periventricular area as well as other areas of the white matter throughout the CNS. Lesions in the corpus callosum are particularly characteristic, since this is an area of relative avascularity, suggesting a demyelinating lesion instead of a vascular etiology. A previous retrospective study reported that 27% of 150 patients with multiple sclerosis had a positive ANA (261). However, a more recent, prospective study found that only 1 of 48 multiple sclerosis patients had a positive ANA (262). Multiple sclerosis patients with positive antinuclear antibodies should not have autoantibodies against specific antigens such as SS-A, SS-B, Smith, ribonuclear protein, or dsDNA, nor should they have hypocomplementemia. Recently, two studies reported that multiple sclerosis patients occasionally have anticardiolipin antibodies (263 ,264). Notably, these MS patients frequently had transverse myelitis, which is a known manifestation of antiphospholipid antibody syndrome. In summary, any multiple sclerosis patient with a positive antinuclear antibody, hypocomplementemia, or antiphospholipid antibodies should be re-evaluated for an alternative diagnosis such as

primary antiphospholipid antibody syndrome, SLE, or Sjögren's syndrome.

Patients with antiphospholipid antibodies can have multiple sclerosis-like presentations (257 ,265). They frequently have transverse myelitis with or without optic neuritis. They may have livedo reticularis of the skin. The MRI of these patients may be indistinguishable from multiple sclerosis (257 ,266), however, the pathophysiology is felt to be different. Patients with antiphospholipid antibodies develop their lesions because of vascular occlusion, whereas multiple sclerosis patients develop demyelinating plaques. Laboratory evaluation shows the patients have the lupus anticoagulant and/or anticardiolipin antibodies (IgG or IgM). When tested, they also have anti-$ß_2$ glycoprotein-1 antibodies, which are felt to be more specific for antiphospholipid antibody-mediated disease. CSF evaluation reveals the majority of these patients have normal IgG indexes, negative oligoclonal bands, and negative myelin basic protein results (267 ,270 ,271). In SLE patients (irrespective of antiphospholipid antibody status) who have oligoclonal bands in their CSF, the average number of bands is less than the number seen in MS patients and may disappear with immunosuppressive therapy, which does not occur in multiple sclerosis (268 ,269 ,270 ,271).

The therapy of patients with lupoid sclerosis differs from multiple sclerosis therapy. Both patient populations may respond to immunosuppressive therapy. However, SLE patients with lupoid sclerosis because of vascular occlusion from antiphospholipid antibodies are best treated with anticoagulation. Therapies used for multiple sclerosis such as ß interferon or Copaxone have not been evaluated in lupoid sclerosis, but are unlikely to be effective.

Seizures

Seizures occur in 10% to 20% of patients with SLE (Table 37-3). They may occur prior to the development of other symptoms of SLE or at any time during its course (51 ,53 ,148 ,272 ,273). Generalized major motor and partial complex seizures are most common, although any kind of seizure can occur. Seizure episodes are usually self-limited, although status epilepticus can occur and frequently signals a preterminal event. Seizures may occur in isolation or accompany other neurologic symptoms.

The etiology of seizures in NP-SLE is multifactorial. Antineuronal antibodies, focal ischemia and infarcts because of vascular occlusion from thrombosis and emboli, hemorrhage, and cytokine or neuroendocrine effects on the seizure threshold have all been implicated. Several studies have shown an association between antiphospholipid antibodies and seizures in SLE patients (53 ,274 ,275 ,276). There is an increased risk of seizures, seizures with strokes, and recurrence of seizures in patients with higher titers of antiphospholipid antibodies (272 ,276). Liou et al. have demonstrated that anticardiolipin antibodies from SLE patients with seizures can inhibit the gamma-aminobutyric acid (GABA) receptor-ion channel complex system, and this may increase cellular excitability (277). Chapman et al. reported that antiphospholipid antibodies may bind, permeabilize, and depolarize brain synaptoneurosomes, possibly leading to neuronal dysfunction by a nonthrombotic mechanism (109). However, most seizures in patients with antiphospholipid antibodies are probably a result of cerebral ischemia from cerebral microinfarctions. Secondary causes of seizures include: infections, medication effects, metabolic disturbances, hypoxemia, and hypertension, which must be ruled out in all SLE patients with seizures.

Most patients with seizures because of NP-SLE will respond to anticonvulsant medications. Although some anticonvulsants have been shown to cause a positive antinuclear antibody and rarely clinical SLE, this is not a reason to withhold these medications when they are indicated for patients with established lupus. Seizure control is important since recurrent seizures increase the vulnerability of neurons to additional injury. Consequently, in patients with status epilepticus, recurrent seizures, or other neurologic manifestations, corticosteroids and other immunosuppressive medications may be used. SLE patients with high-titer antiphospholipid antibodies and seizures should also receive anticoagulation, especially if the brain MRI shows areas of microinfarction.

Aseptic Meningitis

SLE patients with aseptic meningitis present with fever, headache, meningeal signs, and CSF pleocytosis with normal CSF glucose and protein less than 100 mg/dL (278 ,279 ,280). The pleocytosis is most commonly less than 200 to 300 cells/mm^3 and predominantly lymphocytes. Rarely, significantly higher cell counts with a neutrophil predominance can occur in severely ill patients. Infectious meningitis of any cause (281), subarachnoid hemorrhage, carcinomatous meningitis, sarcoidosis, and medication effects, such as from nonsteroidal anti-inflammatory drugs (NSAIDs) (ibuprofen, tolmetin, indomethacin, and sulindac) as well as from intravenous gammaglobulin and azathioprine, must be excluded (282). The etiology of aseptic meningitis in NP-SLE is unclear, but patients usually respond to corticosteroid therapy.

Cranial Neuropathies

Cranial neuropathy occurs in 3% to 16% of SLE patients during the course of their disease. It usually occurs during active SLE, can be transient, and usually responds to corticosteroid therapy. Ptosis (283), third and sixth nerve palsies (284 ,285 ,286), internuclear ophthalmoplegia (287), trigeminal neuralgia (288 ,289), and facial nerve palsies are the most common. Optic neuropathy causing blindness (290), tinnitus, vertigo, and sensorineural hearing loss are less common symptoms (291). The etiology of cranial neuropathies include vascular occlusion and focal meningitis. Autopsy studies have demonstrated lesions in the brainstem as well

as the peripheral part of the cranial nerves (56). Some of these neuropathies have been associated with vasculitis and others with thrombosis associated with antiphospholipid antibodies (292). Keane reported a retrospective study describing eye movement abnormalities in 113 hospitalized SLE patients seen by a neuro-ophthalmology service over a 25-year period (293). Of 55 oculomotor abnormalities, 33 involved limitation of eye movements or abnormal eye position at rest. Many of the abnormalities were subtle or transient. Sixteen of these 33 patients had evidence of brainstem infarcts causing cranial nerve dysfunction.

Other unusual presentations or causes of cranial nerve dysfunction in SLE have been reported. These include bilateral facial nerve palsy caused by angioedema (294), painful ophthalmoplegia (295), Brown's syndrome (296 ,297), Miller-Fisher syndrome (298), cavernous sinus thrombosis (299), and visual disturbances because of lymphocytic hypophysitis (300).

Peripheral Polyneuropathies

Peripheral nervous system involvement occurs in 2% to 21% of SLE patients (Table 37-3). Two cross-sectional studies of 30 and 31 patients, respectively, both found a 6.5% prevalence of peripheral sensorimotor neuropathy on extensive neuromuscular testing (301 ,302). The most common presentation is a distal sensorimotor neuropathy (66% of patients) (301 ,302 ,303 ,304 ,305). Less commonly, patients can have mononeuritis multiplex (14 ,306 ,307), acute or chronic polyradiculopathy (308 ,309 ,310), and rarely, a plexopathy (311). Peripheral nervous system involvement can be the initial presentation of SLE (303 ,306) and has been reported to occur in pediatric SLE patients (312). Symptoms can be severe or subtle and overlooked by the clinician.

Patients with distal, symmetric, peripheral polyneuropathy can present with a mild to severe sensory or less commonly sensorimotor fiber involvement. Patients usually complain of numbness and dysesthesias. Neurologic testing shows cutaneous hypesthesia to pinprick, light touch, and temperature stimuli. A recent controlled immunohistologic study of skin biopsies has demonstrated involvement of small, nonmyelinated afferent nerve fibers (313). Patients with significant paresthesias and abnormal nerve conduction tests are treated with glucocorticoids and antiseizure medications/tricyclic antidepressants. Patients with mild symptoms and/or normal electrodiagnostic studies are treated symptomatically with neuroleptics since 67% will not deteriorate on follow-up (314).

Less commonly, there is large, myelinated afferent fiber involvement, which manifests as deficits of vibratory and proprioceptive sense, areflexia, and sensory ataxia (315) with variable motor dysfunction. When motor axons are affected, weakness and muscle atrophy are seen. Electrodiagnostic studies usually show features of a mixed axonal and demyelinating neuropathy. The pathogenesis of the peripheral neuropathy is unclear. Antineuronal antibodies and vasculitis (304 ,305) from deposition of immune complexes have both been implicated (316).

Mononeuritis multiplex presents as multifocal and random dysfunction of individual, noncontiguous nerve trunks. Patients frequently present with development of sensorimotor deficits in the upper or lower extremities (wrist or foot drop) with an asymmetric distribution (Fig. 37-3). Occasionally, it can be widespread and mimic a distal, symmetric polyneuropathy. It typically occurs in the setting of active SLE, often with other neurologic abnormalities (308). Neurodiagnostic studies usually show an axonal pattern with reduction in amplitude of evoked compound action potentials with relative preservation of nerve conduction velocities. The etiology is felt to be a vasculitis of the vasonervorum (316 ,317 ,318), although this can only be demonstrated on sural nerve biopsy in 50% of cases. Aggressive therapy with corticosteroids and intravenous pulse or daily oral cyclophosphamide with or without plasma exchange is recommended (307 ,308 ,318). Recovery of nerve function takes up to 1 year.

There have been few reported cases of SLE patients with an inflammatory polyradiculoneuropathy (220 ,308 ,309 ,310). There are two forms: the acute form resembles Guillain-Barré syndrome and the chronic form resembles chronic, inflammatory, demyelinating polyradiculoneuropathy (CIDP). Patients presenting acutely have an ascending, predominantly areflexic motor paralysis, which peaks in 10 to 14 days. There is little or no sensory loss. There is little loss of cutaneous sensation since small, nonmyelinated fibers are not involved. Involvement of large myelinated afferent fibers leads to loss of proprioception and vibratory sensation.

There can be an associated autonomic dysfunction in some patients. There is no sphincter disturbance, which helps separate it from transverse myelitis. CSF examination reveals an elevated total protein with a white blood cell count less than 50. Electrodiagnostic studies reveal a demyelinating pattern with slowing of nerve conduction velocities, dispersion of evoked compound action potentials, conduction block, and marked prolongation of distal latencies. The pathogenesis is unknown. Unlike Guillain-Barré syndrome without SLE, patients have been successfully treated with corticosteroids. Recovery can occur within weeks if there has been no neuronal damage. There has been limited experience with the use of plasmapheresis (three cases) or intravenous gammaglobulin (no cases) in SLE patients with Guillain-Barré-like symptoms (308). Rarely, a patient presenting with acute polyradiculopathy will have an axonal pattern on electrodiagnostic studies and evidence of vasculitis (319). These patients should be treated with corticosteroids and cyclophosphamide. Recovery will take many months, since there is axonal damage.

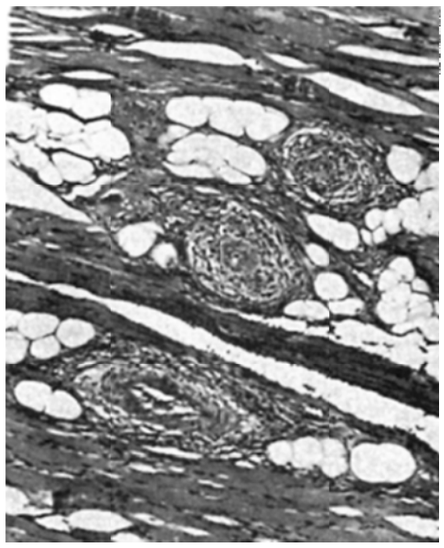

Figure 37-3. Vasculitis in gastrocnemius muscle biopsy of a patient with severe peripheral neuritis of legs and bilateral footdrop (H&E; 150).

SLE patients with chronic, demyelinating polyradiculopathy resembling CIDP can present with recurrent episodes of acute, Guillain-Barré syndrome-like symptoms, a mononeuritis multiplex-like pattern, or as a symmetric polyradiculopathy evolving over weeks to months. Electrodiagnostic studies frequently are confusing, showing a mixed axonal-demyelinating pattern. Nerve biopsy is usually not helpful, but may show inflammation. Therapy includes corticosteroids, plasmapheresis, cyclophosphamide, and intravenous gammaglobulin (310).

There are multiple secondary causes of peripheral nervous system involvement, which must be ruled out before attributing peripheral nerve dysfunction to NP-SLE. Uremia, diabetes mellitus, drug toxicities, vitamin deficiencies, heavy metal/solvent exposure, cancers and paraproteinemias, viral and other infections, sarcoidosis, alcohol and other toxins, hereditary neurologic diseases, and other causes must be considered and evaluated for in the SLE patient.

Autonomic Disorders

The autonomic nervous system is probably more commonly involved in SLE patients than previously reported. Acute autonomic neuropathy with profound dysfunction of the parasympathetic and/or sympathetic nervous system has rarely been reported in SLE (320 ,321). Gastrointestinal (constipation), cardiovascular (orthostatic hypotension), genitourinary (sphincter/sphincteric, erectile/ejaculatory dysfunction), sweating (anhydrosis and heat intolerance), and pupillary abnormalities are evident and, when severe, respond to corticosteroids.

Sensitive tests of autonomic function show that mild dysfunction may be present, although clinically unappreciated. Recently, Straub and colleagues reported that 29% of 31 SLE patients had abnormal pupillometry, reflecting lesions of the pupillary parasympathetic nervous system (302). This may be a result of cytokine effects on the hypothalamic autonomic nervous system (322). Additionally, 9.7% of these 31 patients had abnormal cardiovascular autonomic nervous system findings using a standard battery of tests, age-matched norms, and modern definitions of autonomic dysfunction. This is in agreement with the 2% to 13% prevalence found in two controlled studies of 34 and 23 outpatient SLE patients, respectively (323 ,324). Two recent reports found a 20% to 45% prevalence of cardiovascular autonomic nervous system dysfunction although abnormalities on testing did not correlate with symptoms (325 ,326). Of note, corticosteroids can mask cardiovascular autonomic changes resulting from SLE (327).

Myasthenia Gravis and Related Disorders

Myasthenia gravis and SLE may coexist in the same patient (328). There have been over 50 reported cases. Myasthenia typically precedes the onset of SLE in the majority of these patients. In some cases, SLE develops following thymectomy for the treatment of myasthenia gravis (329). Patients have typical manifestations of myasthenia with neuromuscular fatigue and weakness of bulbar or other voluntary muscles with repetitive muscular contractions. There is no impairment of sensation or loss of reflexes. Antibodies to the acetylcholine receptor can be demonstrated in 85% of myasthenia patients and are felt to cause neuromuscular symptoms by reducing the number of acetylcholine receptors at the neuromuscular junction. Diagnosis is made clinically and confirmed with electromyography and repetitive peripheral nerve stimulation at a rate of two per second showing a characteristic decremental response, which is reversed by the acetylcholinesterase drugs, edrophonium or neostigmine (see Chapter 40).

There have been a few SLE patients reported with Lambert-Eaton myasthenic syndrome (LEMS) (330). Patients present with weakness and hyporeflexia, which improves with exercise. Neurodiagnostic studies show a myopathic electromyogram (EMG) with low-amplitude compound muscle action potential, which increases in amplitude after exercise. High-frequency, repetitive stimulation demonstrates a 50% or more increment in the amplitude of the compound motor action potential. There is no improvement of clinical or EMG findings with anticholinesterase drugs. The etiopathogenesis is suspected to be an IgG antibody against the voltage-gated calcium channels in the presynaptic neuromuscular junction. Plasmapheresis and immunosuppressive medications are effective therapy. There has been one case report of an SLE patient with neuromyotonia (331). This patient presented with persistent myokymia at rest, both clinically and on EMG. The suspected pathogenesis is an IgG antibody against the voltage-gated potassium channels in the presynaptic neuromuscular junction.

Other Neuropsychiatric Lupus Syndromes

There have been other NP-SLE syndromes reported. Schnider et al. reported a patient who developed the acute onset of severe amnesia. MRI of the brain showed isolated hippocampal damage (332). It is unknown if antibodies against the NMDA receptor contributed to the atrophy. A case of limbic encephalopathy because of NP-SLE was reported in a young woman with SLE (333). The patient had fever, headache, encephalopathy, generalized seizures, and antiribosomal P antibodies in her CSF. All tests for herpes simplex and paraneoplastic syndromes were negative. A case of the syndrome of inappropriate antidiuretic hormone (SIADH) was reported as the initial manifestation of SLE in an elderly woman with antiribosomal P antibodies (334). Other cases of NP-SLE with SIADH have been reported (335). Finally, there have been several reported cases of reversible posterior leukoencephalopathy (336 ,337). These patients present with the sudden onset of neurologic symptoms that mimic other neurologic conditions. Imaging shows reversible lesions in the posterior cortex on diffusion-weighted MRI as a result of vasogenic edema. This manifestation has been associated with severe hypertension, uremia, thrombotic thrombocytopenia purpura (TTP), and use of cytotoxic medications. Full recovery usually occurs with control of the precipitating risk factors.

NP-SLE in Children and the Elderly

Several groups have examined NP-SLE in children. Yancey et al. (338) found a 43% incidence in 37 children, and their literature review of 11 pediatric studies found a 33% incidence in 353 children (see Chapter 43). A recent 6-year prospective study of 75 pediatric SLE patients found that 95% had evidence of NP-SLE at some time using the ACR NP-SLE nomenclature (168). If only serious manifestations were considered then the prevalence of NP-SLE fell to 76%. Not surprisingly, NP-SLE was present in twice as many hospitalized patients compared to SLE outpatients. Adolescent SLE patients with antiphospholipid antibodies, particularly the lupus anticoagulant, are most at risk for strokes and need life-long anticoagulation to prevent recurrences (339 ,340).

CNS involvement in older age groups (i.e., >50 years) is reported to be milder and less frequent with an incidence between 6% and 19% (341 ,342 ,343). In one report, however, 9 of 10 patients who were diagnosed with SLE at an age of older than 50 years had neuropsychiatric manifestations. Of these, 5 had peripheral neuritis, 3 had cerebellar ataxia (344), and nearly all were steroid responsive. Two percent of 254 individuals, who were admitted to a psychogeriatric center in England over a 2-year period, turned out to have SLE (345).

Differential Diagnosis of Secondary Causes of CNS Dysfunction in SLE Patients

Secondary causes of CNS dysfunction in SLE patients must always be ruled out before attributing symptoms to primary NP-SLE (Table 37-4) (42 ,43 ,44). Prospective studies point out that 50% to 78% of neurologic events are caused by secondary factors (44 ,54). The most common secondary causes include infections, medications, and metabolic disturbances. Equally important is the clinician must realize that the presence of an antinuclear antibody in a patient with neurologic symptoms does not imply that the patient has NP-SLE or, for that matter, SLE at all (346 ,347). Many other possibilities must be considered in the differential diagnosis.

Infections

SLE patients have an increased susceptibility to bacterial, viral, fungal, and parasitic infections because of disease and medication effects on the immune system (281) (see Chapter 45). The neurologic manifestations of CNS infection can include: confusion, lethargy, headache, neck pain, seizures, psychosis, and fever with or without focal or generalized sensory and motor deficits (42 ,43 ,44). Nearly every organism has been reported to infect patients with SLE (281). Tuberculosis, bacterial endocarditis, herpes simplex encephalitis, bacterial meningitis (Neisserial, *Listeria*, *Nocardia*, *Salmonella*, and syphilitic), and opportunistic central nervous system infections (*Toxoplasma*, *Aspergillus*, amoebic, and Cryptococcal) have all been reported as potentially mistaken for NP-SLE. Human polyoma virus JC has been demonstrated as the cause of progressive multifocal leukoencephalopathy in SLE patients (348).

The most critical test to exclude CNS infection is a lumbar puncture with CSF examination. Although CSF pleocytosis and elevated protein also can be seen in cerebral vasculitis, an associated low glucose, positive Gram stain and/or cultures, or polymerase chain reaction tests for viruses are diagnostic of CNS infections. CT scan with contrast or brain MRI may help to locate an abscess or focal area of involvement. Whenever infection is likely, broad-spectrum antibiotics that cross the BBB should be initiated. After that, corticosteroid doses can be increased to treat possible NP-SLE while cultures are pending or to prevent Addisonian crisis during stress. Unfortunately, unsuspected CNS infections are all too commonly first diagnosed at time of postmortem examination.

Medications

Patients with lupus often take medications that are known to have CNS toxicity. Corticosteroids have been reported to cause psychosis, depression, mania, and delirium (349 ,350 ,351). Notably, mood alteration (depression/mania) is

more common than psychosis. The majority of patients who develop psychiatric disturbances will do so within 2 weeks of starting or increasing the corticosteroid dose. Factors that have predicted psychiatric side effects from corticosteroids include high-dose intravenous methylprednisolone, doses of oral prednisone greater than 40 mg/day, and female gender. Age, previous psychiatric illness, and prior history of steroid psychosis were not predictive of corticosteroid-induced psychiatric side effects (350). Steroid psychosis, however, is extremely uncommon in pediatric SLE patients. Most patients with steroid psychosis improve within days to a few weeks by lowering and dividing the dose of corticosteroids. Antipsychotic medications (chlorpromazine, risperidone), lithium, and electroconvulsive therapy have been used successfully. Tricyclic antidepressants make symptoms worse and should be avoided (352). There is little experience with the use of selective serotonin reuptake inhibitors.

Antimalarial therapy has been associated with a variety of neurologic symptoms, including headaches, irritability, seizures, and neuromyopathy (see Chapter 59). NSAIDs can cause a variety of CNS side effects (353). Ibuprofen and rarely other NSAIDs have caused aseptic meningitis, particularly in SLE patients (282). Acute psychosis with disorientation, paranoia, or hallucinations can occur with indomethacin or sulindac. The indolacetic acid derivatives (indomethacin, tolmetin, and sulindac) can also induce headache. Cognitive dysfunction with memory impairment and depression can occur with indomethacin and less commonly with other NSAIDs, especially in elderly patients. Antihypertensive medications can cause fatigue and depression. Azathioprine can cause aseptic meningitis; intravenous gammaglobulin has also been reported to cause aseptic meningitis (282) and strokes (354). All treating physicians must obtain a careful drug history from their patients. Nearly all of these medications can be withheld for brief periods to determine causation.

Thrombotic Thrombocytopenic Purpura

TTP is characterized by fever, thrombocytopenia, microangiopathic hemolytic anemia, neurologic symptoms, and renal involvement. The thrombocytopenia and hemolytic anemia are nonimmune mediated, which helps separate this from SLE. There have been over 30 cases of TTP and SLE occurring in the same individual (355). In the majority (62%) of cases, SLE preceded the development of TTP, while in 17% the two diseases developed simultaneously. Rarely, TTP preceded the onset of SLE. In 50% of cases, TTP complicated the course of active SLE, whereas in the remaining, it developed in SLE patients with inactive disease.

The etiology of TTP occurring in SLE may be similar to idiopathic TTP. Acute TTP has been found to be a result of an IgG autoantibody against the metalloprotease responsible for cleavage of the monomeric subunits of von Willebrand factor (356). This allows for the accumulation of unusually large multimers of von Willebrand factors secreted by endothelial cells into the plasma. These multimers bind to platelet glycoprotein receptors, causing platelet adhesion and microthrombi. The treatment of acute TTP in SLE patients includes plasmapheresis to remove the autoantibody and large multimers of von Willebrand factor, followed by fresh frozen plasma to replace the metalloprotease. Antiplatelet agents, corticosteroids, and/or immunosuppressive drugs have been used, but are not as effective as plasmapheresis and plasma replacement. However, corticosteroids and immunosuppressives may be needed to prevent recurrence by suppressing autoantibody formation. Precipitating causes of idiopathic TTP include drugs such as ticlopidine and infection. These, however, have rarely been reported in patients with SLE complicated by TTP (355).

Other Causes of CNS Dysfunction

Other secondary causes of CNS dysfunction that must be excluded include: hyponatremia, hypercalcemia, uremia, hypoxia, accelerated hypertension, fever, hypothyroidism, cerebral lymphoma (357), and subdural hematoma (358), as well as others. SLE patients have accelerated atherosclerosis, which can lead to stroke and hypertension, causing intracerebral hemorrhage. Berry aneurysms have been reported in SLE patients and can cause subarachnoid hemorrhage (94 ,95 ,96).

Fibromyalgia is commonly observed in SLE patients. Pistiner et al. reported that 20% of their SLE patients fulfilled established criteria for fibromyalgia (23). A recent review of fibromyalgia in SLE emphasizes these findings (359). Fatigue, myalgias, paresthesias, insomnia, and headaches are frequent in patients with fibromyalgia. Additionally, reactive depression with or without fibromyalgia is also commonly found in SLE patients. Living with SLE can involve lifestyle adjustments and create stress. This can lead to depression, anxiety, and functional symptoms such as sweating, palpitations, diarrhea, and hyperventilation, which are observed with increased frequency in patients with SLE (360). It is important to not confuse these symptoms with active NP-SLE.

Sleep apnea can also occur in SLE patients. Corticosteroid therapy leads to weight gain and weakness of respiratory muscles. This can lead to apnea/hypoventilation during sleep. Patients with sleep apnea complain of fatigue, headaches, and mental clouding, which can be misdiagnosed as NP-SLE. This is a common and underdiagnosed cause of mild CNS symptoms in SLE patients.

Positive ANA Without Lupus

There are patients with nonspecific complaints such as myalgias, arthralgias, fatigue, and headache, who are found by their primary care physician to have a low-titer antinuclear

antibody without other objective or laboratory findings of lupus. These patients may be sent for rheumatologic consultation to confirm a diagnosis of SLE or NP-SLE as a cause of their symptoms (346). Furthermore, there are patients with a drug-induced ANA from procainamide, phenothiazines, or other medications who may have or develop neuropsychiatric symptoms from causes other than SLE or drug-induced SLE (361). Consequently, it is imperative to first confirm a diagnosis of SLE in any patient being sent for consultation to rule out NP-SLE.

Clinical and Laboratory Evaluation

A methodological work-up is essential for the patient with SLE who presents with neuropsychiatric manifestations (26 ,362 ,363). A careful and thorough history and physical examination including a complete neurologic and mental status evaluation must be done on each patient. Additionally, a variety of laboratory, CSF, neurodiagnostic studies, and when appropriate, cultures of bodily fluids are done to assess disease activity and to exclude other diseases, which can cause neurologic symptoms. Earlier studies emphasized that certain clinical signs, such as retinal and dermal vasculitis or livedo reticularis, were more common in NP-SLE patients, particularly those with cerebrovascular disease (17). Furthermore, although NP-SLE can be the initial or sole active manifestation of SLE, many studies have reported that NP-SLE frequently occurs when SLE is clinically and serologically active (17 ,18 ,19 ,21 ,27). However, in all SLE patients with CNS dysfunction, additional tests will be necessary to confirm an NP-SLE diagnosis and exclude other causes (Tables 37.5 ,37.6 ,37.7).There is no single blood test that can diagnose NP-SLE. *After excluding secondary causes, the diagnosis of NP-SLE can only be confirmed if a patient's neuropsychiatric symptoms can be corroborated with objective abnormalities in neuropsychological, spinal fluid, EEG, and/or imaging studies*. Recently, an international, multidisciplinary committee made recommendations of the basic laboratory evaluation and diagnostic imaging which should be obtained on all patients suspected of having NP-SLE (Table 37-5) (10) (Fig. 37-4).

Table 37-5: Laboratory Evaluation and Diagnostic Imaging of SLE Patients with Neuro-Psychiatric Manifestations

Complete blood count/peripheral blood smear
Chemistries: electrolytes, creatinine, glucose
Liver-associated enzymes
Urinalysis
C3/C4 and/or CH_{50}
Anti-dsDNA antibodies
Antiphospholipid antibodies/anti $ß_2$ glycoprotein I
Cerebrospinal fluid: cell count, protein, glucose, IgG index, oligoclonal bands, VDRL
Brain MRI with gadollinium
Electroencephalogram
Other tests when indicated
Serum and CSF antineuronal antibodies
Computerized tomography of brain
Echocardiogram
Magnetic resonance angiogram
Angiogram
Tests for hypercoagulability: protein C, protein S, SAT III, prothrombin mutation, factor V Leiden, homocysteine
Antiribosomal P antibodies
Cryoglobulins
Other tests—investigational
Specific serum and CSF antineuronal antibodies
CSF levels of cytokines, matrix metalloproteinases, or proteins indicating neuronal damage
Single-photon emission tomography
Magnetic resonance spectroscopy
Positron emission tomography

Adapted from ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature. The American College of Rheumatology Nomenclature and Case Definitions for Neuropsychiatric Lupus Syndromes. *Arthritis Rheum* 1999;42:599–608.

Table 37-6: Frequency of Abnormal Laboratory Tests Commonly Used in the Evaluation of Neuropsychiatric Lupus Erythematosus

Test	Frequency of Abnormal Test Result Range (%)	Comment
Serologic		
Antineuronal antibodies	30–92	Diffuse manifestations
Antineurofilament antibodies	58	Diffuse manifestations
Antiribosomal-P antibodies	45–90	Psychosis/depression
Antiphospholipid antibodies	45–80	Focal manifestations, strokes
Cerebrospinal fluid		
Routine		
Pleocytosis	6–34	Rule out infection and NSAID meningitis
Increased protein	22–50	Nonspecific
Low glucose	3–8	Rule out infection, transverse myelitis
Special		
Antineuronal antibodies (IgG)	40–90	Diffuse manifestations, present in 40% with focal manifestations
Elevated O albumin	8–33	Break in blood-brain barrier
Elevated IgG/IgM index	25–66	Diffuse manifestations
Oligoclonal band (2 bands)	20–82	Diffuse manifestations

Adapted from West SG. Neuropsychiatric lupus. *Rheum Dis Clin North Am* 1994;20:129–158.

Table 37-7: Frequency of Abnormal Diagnostic Tests Commonly Used in the Evaluation of Neuropsychiatric Lupus Erythematosus

Test	Frequency of Abnormal Test Results Range (%)	Comment
Electroencephalogram	54–85	No specific abnormality. SLE pts without CNS Sxs can have abnormal EEG (48%).
Neuroimaging Procedures		
Brain scan	8–19	Some studies report higher prevalence of abnormalities.
CT scan	27–71 (atrophy)	Atrophy may be due to corticosteroids.
	10–25 (infarct or hemorrhage)	CT scan will miss 20%–25% of definite clinical infarcts.
MRI scan		
All NPLE pts	77	No specific lesion, atrophy (28%–71%).
NPLE pts, diffuse Sxs only	Less than 50	More likely abnormal if obtain within 48 hrs of treatment
NPLE pts, focal Sxs	Up to 100	
SLE pts, CNS Sxs unrelated to NPLE	31	
SLE pts (<age 45), no hx CNS Sxs	Less than 5–10	
SPECT scan	44–88	Up to 67% of SLE pts with CNS events unrelated to NPLE have abnormal scans.
Angiography	10	More likely abnormal in embolic strokes.
Echocardiography	40	Definite valvular lesions more common in stroke pts. May have association with antiphospholipid antibodies.

CNS, central nervous system; Sxs, symptoms; pts, patients; EEG, electroencephalogram; CT, computed tomography; MRI, magnetic resonance imaging; NPLE, neuropsychiatric lupus erythematosus; SPECT, single-photo-emission computer tomography; hx, history.

Adapted from West SG. Neuropsychiatric lupus. Rheum Dis Clin North Am 1994;20:129–158.

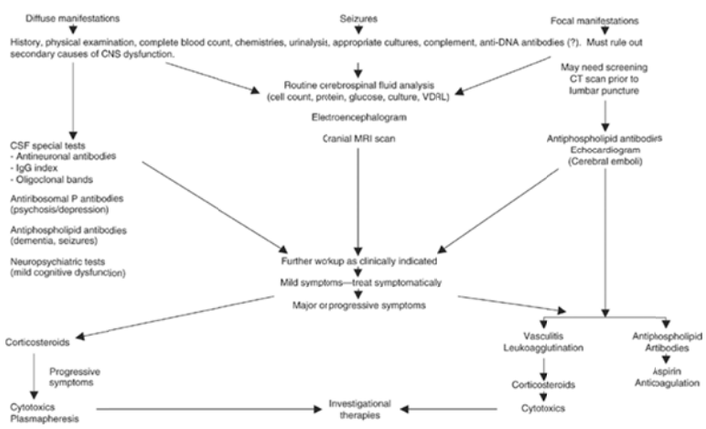

Figure 37-4. Algorithm for the evaluation and treatment of systemic lupus erythematosus patients with neuropsychiatric lupus erythematosus.

Clinical Laboratory Tests

Blood tests include a complete blood count, including platelet count and peripheral smear. The blood smear should be examined for schistocytes and thrombocytopenia to exclude TTP. Chemistries including electrolytes, creatinine, glucose, and liver-associated enzymes are obtained to exclude metabolic abnormalities that can cause neurologic dysfunction. A urinalysis should be obtained for disease activity and to rule out infection. Complement (C3/C4 or CH_{50}) determinations and anti-dsDNA antibodies should be obtained to assess disease activity. Testing for antiphospholipid antibodies includes lupus anticoagulant, anticardiolipin antibodies, and anti-$ß_2$ glycoprotein-1 antibodies. Other tests for hypercoagulable states, including factor V Leiden, protein C and S levels, serum antithrombin III levels, and prothrombin 20210A mutation may be indicated in selected patients. Most patients with SLE will have an elevated erythrocyte sedimentation rate and a normal or mildly elevated C-reactive protein. A significantly elevated C-reactive protein (>6 mg/dL) usually indicates systemic vasculitis or infection. A fasting lipid profile and homocysteine levels are obtained to establish vascular risk factors. Serum antilymphocyte and antineuronal antibodies are considered investigational.

Antiribosomal P Antibodies

Antibodies to the C-terminal region of cytoplasmic ribosomal P protein are found in 12% to 16% of SLE patients and are among the most specific tests for SLE (169 ,170 ,172). Bonfa et al. first observed these antibodies in 18 of 20 SLE patients with psychosis/major depression (169). Several groups have related antiribosomal P antibodies to psychosis and severe depression, but others have failed to confirm this association (171 ,172). Some investigators have found antiribosomal P associated with CNS disease in general, as opposed to psychosis in particular (364 ,365). Serum levels of the antibody may correlate with the severity of the psychosis in selected patients (26 ,39 ,366 ,367), but also vary widely over time without any clinical associations. The antibody usually is not found in the CSF (26 ,39), although it has been in some patients (368). SLE patients with mild depression and/or cognitive dysfunction do not have elevated ribosomal P levels (162 ,163).

Iverson and Reichlin have summarized the available evidence concerning the value of testing for antiribosomal P antibodies (172 ,369). Both noted that studies supported the specificity of antiribosomal P antibodies for SLE, and that these antibodies are not found in patients with neuropsychiatric conditions who do not have SLE. Additionally, several

studies have shown that these antibodies are more prevalent in Oriental patients with SLE than Caucasians or African Americans, regardless of whether these patients had neuropsychiatric manifestations. Combining the studies, Iverson estimated that the sensitivity of antiribosomal P antibodies to neuropsychiatric manifestations was 0.64 to 0.66, the positive predictive value was only 0.29, and the negative predictive value was 0.90. Therefore, he supported the opinion of Teh (37) that, “There is no value in measuring anti-P antibodies, either as a single determinate for identifying patients with lupus psychosis (or depression) or sequentially as a predictor of impending relapse of the lupus psychiatric disorder.” This opinion, however, is not supported by all clinicians who care for patients with NP-SLE (26 ,39). Some feel that the high specificity of this antibody for SLE makes it particularly useful as a diagnostic test in neuropsychiatric cases without a definite diagnosis of SLE (10). Furthermore, in an individual patient who does have NP-SLE and antiribosomal P antibodies, the titer of antibody may become undetectable with successful therapy (39 ,170).

The mechanism of how an antibody against a cytoplasmic antigen can cause CNS dysfunction is unclear. However, ribosomal P antigens have been demonstrated on the surface of endothelial cells (370) and on the surface of human neuroblastoma cells (108). This suggests that these autoantibodies may bind to membrane surface antigens, instead of cytoplasmic ribosomal P proteins. Binding of antiribosomal P to neuronal cells might directly participate in neuronal dysfunction. The binding of antiribosomal P to these surface antigens might explain why antiribosomal P antibodies are not found free in the CSF of patients with NP-SLE, while antiribosomal P antibodies are found in their sera.

Antilymphocyte Antibodies

Antilymphocyte antibodies are not specific for SLE and can occur in other illnesses, including infections, malignancy, inflammatory bowel disease, and multiple sclerosis. A subset of antilymphocyte antibodies, however, cross-react with neurons. Fever, neuropsychiatric symptoms, skin lesions, and hematologic abnormalities are the most common manifestations in patients with SLE and serum lymphocytotoxic antibodies (371 ,372). Denburg et al. has related cognitive dysfunction to the presence of serum IgM lymphocytotoxic antibodies in over 445 patients evaluated (159 ,373). This may reflect the presence of the antineuronal subset of antilymphocyte antibodies. In a prospective study, Temesvari et al. (374) found a correlation between fluctuation in serum lymphocytotoxicity and relapses or remissions of neuropsychiatric involvement in individual patients with SLE (see Chapter 28).

Serum Antineuronal Antibodies

Serum antineuronal antibodies are more common in NP-SLE patients (30% to 92%) than in SLE patients without CNS lupus (5% to 20%) (31 ,32 ,100 ,101 ,102 ,103 ,104 ,105 ,375). They are neither as sensitive nor as specific as CSF antineuronal antibody measurements. Nevertheless, neuroblastoma-binding serum autoantibodies are particularly frequent in NP-SLE patients with diffuse presentations, such as encephalopathy and cognitive dysfunction (32 ,33 ,375). A limited number of studies have investigated the fine specificity of some of these antibodies (376). Several membrane antigens have been identified as targets of these antineuronal antibodies. In neuroblastoma cells, a 97-kilodalton membrane protein of unknown function is the target antigen for autoantibodies from a limited number of NP-SLE patients (377). An immunodominant, C-terminal epitope of ribosomal P antibody has been demonstrated on the surface of human neuroblastoma cells (108 ,378). Hanson et al. have found antibodies against a 50-kilodalton antigen in the plasma membrane of brain synaptic terminals in 19 of 20 NP-SLE patients (102), whereas Denburg and Behmann documented the presence of antilymphocyte/antineuronal antibodies against a 52-kilodalton antigen in patients with NP-SLE (379). Recently, Galeazzi et al. and others have found antiganglioside antibodies (GM1) or antigalactocerebroside antibodies more commonly in SLE patients with NP-SLE (380 ,381 ,382 ,383). Several cytoplasmic antigens including ribosomal P, neurofilaments, and L-fimbrin have been reported as target antigens. Serum antineurofilament antibodies, which react against cytoskeletal neurofilament triplet protein antigens, were found in 58% of patients with diffuse neuropsychiatric manifestations, compared to 20% with focal symptoms and 18% of controls (103). Antibodies to L-fimbrin expressed in cytoplasmic microfilaments of leukocytes have also been found increased in patients with NP-SLE (384). Recently neural tissue-specific autoantibodies against glial fibrillary acidic protein and microtubule-associated protein-2 have been associated with various NP-SLE manifestations (104 ,105). Finally, a subset of anti-dsDNA antibodies have been shown to cross-react with the NR2 subtype of the NMDA glutamate receptor (111) and has been associated with depression and cognitive difficulties (164). The significance of these targets, or how antibodies against them cause disease, is unclear. Notably, each of these autoantibodies is more common in patients with diffuse, as opposed to focal, manifestations of NP-SLE.

Antiphospholipid Antibodies

Antiphospholipid antibodies are a heterogenous group of autoantibodies associated with thromboembolic events. The lupus anticoagulant and anticardiolipin antibodies are the ones best characterized. Lupus anticoagulant prolongs phospholipid-dependent coagulation assays, such as the activated partial thromboplastin time and the kaolin clotting time. Subsequently, more sensitive assays, particularly the dilute Russell viper venom time (dRVVT), have been used to detect and/or confirm the presence of a lupus anticoagulant, even in patients with normal activated partial thromboplastin time (PTT) and clinical features of the

antiphospholipid antibody syndrome. Anticardiolipin and anti-β_2 glycoprotein I (β_2GPI) antibodies are detected by a standard enzyme-linked immunoabsorbent assay. In the meta-analysis by Love and Santora of 29 retrospective studies comprising greater than 1000 patients with SLE, the average prevalence of lupus anticoagulants and anticardiolipin antibodies were 34% and 44%, respectively, with an overall incidence of thrombotic complications of 28% (385). Many studies combine primary antiphospholipid antibody syndrome patients with SLE patients with antiphospholipid antibodies, making interpretation of these studies difficult to apply to SLE patients only (386).

There have been several neurologic syndromes associated with antiphospholipid antibodies in patients with lupus (53 ,292). The most common are stroke, cerebral venous sinus thrombosis (78), ocular ischemia, sensorineural hearing loss (291), dementia, seizures (387), chorea, and transverse myelopathy (204). Each one is felt to be a result of a thromboembolic event resulting in vascular occlusion. A major clinical question is why do some patients with antiphospholipid antibodies develop these neurologic syndromes, whereas others do not. SLE patients at greatest risk are those who have the lupus anticoagulant and/or high-titer IgG (and possibly IgM and IgA) anticardiolipin antibodies with β_2GPI specificity. SLE patients with antiphospholipid antibodies of different specificities have an increased risk of cerebral infarction compared to patients with a single antiphospholipid antibody. The presence of combinations of lupus anticoagulant, anticardiolipin/β_2GPI, and/or antiphosphatidylserine/prothrombin antibodies was strongly associated with stroke (388). Clinically, patients with antiphospholipid antibodies who have a history of a previous thromboembolic event, livedo reticularis, thrombocytopenia, or active lupus (vasculitis, hypocomplementemia, elevated anti-dsDNA antibodies) are at increased risk for thrombosis (389 ,390 ,391). Furthermore, approximately one third of patients with antiphospholipid antibodies have abnormal echocardiograms which demonstrate left-sided valvular lesions, which are a potential cardiac source for an embolic stroke (88 ,198 ,199 ,392). SLE patients with antiphospholipid antibodies, who have cerebral microemboli detected by transcranial Doppler ultrasound, are more likely to develop cerebrovascular ischemic events (393). Up to 30% of SLE patients who develop a thromboembolic event are likely to develop a recurrent episode within one year of the initial event (394). The initial type of thromboembolic event (arterial versus venous) is the most likely type of event to recur in a given patient, although usually not in the same vascular territory (395).

The ability of antiphospholipid antibodies to cause thrombosis is a result of a complex interaction between these antibodies, brain endothelial cells, and cerebral hemostasis, which is poorly understood (396 ,397). Brain endothelial cells display different functional and phenotypic characteristics compared to endothelial cells at other anatomic sites. β_2GPI appears to be expressed in higher amounts on brain endothelial membranes. Annexin V has increased importance in preventing thrombosis in the brain. Antiphospholipid antibodies with anti-β_2GPI specificity with or without anti-endothelial cell antibodies can selectively damage and/or activate these cells leading to prothrombotic consequences. Other mechanisms such as complement activation have also been proposed (398). Notably, certain clinical conditions such as surgery and infection can further increase the risk of thrombosis perhaps through the release of tissue factor. Finally, other cerebrovascular risk factors can be additive to the thrombotic risk conferred by antiphospholipid antibodies (399 ,400). Cigarette smoking, hyperlipidemia, hypertension, diabetes mellitus, and hyperhomocystinemia are all correctable risk factors, which need to be identified and treated.

There are SLE patients with neurologic thromboembolic events who will lack antiphospholipid antibodies on testing during the acute event. This may be a false-negative result possibly because of consumption of the antibody during the acute thrombotic episode. Consequently, these patients should be retested for these antibodies 6 to 8 weeks after the initial thrombotic event, since some patients will become positive for antiphospholipid antibodies at this time. However, approximately 10% of patients with an antiphospholipid antibody-like syndrome will remain negative for the lupus anticoagulant and IgG/IgM anticardiolipin antibodies, even with repeated testing. Some of these patients may demonstrate IgA anticardiolipin antibodies, antibodies against other negatively-charged noncardiolipin phospholipids, such as phosphatidylserine and phosphatidylinositol, or antibodies against specific phospholipid-binding proteins, including β_2GPI, prothrombin, protein C, protein S, thrombomodulin, and kininogens (401). It is not clear how, or if, these autoantibodies put SLE patients at increased risk for thromboembolic events.

CSF Tests

CSF analysis is useful in all SLE patients with a change in neurologic status, particularly to exclude infection or other secondary causes of CNS dysfunction. In patients with NP-SLE, CSF results may be unremarkable. However, patients with NP-SLE may have abnormalities helpful in confirming the diagnosis and guiding management. The NP-SLE consensus panel recommended that routine CSF tests, IgG index, and oligoclonal bands be determined on all patients suspected of having NP-SLE (10).

Routine CSF Tests

Routine CSF tests include cell count with differential, protein, glucose, Gram stain, other special stains, VDRL, and cultures. Pleocytosis and elevated protein are found in some patients with active NP-SLE. Protein abnormalities were found to be more common (range, 22% to 50%) than pleocytosis (range, 6% to 34%) in 10 studies analyzing spinal fluid findings in 250 patients with NP-SLE

(11 ,17 ,18 ,21 ,22 ,118 ,402). Neutrophilic pleocytosis with elevated protein suggests cerebral vasculitis with ischemia, if infection is ruled out (403). Patients with antiphospholipid antibodies and neurologic thromboembolic events frequently have elevated protein with mild or no pleocytosis.

The CSF glucose level is rarely decreased in NP-SLE. It has been reported low in between 3% and 8% of patients (18 ,21 ,22). Patients with acute transverse myelopathy have been reported to have hypoglycorrhea more than patients with other manifestations of NP-SLE (205), although they frequently have normal CSF glucose levels. CSF pleocytosis, elevated protein levels, and low glucose should always raise suspicion of an acute or chronic infection before attributing these abnormalities to NP-SLE.

CSF Immunologic Tests

Several studies have noted that CSF IgG levels are elevated in 69% to 96% of NP-SLE patients (22 ,32 ,402). Bluestein and Zvaifler found that a CSF IgG level greater than 6 mg/dL almost always indicated NP-SLE, but was present in only 40% of their patients (32). An elevated CSF Q-albumin ratio, indicating a break in the BBB, has been noted in up to one third of patients, especially those with progressive encephalopathy, transverse myelitis, and strokes (26 ,118 ,119). Several groups have now confirmed that an elevated IgG index and/or IgG oligoclonal bands are seen in up to 80% of patients, particularly those with diffuse manifestations, such as encephalopathy and psychosis (22 ,26 ,118 ,119 ,120 ,122). Hirohata et al. have also found IgM and IgA indices elevated (120). Patients with focal manifestations, such as stroke because of antiphospholipid antibodies, typically do not have an elevated IgG index or oligoclonal bands, unless they also have a coexistent encephalopathy (complex presentation) (26). These abnormalities have been shown to normalize in some patients after successful therapy (26 ,121).

CSF Antineuronal Antibodies

Bluestein et al. (31 ,32) found IgG antibodies to neurons in the CSF of 74% of 28 SLE patients with CNS involvement, as compared to only 11% of 18 SLE patients without CNS disease, using a radioimmunoassay with SK-N-SH neuroblastoma cells as the target. Furthermore, 90% of the patients with diffuse manifestations of psychosis, encephalopathy, or generalized seizures had elevated IgG antineuronal antibodies, compared to only 25% of patients with focal manifestations of hemiparesis or chorea. Notably, the antineuronal antibody was concentrated eightfold in the CSF, relative to its concentration in paired serum samples. In contrast, Kelly and Denburg, using a less sensitive hemadsorption assay, detected IgG antineuronal antibodies in only 17% of their patients with NP-SLE, using SK-N-ML neuroblastoma cells as a target (35). They correlated the presence of these antibodies with cognitive dysfunction; diffuse, nonfocal CNS manifestations; abnormal Q-albumin ratios; and active lupus with elevated anti-dsDNA antibody levels (33 ,34 ,35).

More recently, West et al. and Isshi and Hirohata, using sensitive enzyme-linked immunoabsorbent assays (ELISAs) with neuroblastoma cells as the target antigen, found a high percentage of their patients with active NP-SLE had antineuronal antibodies in their CSF. West et al. (26) reported that 12 of 40 (30%) SLE patients with diffuse manifestations had antineuronal antibodies, while Isshi and Hirohata (39) found 39 of 41 (95%) NP-SLE patients had CSF IgG antineuronal antibodies. Moreover, Isshi found in the 1 patient they followed serially that the CSF antineuronal antibody activity markedly fell with successful therapy, but increased along with an elevation of CSF IL-6 during an exacerbation of NP-SLE (39).

Miscellaneous Determinations

Several other laboratory and immunologic tests have been reported to be helpful and/or abnormal in NP-SLE. Brook et al. found CSF lactic acid to be normal in those with NP-SLE, whereas it is increased in patients with bacterial meningitis (404). Occasionally, LE cells can be found in the CSF in patients with NP-SLE, but rarely are they looked for and documented. Myelin basic protein has been reported, particularly in myelopathy patients. Other CSF serologic tests reported to be elevated in NP-SLE include: immune complexes (22), substance P (405), B_2-microglobulin levels, cyclic GMP levels (406), prostaglandin E2 (130), quinolinic acid (407), and CD4+ T cells. Most of these are single center reports or abstracts and generally not available to clinicians.

A past study reported that C4 levels in CSF decrease in those with acute NP-SLE (408). The C4 level turned out to be unstable in CSF, difficult to measure, and its value unconfirmed in other reports (402 ,409 ,410 ,411). However, intrathecal C4 synthesis may be increased in NP-SLE patients with diffuse manifestations (412). Another group reported increased levels of C5b-9 in the CSF of patients with active NP-SLE, suggesting intrathecal complement activation (413). Autoantibodies have rarely been reported in the CSF, including: antiribosomal P (368), antideoxyribonucleoprotein (414), anti-dsDNA (402 ,415), anticardiolipin antibodies, and anti-Ro/anti-La (415). Other groups have failed to detect some of these antibodies (26 ,170).

Several cytokines have been reported to be elevated in the CSF of active NP-SLE patients. Several groups have found elevated IL-6 levels in patients with NP-SLE (127 ,128 ,129 ,130). This is of interest since IL-6 can contribute to B cell differentiation into antibody-secreting plasma cells. Recently, Isshi and Hirohata reported a patient whose CSF IL-6 level and antineuronal antibodies increased during an exacerbation of NP-SLE, whereas the serum antineuronal antibody levels did not increase, suggesting intrathecal synthesis of antineuronal antibodies (39). Other cytokines reported to be elevated in the CSF of NP-SLE patients are IL-1 (127), IL-8 (129), IFN-α (416), and the chemokine CXCL10 (417).

Levels of glial fibrillary acidic protein and neurofilament triplet protein are 3 to 7 times higher in the CSF of patients with NP-SLE compared to controls (129). These levels correlated with the degree of abnormalities found on brain MRI. Measurements of these are evidence of neuronal damage and may be useful in the future.

Summary

When a lumbar puncture is performed in SLE patients with CNS dysfunction, the CSF tests that should be ordered are: cell count with differential, glucose and protein levels, VDRL, and Gram stain and cultures. Additionally, CSF should be sent for antineuronal antibodies and a "multiple sclerosis panel," which includes a CSF IgG level, Q-albumin ratio, IgG index, oligoclonal bands, and a calculated IgG synthesis rate. Patients with diffuse manifestations frequently have elevated antineuronal antibodies and/or an elevated IgG index and oligoclonal bands, suggesting immunologic activity (26 ,39). Patients with only focal manifestations usually do not have antineuronal antibodies, elevated IgG index, or oligoclonal bands, but may have an elevated Q-albumin ratio because of disruption of the BBB. Patients with pleocytosis and elevated protein levels with negative cultures frequently have acute inflammation from vasculitis causing their focal symptoms. In contrast, patients with antiphospholipid antibodies causing thrombosis and focal symptoms usually have elevated protein levels, but mild or no pleocytosis in their CSF. Infection must be ruled in all patients with CNS dysfunction.

Neuroimaging Studies

There have been dramatic advancements in neuroradiographic procedures for a variety of neurologic diseases. Brain imaging is an important part of the evaluation of SLE patients with neurologic dysfunction. Several recent, excellent review by leaders in the field have summarized the scientific basis for the use of neuro-imaging modalities in NP-SLE, point out their limitations, and make recommendations for their use (418 ,419 ,420). Chapter 38 presents an in-depth discussion of the various neuro-imaging modalities.

Angiography

Cerebral angiography is frequently normal in NP-SLE patients, even in those with cerebral infarction on MRI (63 ,64 ,65 ,66 ,421 ,422 ,423 ,424). This lack of sensitivity may be explained by the small size of vessels affected by lupus vasculopathy. Occasionally, vasculitis of larger-size arteries or cerebral emboli can be documented. However, angiograms are an invasive procedure with possible morbidity. A recent study reported angiograms in patients with suspected vasculitis were associated with transient neurologic deficits in 11.5%, which were persistent in 0.8% (425). MR angiography is a noninvasive alternative that can demonstrate abnormalities in medium to large vessels. In patients with suspected emboli, carotid Doppler and echocardiogram (including transesophageal technique) should be done to rule out a source of emboli.

Summary

Currently, MRI with gadolinium is the only imaging modality recommended for the evaluation of NP-SLE (10). CT scan is useful to rapidly rule out a large infarct or hemorrhage in an SLE patient with acute neurologic deterioration. MRI is superior to CT scan for detecting edema, infarcts, and hemorrhage. However, there is no MRI finding that is specific for NP-SLE. Furthermore, patients with NP-SLE, particularly those with diffuse manifestations, may have a normal conventional MRI. Conversely, SLE patients without NP-SLE may have abnormalities on MRI, which may be misinterpreted as NP-SLE. Thus, the results of MRI must be interpreted along with the clinical and other laboratory findings to establish a diagnosis of NP-SLE (26). Other MRI techniques, including MR relaxometry, MR spectroscopy, diffusion MRI, perfusion MRI, magnetization transfer imaging, and transcranial Doppler ultrasound are presently research tools and should be limited to selected cases of symptomatic patients with a normal conventional MRI. In the future, they may increase the sensitivity and specificity of conventional MRI and prove that all patients with NP-SLE will have demonstrable abnormalities. Although MRS cannot be used to specifically diagnose NP-SLE, it is very sensitive and specific for brain injury and may be very useful to confirm an organic basis for neurocognitive dysfunction in a patient suspected of having mild NP-SLE (418 ,419 ,420).

PET and SPECT scans are very sensitive but lack specificity, thus limiting their value in the diagnosis of NP-SLE. These modalities should be considered research neuro-imaging procedures and not used in the clinical evaluation of patients with NP-SLE. However, a normal SPECT scan may rule against significant brain pathology in an SLE patient with nonspecific neuropsychiatric symptoms.

Electroencephalography

Conventional EEG is abnormal in 60% to 91% of adult and pediatric patients with NP-SLE (17 ,18 ,19 ,20 ,21 ,22 ,26 ,338). Recently, EEG abnormalities have been found to correlate with the presence of antiphospholipid antibodies even in the absence of brain MRI abnormalities (426). The most common finding is diffuse slowing with increased β and δ background activity. Focal abnormalities and seizure activity can also be seen. Unfortunately, the EEG findings are not specific for NP-SLE, and other disorders, including metabolic encephalopathies and drug effects, can give similar findings. Furthermore, up to 50% of SLE patients without active NP-SLE can have abnormal EEGs. Consequently, a single abnormal EEG has limited diagnostic value for NP-SLE.

On occasion, however, an EEG may be very helpful, revealing unsuspected seizure activity that was not apparent clinically. EEGs may be able to distinguish steroid-induced psychosis from NP-SLE.

Quantitative electroencephalography (Q-EEG) has been found to be more sensitive and specific compared to conventional EEG (427 ,428). Q-EEG is abnormal in 87% of patients with definite NP-SLE, 74% of patients with probable NP-SLE, and 28% of SLE patients without neuropsychiatric symptoms. Serial Q-EEG reportedly show improvement with therapy.

Visual, brainstem, auditory, and somatosensory evoked potentials have been reported to be useful in detecting subtle cortical dysfunction not detected with conventional EEG (429 ,430 ,431 ,432). Others have failed to demonstrate evoked potential to be of value (433). Further controlled studies on these modalities are needed to establish their usefulness in clinical practice.

Treatment

The therapy of NP-SLE differs depending upon the clinical presentation and suspected pathogenesis (434). A thorough clinical evaluation and appropriate diagnostic evaluation of any SLE patient with new neuropsychiatric symptoms is important to establish the extent of neurological impairment and brain injury, in order to assess future progression and response to therapy. Secondary causes of CNS dysfunction should be excluded quickly and all unnecessary medications should be stopped. Therapy should not be delayed pending test results. If it is unclear whether the CNS dysfunction is a result of primary NP-SLE or a secondary cause, then the patient should be treated for both until diagnostic test results return (Fig. 37-4).

CNS Manifestations

The treatment of NP-SLE is empiric since there have been few controlled clinical trials. The therapy should be tailored to the severity of the presentation and suspected etiology. Patients with mild, diffuse manifestations such as headaches, anxiety/dysphoria, paresthesias, or an isolated seizure may only need analgesics, psychotropic medications and psychologic support, or antiseizure medications, respectively, and observed closely for any neurologic progression. A particularly difficult clinical situation is the SLE patient with a complaint of cognitive dysfunction but a clinically normal mental status examination. In these patients, serial psychometric testing may be helpful in establishing the presence, extent, and progression, if any, of impairment. Secondary causes such as medications, thyroid disease, depression, and especially sleep apnea need to be excluded. Treatment should be supportive, including memory aids, unless progression can be documented. The use of immunosuppressive therapy in this clinical situation is limited (435).

NP-SLE patients with severe or progressive, diffuse presentations such as acute confusional state, psychosis, severe depression, and coma may benefit from immunosuppressive medications. Most clinicians recommend 1 mg/kg/day of prednisone in divided doses. For the most severe cases, pulse intravenous methylprednisolone (1 g daily for 3 days) may be beneficial (436 ,437 ,438). Failure to respond within a few days may necessitate doubling of the prednisone dose. Another alternative is to switch from prednisone to dexamethasone (12 to 20 mg qd), which penetrates the BBB better than other corticosteroid preparations. Continued failure to respond is an indication to add cytotoxic medications and/or a trial of plasmapheresis, particularly for comatose patients. Pulse intravenous cyclophosphamide (0.75 to 1.0 g/m^2) given every 3 to 6 weeks has been reported to be beneficial in both adult and pediatric patients (439 ,440 ,441 ,442 ,443 ,444). Azathioprine has also been used, but with less impressive results. Patients with psychosis or depression should receive appropriate psychotropic medications to aid in the subsequent tapering of immunosuppressive medications.

Other methods of cyclophosphamide administration have been reported. A recent open-labeled study of 13 patients with lupus psychosis showed a favorable outcome in all patients treated with oral cyclophosphamide for 6 months followed by maintenance therapy with azathioprine (445). Two other trials have examined lower doses of intravenous cyclophosphamide. One trial used 500 mg every 2 weeks for three pulses followed by monthly 500-mg pulses for 6 months (446). The other used only 200 to 400 mg monthly (447). Both regimens were effective, had fewer side effects, but higher relapse rates compared to standard monthly therapy doses as used by NIH investigators in the lupus nephritis trials (0.75 to 1.0 g/m^2).

NP-SLE patients presenting with focal manifestations demand an immediate and aggressive evaluation. If vasculitis is suspected, corticosteroids in high doses similar to patients with severe, diffuse manifestations are used. Cytotoxic medications should be used early in patients with vasculitis. Clinical experience suggests that cyclophosphamide is more effective than azathioprine, methotrexate, cyclosporine, mycophenolate mofetil (448), or IV pulse methylprednisolone (449). Once the patient's NP-SLE is controlled on cyclophosphamide, another cytotoxic medication may be substituted to maintain remission. Whether chronic antiplatelet therapy prevents thrombosis or atheroma formation in the damaged vessel is unknown, but often used.

Many patients with focal manifestations of NP-SLE have antiphospholipid antibodies. Since the suspected pathogenesis is thrombosis and not vasculitis, patients are treated with antiplatelet drugs, hydroxychloroquine for its mild anticoagulant effect, and/or anticoagulation. In patients with large or cardioembolic strokes, excessive heparinization is dangerous and may cause hemorrhage into the infarcted area. Consequently, particularly in patients with an elevated PTT because of the lupus anticoagulant,

heparin levels should be followed as well as serial CT scans. Although the intensity of warfarin therapy has been debated (450), most experts recommend lifelong warfarin at an INR of 3.0 to 3.5 for cerebral arterial thrombosis (451 ,452). Patients with the lupus anticoagulant should also have periodic factor II and chromogenic factor X levels followed and maintained at 15% to 20% of normal to assure adequate anticoagulation (453). Patients who continue to thrombose on anticoagulation may respond to intravenous gammaglobulin or plasmapheresis with immunosuppressive therapy (440).

NP-SLE patients with seizures should be treated with antiseizure medications. Patients presenting in status epilepticus or who have recurrent seizures should be treated with high-dose prednisone. Patients with seizures, cerebral infarcts, and moderate to high titers of antiphospholipid antibodies should be anticoagulated once seizures are controlled, although they are at increased risk for falls and cerebral trauma (53). Antiseizure medications may have side effects, which can mimic active lupus. Phenytoin can cause fever, adenopathy, and leukopenia. Ataxia and other neurologic symptoms can occur if the phenytoin level rises above therapeutic levels. Carbamazepine can cause severe leukopenia and must be monitored closely. SLE patients with seizures should remain on antiseizure medications for at least 1 year. If they have no recurrence of seizures, a normal MRI, and normal EEG, then antiseizure medications can be withdrawn, and the patient followed closely. Vehicle driving restrictions should be enforced.

Some patients with NP-SLE will not respond to, may not tolerate, or will have contraindications to aggressive therapy. In these patients, a variety of other therapies have been tried. One novel approach is intrathecal therapy for patients with diffuse NP-SLE. Funauchi et al. recently reported two SLE patients with a good response to intrathecal corticosteroids (454). Other investigators have used intrathecal methotrexate and dexamethasone (10 mg of each, weekly for 3 weeks). Two reports totaling 27 patients with diffuse manifestations of NP-SLE received this therapy with over 90% of patients responding (30 ,455). These observations have recently been confirmed where 22 of 24 NP-SLE patients unresponsive to oral corticosteroids responded to intrathecal therapy (456). Another therapy is intravenous immunoglobulin (IVIG). Six of seven patients with severe, acute NP-SLE responded to IVIG (457 ,458). IVIG should be given at a dose of 400 mg/kg/day for 5 consecutive days, instead of 1,000 mg/kg/day for 2 days, in order to lessen the chance of side effects such as thrombosis, fluid overload, renal function deterioration, and aseptic meningitis. A third therapy is plasmapheresis (459 ,460 ,461 ,462 ,463). For a critically ill patient, 40 to 60 mL of plasma/kg/day is removed for up to 5 consecutive days. For a more stable patient, 40 mL of plasma/kg/day three times a week for 3 weeks constitutes a therapeutic trial. Oftentimes, only 1 week of plasmapheresis is needed to stabilize the patient. Plasmapheresis is particularly useful for patients with cerebral vasculitis to allow time for the corticosteroids and cytotoxic medications to take effect. It is also used in patients with recurrent cerebral infarcts, associated with antiphospholipid antibodies, who fail anticoagulation. However, some investigators are using IVIG in this clinical situation to avoid the catheter-related thrombosis frequently seen in these patients (440 ,464). Notably, immunosuppressive medications are continued during plasmapheresis to prevent rebound antibody production and/or disease flare. Frequent side effects of plasmapheresis are catheter-related problems and infection (465). Patients developing hypogammaglobulinemia should receive replacement immunoglobulin to help prevent infection. Another use for plasmapheresis is in SLE patients with hyperviscosity syndrome (viscosity greater than 4) from circulating immune complexes, severe hypergammaglobulinemia, or cryoglobulinemia (cryocrits greater than 8%) who have symptoms of cerebral insufficiency. Finally, plasmapheresis with fresh frozen plasma replacement is the best therapy for SLE patients with TTP (466). Other therapies reported in isolated case reports to be successful in steroid-unresponsive NP-SLE are B cell depletion therapy with antiCD20 (467), hyperbaric oxygen for cognitive impairment (468), and intrathecal plasmapheresis (469). Hematopoietic stem cell transplantation (470) or high-dose cyclophosphamide therapy (471) may be considered for patients with severe and resistant NP-SLE.

Difficult Clinical Situations

Several difficult clinical situations warrant further comment. First is the SLE patient on corticosteroids who presents with neuropsychiatric symptoms that could be NP-SLE versus steroid psychosis. One approach is to double the dose of corticosteroids for 3 days while awaiting test results. If the psychotic episode is a result of NP-SLE, it will respond to this therapy. Failure to improve lessens the likelihood of NP-SLE and the corticosteroids should be tapered to half of the original dose. If corticosteroids cannot be tapered, then antipsychotics such as haloperidol or lithium can be used (350 ,472). Tricyclic antidepressants should be avoided (352).

A second situation is the young (<40 yr) SLE patient with mild cognitive complaints who is found to have multiple small T2-weighted lesions in the cerebral white matter on brain MRI. Patients in whom cognitive dysfunction is confirmed by formal neuropsychiatric testing should receive antiaggregant therapy (aspirin 75 to 100 mg/d) and/or hydroxychloroquine especially if antiphospholipid antibodies are present (53 ,473). Patients who fail to respond to this therapy as evidenced by progression of cognitive dysfunction and/or accumulation of brain lesions on MRI may benefit from oral anticoagulation with warfarin. A third situation is the SLE patient with dementia from prior NP-SLE or from infarctions related to antiphospholipid antibodies. The dementia in these patients will not respond to corticosteroids and, in fact, may worsen. SLE patients with stable dementia should not be automatically assumed

to have active NP-SLE and therefore should not be treated aggressively with immunosuppressive medications.

Two other difficult clinical situations are transverse myelitis and chorea. Transverse myelitis should be treated aggressively with corticosteroids and cyclophosphamide (204 ,224 ,225 ,226). Patients presenting acutely should receive intravenous pulse methylprednisolone followed by high-dose prednisone and intravenous monthly cyclophosphamide. Patients with chorea usually respond to corticosteroids and haloperidol. Those that do not respond may need cytotoxic medications and/or plasmapheresis (244).

Headaches can be a chronic problem in SLE patients. Initial studies showed that headaches associated with SLE flares would respond to corticosteroids. However, most headaches in SLE patients occur without an SLE flare and do not respond to corticosteroids. Usually symptomatic therapy for migraine or tension headaches is beneficial. In patients with chronic refractory headaches secondary causes such as sleep apnea and saggital vein thrombosis must be excluded. Anecdotal reports have described improvement of headaches in patients with antiphospholipid antibodies who received anticoagulation. However, a recent controlled trial did not show any benefit (474).

Several other neurologic syndromes, including stroke, transverse myelitis, chorea, seizures, and multiple sclerosis-like syndromes, have been associated with antiphospholipid antibodies as well as other pathogenetic mechanisms. When a patient presents with one of these manifestations, the antiphospholipid antibody results may take a few days to return. In the interim, we have treated these patients with corticosteroids and antiplatelet drugs until results of antiphospholipid antibodies return, particularly since vasculitis can coexist with antiphospholipid antibody-associated thrombosis (74). If the antiphospholipid antibodies are positive, the next decision is whether to continue with antiplatelet drugs or to anticoagulant. One approach has been to anticoagulate those patients with the lupus anticoagulant or high-titer (greater than 40 to 50 GPL units) IgG anticardiolipin/anti-$ß_2$ glycoprotein-1 antibodies and/or other manifestations of the antiphospholipid antibody syndrome, including livedo reticularis, previous miscarriages, previous thrombotic episodes, and mild thrombocytopenia (452).

Peripheral Nervous System Manifestations

SLE patients with mild, nonprogressive paresthesias require only symptomatic therapy. Patients with cranial, peripheral, or autonomic neuropathy are treated with high-dose corticosteroids initially. Patients with Guillain-Barré or CIDP frequently have IVIG or plasmapheresis as additional therapy. Patients with mononeuritis multiplex because of vasculitis should also receive cytotoxic therapy such as cyclophosphamide. When using cyclophosphamide in patients with peripheral/autonomic nervous system involvement, it is important to determine if the patient has a neurogenic bladder, which may not eliminate the cyclophosphamide metabolites leading to hemorrhagic cystitis. SLE patients with myasthenia gravis are treated with medications that increase the concentration of acetylcholine at the neuromuscular junction. Other therapy is similar to patients, without SLE, who have myasthenia. The role of thymectomy is controversial since SLE has been reported to flare after the thymus has been removed (329).

Prognosis

The prognosis for NP-SLE patients remains guarded. Recent studies have shown that the overall clinical impact of NP-SLE has a negative impact on quality of life as indicated by lower scores on subscales of the SF-36 as well as a higher frequency of disability (475). Although many NP-SLE patients with major diffuse symptoms of NP-SLE appear to recover, studies using psychometric testing demonstrate that many patients are left with cognitive dysfunction suggesting residual CNS damage (165). Patients with focal manifestations may stabilize, but usually do not reverse their deficits during therapy. Notably, individual neuropsychiatric manifestations differ in their prognostic implications.

Few studies have prospectively followed NP-SLE patients over time. Several studies have shown that mild cognitive deficits detected by formal testing do not appear to progress or adversely affect a person's quality of life over time in the majority of patients (152 ,153 ,154). Patients with major NP-SLE manifestations have a less optimistic prognosis. Recurrences of NP-SLE episodes occur in 20% to 40% of NP-SLE patients, leading to more residual dysfunction. Using the SLICC/ACR Damage Index, Petri has found neuropsychiatric damage steadily accrues and is the second-leading organ system that is damaged in the Hopkin's lupus cohort (476). However, a recent study by Karassa et al. is more favorable (477). They followed 32 SLE patients who had been hospitalized for NP-SLE prospectively for 2 years and found that there was substantial improvement (69%) or stabilization (19%) in most cases. Patients with recurrent episodes of NP-SLE and those with antiphospholipid antibodies generally did worse.

With dialysis and transplantation enabling lupus patients with nephritis to survive longer, NP-SLE and its therapy may now be the leading cause of death with a 7% to 19% mortality rate (21 ,30 ,478 ,479). Previous studies indicate that seizures, especially status epilepticus, stroke, and coma are particularly poor prognostic signs (14 ,17 ,478 ,479), demanding aggressive evaluation and treatment to help prevent residual neurologic damage or death. Whether the therapy improves or contributes to long-term morbidity and mortality from conditions like atherosclerosis and cancer is unclear (480). Consequently, the clinician must make every effort to limit the toxicities of therapy by controlling hypertension, treating hyperlipidemia and hyperglycemia, utilizing osteoporosis prophylaxis, administering pneumococcal vaccine, polyvalent (Pneumovax), advising against smoking, treating hyperhomocystinemia with appropriate vitamins, and using medications for *Pneumocystis carinii* prophylaxis (481).

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Chapter 38

Psychopathology of Lupus and Neuroimaging

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Introduction

Involvement of the nervous system by systemic lupus erythematosus (SLE) has been recognized for over 100 years and includes a wide variety of neurologic (N) and psychiatric (P) manifestations. The presentation of neuropsychiatric (NP) disease in individual SLE patients continues to pose diagnostic and therapeutic challenges because of the lack of specificity of the majority of the NP manifestations, uncertainty regarding the pathogenic mechanisms, and a paucity of data to support therapeutic strategies. This chapter will review what is currently known about the more common psychiatric syndromes and cognitive impairment in SLE patients and the role of neuro-imaging in the diagnosis and investigation of NP syndromes.

Classification of Neuropsychiatric Systemic Lupus Erythematosus

The American College of Rheumatology (ACR) classification criteria for SLE (1) include the NP manifestations, seizures, and psychosis. However, it is widely acknowledged that a much broader range of NP disease manifestations occur in SLE patients. Virtually all studies indicate that most NP events reflect involvement of the central nervous system (CNS) compared to involvement of either the peripheral or autonomic nervous systems. A particular NP event may reflect either a diffuse disease process (e.g., psychosis and depression) or focal process (e.g., stroke and transverse myelitis) depending upon the anatomic location of pathology. Although several classifications have been developed for neuropsychiatric systemic lupus erythematosus (NP-SLE) (2 ,3 ,4), most have lacked either definitions for individual manifestations or uniformity in the approach to investigation and diagnosis. In 1999, the ACR research committee produced a standard nomenclature and diagnostic criteria for 19 NP syndromes that are known to occur in SLE patients (5) (Table 38-1). For each of the 19 NP syndromes, potential etiologies other than SLE were identified for either exclusion, or recognized as an “association,” acknowledging that in some clinical presentations definitive attribution is not possible. The identification of other nonlupus causes for NP events in SLE patients is of critical importance and has not been adequately addressed in previous classification systems. Guidelines for reporting NP events were also developed by the ACR research committee and specific diagnostic tests were recommended for each syndrome. Although these criteria were developed primarily to facilitate research studies of NP-SLE, they also provide a practical guide to the assessment of individual SLE patients with NP disease.

Epidemiology of NP-SLE

In a representative selection of studies utilizing the ACR classification criteria for NP-SLE (6 ,7 ,8 ,9 ,10), the overall prevalence of NP disease has varied widely between 37% and 95% (Table 38-2). The most common of the 19 NP syndromes in each of these 5 SLE cohorts were cognitive dysfunction (55% to 80%), headache (24% to 72%), mood disorders (14% to 57%), cerebrovascular disease (5% to 18%), seizures (6% to 51%), polyneuropathy (3% to 28%), anxiety (7% to 24%) and psychosis (0% to 8%). Most of the other NP syndromes were infrequent, with a prevalence of less than 1% in most studies, emphasizing the rarity of many of these NP events in SLE patients.

The attribution of individual NP events to SLE per se or to an alternative etiology remains a challenge. In the absence of a diagnostic gold standard for most of the NP-SLE syndromes, attribution is determined case-by-case on the basis of exclusion using the best available clinical, laboratory, and imaging data. The ACR NP-SLE classification (5) provides a basis for addressing this issue in a systematic manner, because for each NP syndrome there is a comprehensive list of “exclusions” and “associations,” the presence of which may indicate an alternative etiology other than SLE. Utilizing this approach and taking into consideration the temporal relationship between the NP event and the diagnosis of SLE, a recent study has reported that up to 41% of all NP events in SLE patients may be attributed to factors other than lupus (8). This finding is in keeping with data from a Finnish study (6), which concluded that headache, anxiety, mild depression, mild cognitive impairment, and polyneuropathy without electrophysiologic confirmation

should not be considered primary NP manifestations of SLE. The impact of chronic disease was also emphasized by a recent study that reported that NP syndromes commonly associated with SLE may occur with comparable frequency in patients with rheumatoid arthritis (RA) (11), further emphasizing the nondisease-specific nature of many of the NP syndromes in SLE patients. Regardless of attribution, the impact of cumulative NP events in SLE patients is evident from the significant reduction in virtually all domains of the SF-36, a self-report health-related quality-of-life instrument (8) (Fig. 38-1).

Table 38-1: Neuropsychiatric Syndromes in SLE as Defined by the ACR Nomenclature.

Central nervous system	Peripheral nervous system
Aseptic meningitis	Guillain-Barré syndrome
Cerebrovascular disease	Autonomic neuropathy
Demyelinating syndrome	Mononeuropathy
Headache	Myasthenia gravis
Movement disorder	Cranial neuropathy
Myelopathy	Plexopathy
Seizure disorders	Polyneuropathy
Acute confusional state	
Anxiety disorder	
Cognitive dysfunction	
Mood disorder	
Psychosis	

From The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum* 1999;42(4):599–608 with permission.

The results of these recent clinical studies serve to both reinforce previous conclusions about NP-SLE and introduce new concepts. Despite the improved definitions for individual NP syndromes, there continues to be substantial variability in the overall prevalence of NP disease between different populations. Whether this represents inherent differences between study cohorts or a bias in data acquisition remains to be determined. None of the individual NP manifestations are unique to lupus and indeed some occur with comparable frequency in the general population and in patients with other chronic diseases. Thus, in research study design, the inclusion of control groups is critical to determine whether the prevalence of NP disease in SLE patients is in excess of that found in the normal population and in other chronic diseases. Because of the fact that many of the NP syndromes are quite rare (<1%), multicenter efforts will be required to assemble sufficient numbers of patients for study. Current evidence suggests that non-SLE factors likely contribute to a substantial proportion of NP disease in SLE patients, particularly the “softer” NP manifestations such as headache, anxiety, and some mood disorders. Future studies will need to define which of the many NP manifestations have the greatest clinical impact.

Table 38-2: Prevalence of NP Syndromes Using ACR Nomenclature

	Ainiala (2001) Tampere, Finland (6)	Brey (2002) San Antonio, USA (7)	Sanna (2003) London, UK Cagliari, Italy (9)	Hanly (2004) Halifax, Canada (8)	Sibbitt* (2002) Albuquerque, USA (10)
# of patients	46	128	323	111	75
Disease duration (mean ± SD Years)	14 ± 8	8	11 ± 8	10 ± 1	7 ± 8
NP-SLE	91%	80%	57%	37%	95%

*Pediatric onset SLE population

From Hanly JG. Neuropsychiatric lupus. *Rheum Dis Clin N Am* 2005;31:273–298, with permission.

Pathogenesis of NP-SLE

The rationale for identifying the etiology and pathogenic mechanisms underlying NP disease in SLE (Fig. 38-2) is to facilitate the logical development of appropriate and effective therapies. The diversity of NP manifestations, which have been reported in SLE patients, makes it unlikely that

there is a single pathogenic mechanism. However, the exclusion of a substantial proportion of NP events attributable to non-SLE factors, such as complications of the disease, its therapy, or concurrent illnesses, provides a more homogenous clinical population and the greater likelihood of identifying disease specific mechanisms. There are at least three primary immunopathogenic mechanisms that are implicated in NP-SLE, namely vasculopathy of predominantly small intracranial blood vessels, autoantibody production, and the generation of inflammatory mediators.

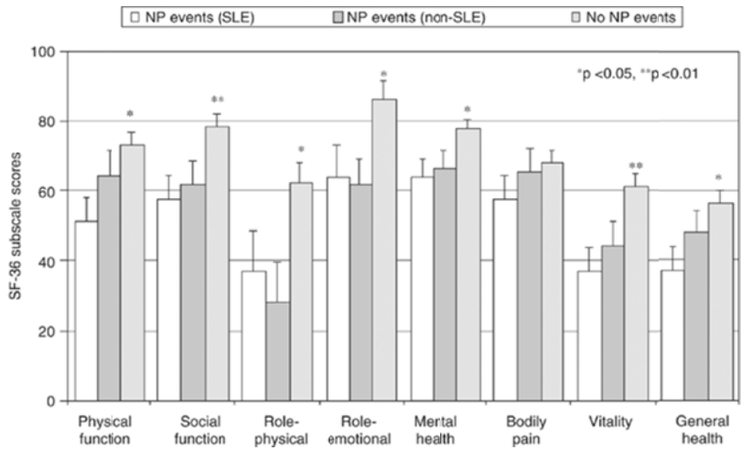

Figure 38-1. Association between cumulative neuropsychiatric events in SLE patients and self-report health-related quality of life as reflected by the SF-36. Derived from Hanly JG, McCurdy G, Fougere L, et al. Neuropsychiatric events in systemic lupus erythematosus: attribution and clinical significance. *J Rheumatol* 2004;31(11): 2156–2162.

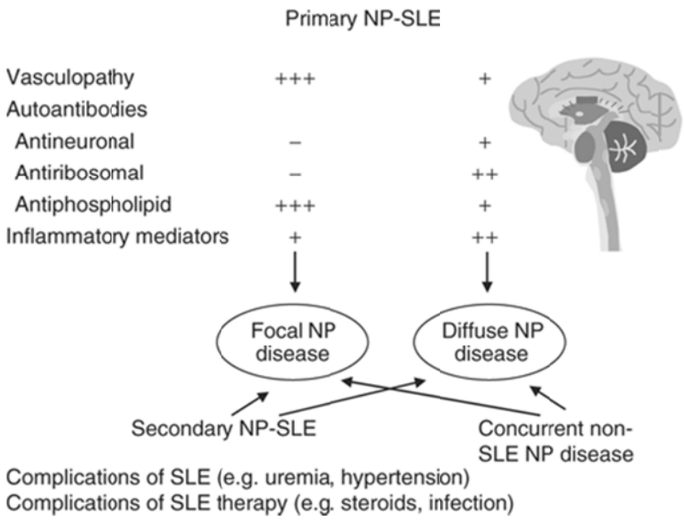

Figure 38-2. Factors contributing to the pathogenesis of neuropsychiatric (NP) disease in SLE. Modified from Hanly JG. Neuropsychiatric lupus. *Curr Rheumatol Rep* 2001;3:205–212.

Vasculopathy

In a limited number of neuropathologic studies (12 ,13 ,14 ,15) the predominant finding was a bland, noninflammatory vasculopathy. In contrast, inflammatory disease of small or large vessels was rare. Brain microinfarcts occurred in close anatomic proximity to the micro-angiopathy (12). Although instructive, the majority of neuropathologic studies in SLE have significant limitations because of a bias in patient selection, a temporal disconnect between the NP event and tissue sampling, and the potential impact of confounding factors, such as infection, hypertension, and corticosteroids on neuropathology.

Autoantibodies

A humoral immune response directed against neuronal antigens, ribosomes, and phospholipid-associated proteins has been implicated in the pathogenesis of NP-SLE. The data from human studies supporting a role for antineuronal antibodies is largely circumstantial. This includes the temporal relationship between clinical events and serologic findings (16), the presence of autoantibodies in the cerebrospinal fluid (CSF) (17) and, to a very limited extent, their identification in neuronal tissues from patients succumbing to the disease (18). Autoantibodies gain access to the CSF of SLE patients by means of passive transfer from the circulation through a permeabilized blood-brain barrier (BBB) (19 ,20) and, independently, by direct intrathecal production (16 ,19). The fine specificity of antineuronal

antibodies has been studied extensively, but in general has not resulted in greater diagnostic specificity.

Most recently, attention has been focused on anti-NR2 glutamate receptor antibodies as a potentially novel system that could explain some of the complexities of NP-SLE. The NMDA (N-methyl-D-aspartate) receptors NR2a and NR2b bind the neurotransmitter glutamate and are present on neurons throughout the forebrain (21 ,22 ,23). The hippocampus, which is the anatomic structure closely linked to learning and memory, has the highest density of brain NMDA receptors (22). In addition to their putative role in learning and memory (24), these receptors display altered expression in major psychoses (25), and if engaged by receptor antagonists, cause hallucinations and paranoia (26). Recent studies (27 ,28) has shown that a subset of anti-DNA antibodies, derived from both murine models of SLE and from a limited number of human subjects with the disease, cross-react with a pentapeptide consensus sequence that is present in the extracellular, ligand-binding domain of NR2 receptors. Moreover, these antibodies induced apoptotic cell death of neurons in vitro and in vivo and were present in the CSF of one SLE patient with progressive cognitive decline. Thus, in contrast to the previously described antineuronal antibodies in SLE, the anti-NR2 glutamate receptor antibodies appear to have a functional consequence leading to neuronal injury in a manner similar to that seen in excitatory amino acid toxicity (29). This effect is mediated via the antigen-binding portion of the antibody (29) and is specifically inhibited by memantine, an NMDA receptor antagonist (27). Moreover, enhanced permeability of the BBB is critical for circulating autoantibodies to enter the CSF and gain access to neuronal cells (27). Although of considerable interest, these findings are preliminary, are largely derived from animal studies and require confirmation in human subjects with NP-SLE. To date, the studies in human lupus examining the association between this subset of antineuronal antibodies and cognitive impairment have yielded conflicting results (30 ,31 ,32).

Antiribosomal P (anti-P) antibodies were first described in SLE patients in 1985 and are quite specific for SLE with a prevalence of 13% to 20% depending upon the ethnic group (33). In 1987, these autoantibodies were first linked to NP-SLE, in particular psychosis (34). Subsequent work either supported, refuted, or extended this initial observation to include depression (33 ,35 ,36 ,37). Potential explanations for the differences in study outcomes include variability in diagnostic criteria for psychiatric disease, variance in the temporal relationship between clinical events and serologic testing and differences in assay technique, particularly antigen preparation and purity. As demonstrated by recent animal work with anti-NR2 glutamate receptor antibodies (27), permeability of the BBB is also a critical factor for anti-P antibodies to exert their putative pathogenic role. One of the largest human studies (36) examined 394 SLE patients, 63 (16%) of whom had anti-P antibodies. There was a significant association with psychosis and depression with odds ratios between 4 and 10. However, because of the low prevalence of clinical events, the positive predictive value was only 13% and 16% for psychosis and depression, respectively. This has important implications for the application of this serologic test in decision making for individual patients. In contrast, a more recent study of 149 patients (37), 12% of whom had anti-P antibodies, did not find an association with any of the NP syndromes as defined by the ACR classification criteria (5).

Additional observations on anti-P antibodies are of interest and may provide insight into their pathogenic mechanisms. One study (38) reported an association between anti-P and antineuronal antibodies and furthermore, demonstrated that anti-P antibodies bind a 38-kd surface protein on human neuroblastoma cells. In another study of 87 SLE patients (39), there was a significant elevation in circulating anti-P antibodies in 34 patients with lupus psychosis, but there was no increase in the level of serum antineuronal antibodies. In contrast, examination of the CSF from the same patients revealed a significant elevation in antineuronal antibodies but not in anti-P antibody levels. These data suggest potential interaction between these two families of autoantibodies in the pathogenesis of NP-SLE and emphasizes the importance of local versus systemic autoantibody production in the causality of a NP event.

Autoimmune antiphospholipid antibodies, which are directed against phospholipid-binding proteins such as $ß_2$-glycoprotein I and prothrombin (40), are associated with predominately focal manifestations of NP-SLE. The most common neurologic disorders are those of vascular origin such as transient cerebral ischemia or stroke, but other associations include seizures, chorea, transverse myelitis, and cognitive dysfunction (9). In a review of over 1,000 SLE patients, NP manifestations occurred in 38% of patients with lupus anticoagulant compared to 21% of patients without these antiphospholipid antibodies (41). The favored pathogenic mechanism for this subset of autoantibodies in NP-SLE is thrombosis within vessels of different caliber and subsequent cerebral ischemia. A procoagulant state may be induced through acquired resistance to protein C and protein S, platelet aggregation, and direct activation of endothelial cells (40). However, the intrathecal production of antiphospholipid antibodies in patients with NP-SLE (19), their association with diffuse cognitive impairment (42 ,43), and in vitro evidence indicating modulation of neuronal cell function (44) and antibody binding to brain tissue (45) all raise the possibility of an alternative and more direct pathogenic mechanism.

Inflammatory Mediators

The potential role of pro-inflammatory cytokines in neuropsychiatric lupus has received increasing attention in recent years. Studies in Japan were the first to report an association between enhanced intracranial production of interleukin (IL)-6 with seizures (46) and interferon-(IFN) α with lupus psychosis (47). Subsequent studies have provided

further evidence for the intrathecal production of IL-6 (48 ,49 ,50 ,51) and have identified other potential candidate cytokines such as IL-10 (51 ,52), IL-2 (53), and IL-8 (50). The sources of intrathecal production of these cytokines include neuronal (47 ,49) and glial cells (47) and the stimulus for, and regulation of, this enhanced cytokine response remains to be determined. Although potentially an epiphenomenon, it may be a consequence of cell activation mediated by autoantibodies within the intrathecal space. However, measuring CSF cytokine levels unselectively in patients with any manifestation of NP-SLE is unlikely to be of value in the diagnostic work-up of individual patients (54).

Other potentially important inflammatory mediators are matrix metalloproteinases (MMPs), a family of endoperoxidases that can degrade extracellular matrix components (55). MMP-9 is a gelatinase and is secreted by a variety of cells in the blood vessel wall including macrophages, T lymphocytes, endothelial cells, and smooth muscle cells (56). Implicated in the pathogenesis of plaque rupture (57), elevated levels have also been associated with other conditions including multiple sclerosis (MS) (58), Guillain-**Barré** syndrome (GBS) (59), and rheumatoid arthritis (RA) (60), as well as SLE (61). A recent study (62) has examined the association between circulating levels of MMP-9 and NP-SLE. Although there was no difference in the levels of MMP-9 between SLE patients and healthy population controls, elevated levels of MMP-9 were associated with NP-SLE and in particular with cognitive impairment. There was a positive correlation between circulating MMP-9 levels and both T1 and T2 lesions on brain MRI scans, which will be discussed in detail later. It is also of interest that increased expression of MMP-9 is found in the disrupted BBB following cerebral ischemia and may facilitate lymphocyte migration into and possibly through the arterial wall (63). Elevated MMP-9 levels have also been detected in CSF samples of patients with NP-SLE compared to SLE patients without NP manifestations and normal controls (64). Furthermore, the positive correlation between CSF MMP-9 levels, pro-inflammatory cytokines, and biomarkers of neuronal and glial degradation (64) supports the suggestion that the enhanced production of MMP-9 is under cytokine control and is responsible for central nervous system (CNS) damage.

Psychiatric Disorders

Psychiatric disorders have been reported to a variable extent in SLE patients (65 ,66) and are likely multifactorial in etiology (67). For example, in a review of 21 studies, Wekking (66) found that the overall prevalence of psychiatric disorders ranged from 17% to 71% with depression being the most common syndrome. They found no consistent relationship with other manifestations of SLE and emphasized the importance of psychosocial stress as an associated factor (66 ,68). Indeed, those studies that have included other chronic disease control groups, such as patients with RA, have reported comparable frequencies and types of psychiatric disorders in control and SLE groups (11). This does not negate the importance of recognizing and managing psychiatric disorders in SLE, however, and one must remember that a particularly tragic outcome of such disorders in SLE patients is suicide (69 ,70). The potential causes of psychiatric disorders in SLE are varied and include acquired brain dysfunction, iatrogenic effects of corticosteroid treatment, psychosocial stressors, and current coping strategies (67). Nevertheless, there is sufficient evidence to support the notion that at least some psychiatric syndromes are primary manifestations of SLE.

The co-occurrence of psychiatric illness and SLE has been well recognized and the diagnostic designation of NP-SLE assumes that psychiatric illness is itself a manifestation of lupus. However, there is a lack of good epidemiologic evidence to support our understanding of these disorders. Many methodologic problems remain including selection bias, lack of adequate comparison groups, differences in case ascertainment criteria (e.g., chart reviews, screening instruments, standardized diagnostic interviews), differences in time-frame and follow-up, failure to account for social and cultural variables, and failure to account for psychiatric illness that predates the diagnosis of SLE.

The *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV)* was published in 1994 by the American Psychiatric Association and was revised to be consistent with the *International Classification of Diseases (ICD) ICD-9-Clinical Modifications (CM)* and *ICD-10*. This has served as the basis for the terminology in the current ACR nomenclature (5) used to describe the psychiatric manifestations of NP-SLE. As outlined in *DSM-IV,* the term “mental disorders” “persists in the title of DSM-IV because we have not found an appropriate substitute” and it does not reflect a viewpoint of a “reductionistic anachronism of mind/body dualism.” Importantly, the term reflects a “clinically significant behavioural or psychological syndrome or pattern that occurs in an individual and that is associated with present distress … or disability or with increased risk of death, pain or disability, or with loss of freedom.” It must not be a “culturally sanctioned response to a particular event.” For those using the *DSM-IV* as a diagnostic manual it is important to recognize that there is “no assumption that each category of mental disorder is a completely discrete entity” and that “specific diagnostic criteria included in DSM-IV are meant to serve as guidelines to be informed by clinical judgement.” From the standpoint of psychiatric syndromes in the context of NP-SLE it is also important that the use of the term “general medical disorders” and “mental disorders” in *DSM-IV* “are merely terms of convenience and should not be taken to imply that there is any fundamental distinction between mental disorders and general medical conditions.”

The ACR NP-SLE nomenclature (5) includes within its taxonomy, acute confusional state, anxiety disorder, mood disorders and psychosis, which are consistent with the *DSM-IV* classifications. These are discussed separately below.

The concept of “cognitive dysfunction” is operationalized in the ACR NP-SLE nomenclature and is discussed later in this chapter.

Acute Confusional State

The use of this term in the ACR nomenclature for NP-SLE (5) has replaced what was previously called “organic brain syndrome.” It is considered synonymous with the *DSM-IV* and *ICD-9* terms “delirium,” and is presumed to also be synonymous with the term “encephalopathy” as often preferred by neurologists. It encompasses a state of impaired consciousness or level of arousal that can progress to coma. Acute confusional states are characterized by a reduced ability to focus, maintain, and shift attention and are accompanied by disturbances of cognition, behavior and mood/affect. Other features include disturbance of sleep-wake cycles and changes in psychomotor behavior. Of the latter, hyperactivity is most easily recognized, whereas lethargy may mask the other symptoms. In the following discussions, the terms “delirium” and “acute confusional state” will be considered synonymous.

The term delirium was first included in the Third Edition of *DSM* and has subsequently been revised. Estimates of the prevalence and incidence of delirium in the general population suffer from many of the same problems associated with epidemiologic studies of NP-SLE itself namely: “evolving criteria, variable case-finding methods, and disparate criteria for subject selection.” This has given rise to considerable variability in prevalence estimates. For example, the prevalence of delirium upon hospital admission among the elderly varies from 11% to 33%, whereas estimates of the incidence during hospitalization vary from 3% to 42% (71). The prevalence of delirium among SLE patients has generally been lower than these estimates and has been reported in 4% to 7% of SLE patients (6 ,8 ,9). Although the review of Jennekens and Kater of five “index studies” listed “organic brain syndrome” as one of the most frequent NP-SLE syndromes (72), all of these were conducted prior to the development of the 1999 ACR nomenclature (5) and undoubtedly included a number of individuals with persistent cognitive impairments unrelated to delirium. In contrast, Brey et al. (7) in their study of the point prevalence of NP-SLE syndromes using the ACR nomenclature (5) for 128 subjects did not report any cases of delirium in their patients.

However, prevalence estimates for delirium are of questionable value. As the name “acute confusional state” implies, delirium develops over a brief period of time (a few days to hours) and may resolve over hours to weeks. Deficits in attention represent a core feature of delirium, but also contribute significantly to the clinical manifestation of cognitive dysfunction that is discussed further below. Distinguishing the acute onset of a delirium in the presence of a previously unrecognized persistent cognitive impairment can prove difficult, because much of this distinction rests on a clinical history of acute onset of symptoms that must be obtained from informants. A key feature of delirium is also fluctuation throughout the day in level of arousal and attentional capabilities. Thus, careful observation is often required to distinguish between delirium and persistent cognitive deficits, whereas single assessments with mental status screening instruments may misdiagnose delirium as dementia. Delirium is more likely to occur if there is pre-existing cognitive impairment (73). Moreover, delirium appears to be an important marker of risk for dementia, in older people without previously recognized cognitive or functional impairment (74). Thus, the presentation of delirium in a patient with SLE, including those situations in which it is associated with pharmacotherapy, indicates the need for careful follow-up. The onset of delirium also emphasizes a need to search carefully for both a clinical history of pre-existing cognitive impairments and residual cognitive impairments after resolution of the acute phase of delirium.

Delirium in association with SLE meets the *DSM-IV* criteria of “delirium due to a general medical condition” (293.0). However, delirium is by nature a multifactorial condition and establishing attribution to SLE is a challenge. Such attribution, according to the ACR nomenclature (5), requires exclusion of CNS infection, metabolic disturbances, substance-induced or drug-induced (or withdrawal) delirium, and any mental or neurologic disorder unrelated to SLE. In their review of clinical syndromes associated with CNS involvement in SLE, Jennekens and Kater (72) list only five potential causes of SLE-induced delirium/encephalopathy, acknowledging that not all causes can be clearly defined, including small vessel vasculitis/vasculopathy, leukoencephalopathy, perivenous spongiform encephalopathy, brain edema as a consequence of cerebral venous thrombosis and the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Although they also exclude “hypertensive encephalopathy” as being a “secondary manifestation,” they state that it “may well be the most frequent cause of delirium/encephalopathy in SLE.”

Anxiety Disorders

Depression and anxiety are common symptoms in lupus and are reported to occur in 24% to 57% of patients (6 ,7 ,8 ,9 ,10 ,37). However, as there are no features of these syndromes that are unique to SLE patients, there is often uncertainty about the etiology and attribution in individual cases.

Anxiety disorders as described in *DSM-IV* include a broad spectrum of behavioral disorders with panic attacks being common among many. In the context of NP-SLE, anxiety disorders are diagnosed on the basis of prominent generalized anxiety or panic attacks or obsessions or compulsions resulting in significant distress or impaired function. An anxiety disorder resulting as a consequence of NP-SLE would be considered as an “anxiety disorder due to a general medical condition” (293.89) according to *DSM-IV*. This requires that the anxiety is “judged to be due to the direct physiological effects of a general medical condition,”

and it is important to make this distinction from an adjustment disorder in which anxiety symptoms result from the stress of having lupus as a medical condition. Although it may be presumed that anxiety arising from an adjustment disorder is the more common presentation of clinically significant anxiety in SLE, there are no studies that have directly addressed this issue. Symptoms of anxiety can also occur in the context of agitation in association with delirium, but such a case could still be a manifestation of NP-SLE. Distinguishing between an anxiety disorder arising from underlying physiologic changes of the CNS versus direct CNS side effects of pharmacologic treatment of SLE or an adjustment reaction to distressing side effects of treatments (e.g., weight gain) can be extremely challenging.

As with all of the "neuropsychiatric" lupus manifestations, attribution of the presenting symptoms is difficult. According to *DSM-IV*, Panic Disorder has an estimated lifetime prevalence of 1.5% to 3.5% and 1-year prevalence of 1% to 2%. Although these figures may not be exceptionally high, panic attacks also have important cultural and gender issues that contribute to the difficulties in attribution for SLE patients because of the overlap with SLE itself. Specifically, Panic Disorder is diagnosed two to three times more frequently among women and the typical onset is from adolescence to the mid-30s, again overlapping with the typical onset of SLE symptoms and its age of diagnosis. Variable prevalence rates of anxiety disorders have been reported in studies of SLE samples. On one extreme is a retrospective chart review of 518 Chinese SLE patients from Hong Kong who had from 1 month to 23 years of follow-up in which apparently only two documented anxiety disorders were identified (75). However, this study stands in stark contrast to a 56% lifetime prevalence of phobia and 12% lifetime prevalence of generalized anxiety reported in Icelandic SLE patients (76). More recently, Sana et al. (9) reported a prevalence of only 7.4% in a sample of 323 SLE patients diagnosed retrospectively, presumably on chart review. Hay et al. (77) had reported a similar 8.2% point prevalence in 73 SLE patients attending a lupus clinic in Manchester, UK, based on responses to the Present State Examination (which determines the presence of anxiety disorders in accordance with *ICD-9* criteria). Chin et al. (78) also reported a similar 7.6% point prevalence of *ICD-9* "anxiety neuroses" in 79 consecutive Malaysian SLE patients. Although these modest prevalence estimates do not detract from the importance of anxiety disorders for those SLE patients experiencing them, it is debatable whether these prevalence rates are significantly higher than the general population. Given this, it may be difficult to consider them as clearly reflecting the consequences of an underlying neuropathologic condition.

In recent studies using the ACR nomenclature (5), Brey et al. (7) reported a 24% prevalence of anxiety disorders in their sample of 128 SLE patients from the San Antonio region who were apparently diagnosed using the Structured Clinical Interview for Psychiatric Diagnosis (79). Similarly, Sibbitt et al. (10) reported a 21% lifetime prevalence of anxiety disorders in 75 "pediatric" (i.e., symptom onset before age 18) SLE cases from New Mexico who were followed for an average of 6 years. Although it is tempting to place greater faith in the accuracy of these latter studies, they do represent selected samples from a relatively constrained geographic region with some racial and cultural variation that may also be relatively unique. Further definitive epidemiologic studies of psychiatric manifestations in SLE using current diagnostic methods are clearly necessary. To date, the studies remain difficult to compare because of case ascertainment differences and because of questions about population base rate differences that may reflect cultural and genetic differences in the populations.

Given these difficulties, one can reasonably ask whether other presenting features can support an association between anxiety disorders and CNS involvement in SLE. While a possible genetic contribution to panic disorders is acknowledged in *DSM-IV*, it is also pointed out that most patients do not have affected first-degree biological relatives. Nevertheless, the possibility that an association between anxiety disorders and SLE arise from a shared underlying genetic basis has been posited (80). A common physiologic mechanism underlying anxiety disorders and SLE is also supported by the presence of "anxiety-like" behaviors in animal models of SLE (80 ,81). However, it is important to distinguish between anxiety symptoms and anxiety disorders, something that must also be considered when examining data derived from self-report screening instruments for mood and anxiety symptoms.

In many respects, studies of anxiety symptoms in SLE must be understood as a line of inquiry distinct from that of studies of anxiety disorders as a manifestation of NP-SLE. This does not diminish their importance, however, and making this distinction evident to patients as well as clinicians may help to separate these possibilities. Based on their prospective study of 23 SLE patients, Ward et al. (82) found that anxiety symptoms changed in parallel with global SLE disease activity as measured by the systemic lupus activity measure (SLAM). Segui et al. (83) also reported a decline in anxiety symptoms associated with the change from active to quiescent disease activity. Although these studies suggest that the distress resulting from active lupus increases anxiety symptoms, such conclusions about causation are tenuous. Ishikura et al. (84) in a sample of 84 female Japanese SLE patients found anxiety symptoms to be associated with lack of knowledge about SLE and its management at the start of treatment. Thus, improved knowledge about SLE and its treatment amongst lupus patients may both reduce the likelihood of future anxiety symptoms and in the process improve the likelihood of recognizing an anxiety disorder that reflects a clinical manifestation of NP-SLE.

Mood Disorders

Mood disorders as described in the ACR nomenclature for NP-SLE (5) include the equivalent of *DSM-IV* major depressive disorder (MDD), that is, one or more major

depressive episodes (2 weeks or more of depressed mood or loss of interest plus at least four other symptoms of depression). Also included is mood disorder with depressive features that is compatible with the *DSM-IV* dysthymic disorder (at least 2 weeks of depressed mood for more days than not plus additional symptoms of depression not meeting the criteria for MDD). As well, mood disorders with manic features and mixed features are included, compatible with *DSM-IV* bipolar disorder (depressive episodes accompanied by manic, mixed, or hypomanic episodes). Such conditions must be distinguished from substance-induced mood disturbances and from *DSM-IV* adjustment disorder with depressed mood (symptoms of depressed mood, tearfulness, feelings of hopelessness developing within 3 months of an identifiable precipitating stressor).

Some recent prevalence studies using the ACR nomenclature (5) have suggested very high prevalence rates for mood disorders among SLE patients. Brey et al. (7) reported major depressive-like episodes in 28%, and mood disorders with depressive features in 19% of their sample of 128 subjects. Mood disorders with manic (3%) and mixed (1%) features were uncommon. Ainiala et al. (85) reported a similarly high prevalence of mood disorders (43%) in their sample of 46 SLE patients. However, a much lower prevalence of mood disorders has been reported by Sanna et al. (9) in 16.7% of their patients, by Hanly et al. in 14.4% (8) and by Mok et al. in 6% (75). Unfortunately, many studies have failed to report the number of SLE patients who were considered to have mood disorders associated with an adjustment disorder. For those reporting the higher prevalence rates, this clearly begs the question. Thus, to date, epidemiologic studies of mood disorders in SLE remain limited despite the common acceptance of an increased prevalence of depressive symptoms in patients with SLE.

Although it is important to distinguish between clinical syndromes of mood disorders such as MDD and depressive symptoms that occur in other contexts, studies using screening and survey methods do not always draw this distinction clearly. Commonly used screening instruments such as the Beck Depression Inventory (BDI) (86) include symptoms, such as fatigue, sleep disturbance, worries about health, concerns about physical appearance and loss of appetite that overlap with those of autoimmune disorders and many other medical conditions. The result of poor specificity can be poor positive predictive value of screening instruments for depression when used in SLE samples (87). Studies comparing the results of various screening measures advance our understanding of these instruments but do little to accurately determine the prevalence of mood disorders per se (87 ,88). Associations between symptoms of depression and other disease manifestations of SLE have been reported in some studies (89 ,90) but not others (84 ,91). However, limited sample sizes and differences in study samples and methodologies make comparisons across studies difficult.

As with anxiety disorders, attribution of mood disorders to a CNS manifestation of SLE is difficult. Patten et al. note that “Young people with long-term medical conditions have a particularly high prevalence of mood disorders” (92). Attributions of mood disorders to SLE require a biologic basis for the association between CNS effects of SLE and mood disturbance. Evidence for this association comes primarily from potential immune system effects on mood states; so-called psychoneuroimmunologic interactions (93). However, complex associations with other social and demographic factors such as knowledge of the disease and social supports (84) must also be considered. As described by Karassa et al. (70) in their study of suicide attempts in a clinic sample of 300 SLE patients, even though increased suicide attempts may be associated with NP-SLE, “we should not underestimate the importance of the psychological factors that coping with life threatening and unpredictable illness creates.” An unpredictable disease course and disabling features are not unique to SLE and it is perhaps not surprising that many of the NP syndromes commonly associated with SLE occur with comparable frequency in RA patients (11) and that appropriately matched groups of SLE and RA patients did not differ on screening measures of symptoms of depression and anxiety.

Psychosis

Using the *DSM-IV* diagnostic criteria, psychosis in the context of NP-SLE represents a psychotic disorder due to a general medical condition (293.0) (i.e., SLE) and is further characterized according to the presence of either delusions (293.81) (false belief despite evidence to the contrary) and/or hallucinations (293.82) (perceptual experiences occurring in the absence of external stimuli) as the predominant symptoms. When present, psychosis must be distinguished from unrelated primary psychiatric disorders such as schizophrenia, which has a similar age of onset as SLE. Also, it is important to recognize that hallucinations and delusions are frequent features of delirium and should be considered as such if they occur strictly within this context. More importantly for SLE patients, perhaps, is the likelihood of an association between psychotic features and pharmacotherapy in SLE including corticosteroids (94 ,95 ,96), antimalarials (97 ,98) as well as nonprescribed drug abuse. Psychosis is reported in up to 8% of SLE patients (6 ,7 ,8 ,9 ,10 ,99). Hanly et al. (8) reported a 3% prevalence, Brey et al. (7) reported a 5% prevalence of psychosis as a NP manifestation of SLE, whereas Sanna et al. (9) reported a 7.7% prevalence. However, Chau and Mok (96) report a 5% incidence of corticosteroid-associated psychosis identified using *DSM-IV* criteria in a 3-year prospective study of 126 SLE patients seen over 3 years. Thus, psychosis appears to be a rare but dramatic manifestation of NP-SLE that must be distinguished carefully from a primary psychiatric illness, delirium, and substance-related disorders. Future epidemiologic studies would benefit from explicit statements about the prevalence and incidence of these conditions in order to provide a better understanding of psychosis as a manifestation of NP-SLE.

Cognitive Function in SLE

Cognition is the sum of mental processes that result in observed behavior. These include reception and perception of external stimuli, processing and manipulation of acquired information, learning and memory, and expression (i.e., observable behaviors including, but not limited to verbal expression). Disturbance of any one of these processes can result in disruption of normal behavior and present as cognitive dysfunction. Cognitive functioning is often conceptualized as consisting of domains of abilities some, but not all, of which have clear neuroanatomical bases. The understanding of these neuroanatomical relationships, to some extent, reflects how broadly or narrowly one defines cognitive domains with those such as attention, memory, or language, being too broad to have simple neuroanatomical correlates. The complexity of cognitive abilities is such that dysfunction may be limited to a particular domain (i.e., focal) or may be extensive enough to cause global impairment, depending on the nature and extent of the domains affected.

When addressing the issue of cognitive dysfunction in SLE, the ACR nomenclature (5) avoided using *DSM-IV* criteria and case definitions. Indeed, no such criteria exist in *DSM-IV,* which addresses cognitive impairment via the construct of “dementia.” Because the case conceptualization of dementia is based on the presenting symptoms of Alzheimer disease, it can be difficult to apply even to persons with significant cognitive impairments arising from stroke (100), let alone persons with more subtle cognitive deficits arising from SLE.

That cognitive problems may be relatively mild and yet have significant functional impact is recognized in the ACR nomenclature (5). Such is known to be the case with multiple sclerosis, a disease with similar demographic characteristics to SLE (101 ,102). Reliance on subjective reports to identify cognitive dysfunction is problematic. Although such complaints are common among SLE patients (103), they also occur in many other clinical contexts and their relationship to objective evidence of cognitive impairment and specific neuropathologic processes remains largely unclear. Still, self-report of cognitive difficulties remains the primary means by which clinicians identify patients who may have significant SLE-associated cognitive dysfunction. The Cognitive Symptoms Inventory is a 21-item, self-report questionnaire designed for patients with rheumatic disease to assess self-perception of one's ability to perform “everyday activities” (104). Although a recent report suggests that this instrument may be useful as a bedside screening tool to identify SLE patients at risk for cognitive impairment (105), it has not been validated in relation to objective measures of cognitive performance. Unfortunately, cognitive status screening instruments developed for use in the diagnosis of dementia also have inadequate sensitivity for the identification of cognitive impairments in SLE (5). Although Leritz et al. (106) using the Mini-Mental State Examination (MMSE) (107) reported that 95% of patients' profiles of responses suggested a “subcortical” pattern of cognitive dysfunction, few of their patients scored within a range typically considered to represent impairment on this measure. Those that did demonstrate problems primarily had difficulties with serial sevens, a task that places demands on the processes of attention, working memory, and mental tracking. The challenge in considering SLE-associated cognitive dysfunction remains how to recognize and document mild, yet significant cognitive impairment.

In clinical practice, “neuropsychology is an applied science concerned with the behavioral expression of brain dysfunction” (108). As described in the ACR nomenclature (5), “documentation by neuropsychological testing” and interpretation “based on normative data appropriate for age, education, sex and ethnic group, wherever possible” is necessary to meet the diagnostic criteria of cognitive dysfunction. The complexity of human behavior is reflected in various classifications of cognitive functions that are examined through neuropsychological assessment with “batteries” of tests used to examine behaviors thought to reflect these functions. As a result, neuropsychological evaluation typically consists of extensive testing that is tailored to objectively detect cognitive dysfunction, especially in those areas identified by the patient as problematic or in those areas likely to be problematic on the basis of the suspected diagnosis. For SLE, the ACR nomenclature (5) has identified eight domains of cognitive functioning of particular importance: simple attention, complex attention, memory, visual-spatial processing, language, reasoning/problem solving, psychomotor speed, and executive functions, at least one of which must be affected in order to meet the case definition of cognitive dysfunction. As with most such classification systems, the distinctions between domains are at best blurred. Lezak (108), for example, excludes “attentional functions” from her classification of cognitive functions, referring to them instead as “mental activity variables” that underlie other cognitive functions. Similarly, “psychomotor speed” or the speed and efficiency with which mentally demanding tasks can be completed, might best be thought of as a “mental activity variable,” whose influence is seen in the performance of tasks that examine other cognitive abilities such as memory, language, visual-spatial processing, or executive functions. These latter domains themselves have numerous subcategories of functions each with accompanying volumes of scientific literature. Nonetheless, the ACR nomenclature (5) provides a valuable framework that establishes the importance of considering both cognitive functions with reasonably well-defined neuroanatomic substrates, and more diffusely represented mental activities that potentially cause the presenting behavioral problems in SLE. This framework then allows for the operationalization of domains of cognitive impairment using a standardized neuropsychological assessment (5).

Attempts to quantify cognitive dysfunction using standardized neuropsychological test batteries and predetermined thresholds for defining abnormal cognition, such as performance greater than two standard deviations below the mean of the general population have been used in

some studies. However, although this “actuarial” approach has the benefit of standardization for population-based studies, it is not ideal for examining complex multidimensional constructs such as cognition in individual cases. For example, a patient with a superior premorbid educational and functional level may report cognitive difficulties, but test at a normal (i.e., average) level compared to the general population. For this individual, however, average test performance may represent a significant decline in their cognitive and functional ability. Furthermore, the common practice of combining individual neuropsychological test scores into index variables can serve to mask deficits in specific areas of cognitive functioning (109). Individual test results may best be expressed in relation to estimated premorbid level of function or competence. Thus, as recognized in the ACR case definition (5), an individualized approach to patient assessment that accounts for psychosocial, demographic, and clinical characteristics is necessary for clinical decision-making.

Cognitive dysfunction, assessed using neuropsychological assessment techniques, has been reported in 12% to 87% of patients (110 ,111 ,112 ,113 ,114 ,115). Since the publication of the ACR nomenclature, at least two studies have applied these case definitions to their general lupus populations and reported prevalence rates of 80% (85) and 11% (9) for cognitive dysfunction. A third study also used the ACR-recommended neuropsychological test battery in conjunction with a new computerized test of cognitive efficiency and identified at least mild cognitive impairment in 78% of SLE patients thereby making it one of the three most commonly identified NP-SLE syndromes in their sample (7). As the majority of SLE patients with cognitive impairment have relatively mild deficits, the careful selection and assessment of cognitive performance in control groups is of critical importance in order to define expected levels of function in comparable groups of healthy individuals and those with other chronic diseases. A variety of neuropsychological testing methods have been used to study SLE patients (77 ,110 ,111 ,113 ,116 ,117 ,118 ,119) with the prevalence estimates for cognitive impairment varying in parallel with the testing methods used, the cut-off or threshold for definition of impairment, and the clinical and demographic characteristics of the study samples (114 ,120). Nevertheless, in spite of the inconsistencies in prevalence estimates for cognitive impairment, recognition that a large proportion of SLE patients may have at least mild cognitive impairment, even without recent disease activity or overt signs of CNS disease (77 ,110 ,113) prompted the emphasis on cognitive assessment in the current ACR nomenclature (5). Whether the development of consensus opinions about the appropriate neuropsychological tests to be used will result in greater consistency in prevalence estimates remains to be seen.

There is no specific or unique pattern of cognitive impairment in SLE but abnormalities include overall cognitive slowing, decreased attention, impaired working memory, and executive dysfunction (e.g., difficulty with multitasking, organization, and planning). Many of these studies provide support for a pattern of cognitive dysfunction consistent with pathology affecting subcortical brain systems (121). For example, the most frequently reported deficits across studies are evident on tests of immediate memory or recall, verbal fluency, attention, information processing efficiency, and psychomotor speed (7 ,113 ,117 ,119 ,122 ,123 ,124). Fisk et al. (118) found that increased SLE disease activity was related to impaired immediate memory and concentration. Denburg et al. (122)) noted that slowed processing is consistent with a diffuse vasculopathy. Loukkola et al. (125) suggested that their findings of predominant problems in psychomotor speed, complex attention, and memory reflect the “nonspecific” nature of CNS involvement in SLE, although this pattern again is consistent with predominant subcortical pathology. A similar “subcortical” pattern is also considered a hallmark of cognitive dysfunction in MS (126). Although SLE and MS are different disease processes, both can present with a spectrum of brain pathology and there are some neuropathologic similarities between the two. For example, diffuse effects on white matter fiber tracts, including demyelination are seen in both MS (127) and in SLE (12 ,128 ,129 ,130 ,131 ,132 ,133). Thus, it is perhaps not surprising that Shucard et al. (134) found SLE and MS patients to have similar cognitive deficits and similar compensatory strategy use when performing a test of information processing speed and efficiency, the Paced Auditory Serial Addition Test (PASAT) (135). MS patients and healthy controls were both known to “chunk” items on this test as reflected by a decreasing percentage of paired correct responses with increasing stimulus presentation rate (136). Shucard et al. (134) found that, as with MS patients, those with SLE showed a significant reduction in the percentage of paired correct responses, even at the slowest presentation rates. This poorer performance by the SLE group was not accounted for by disease duration, disease activity, depression, or fatigue.

Although comprehensive neuropsychological test batteries, using individualized assessment approaches, may have the sensitivity to identify subtle cognitive deficits in SLE, for many individual patients these deficits will be “subclinical” in the context of routine clinical practice. For example, a review of 14 cross-sectional studies of cognitive function in SLE revealed subclinical cognitive impairment in 11% to 54% of patients (120). This latter observation raises a number of important clinical questions. For example, what is the evolution of these cognitive deficits over time and does their detection predict the subsequent development of other more profound clinically overt forms of NP disease? Although cognitive impairment may be viewed as a distinct subset of NP-SLE, it can also serve as a surrogate of overall brain health in SLE patients that may be affected by a variety of factors including other NP syndromes.

Change in Cognitive Function Over Time

Prospective studies that have examined the course of cognitive functioning in SLE patients have not found that the

overall point prevalence of cognitive dysfunction increases over time, with individual subjects oscillating between being impaired and unimpaired at different points in time (124 ,137). For example, in a 5-year prospective study of 70 SLE patients by Hanly et al. (124) using standardized neuropsychological tests the prevalence of overall cognitive impairment in SLE patients fell from 21% to 13% over the period of study. Five patterns of cognitive performance were observed over the 5-year period of this study (Fig. 38-3). Eighty-three percent of patients were either never impaired or had resolution of cognitive impairment without specific therapeutic interventions. An additional 13% of patients demonstrated an emerging or fluctuating pattern of impairment and only 4% (two patients) showed persisting deficits that were stable over time. Thus, patients who were impaired did not demonstrate a marked deterioration in cognitive performance and the most frequent change seen over time was that of improved function. Similar benign changes in cognitive performance over time have been reported by Waterloo et al. (138) in 28 patients over 5 years, by Hay et al. (137) in a 2-year prospective study and by Carlomagno et al. (139).

Attempts have been made to examine predictors of cognitive decline over time. Hanly et al. (124) compared patients who were cognitively impaired at a baseline assessment to those who were not impaired, but found that the differences between groups on tests of recent memory and delayed free recall decreased over the subsequent 5 years. A similar result was reported by Waterloo et al. (138). However, those patients who had had clinically overt NP-SLE at any time in their disease course had a statistically significant decline in memory performance over 5 years when compared to patients without a history of clinically overt NP-SLE (124). These results suggest that although the identification of subclinical cognitive impairment can have important current clinical implications, it is the occurrence of clinically overt NP events that has greater relevance for the accumulation of cognitive impairment over time. Thus, fluctuation in cognitive function in SLE patients appears to be common and although deterioration in cognitive functioning may occur in select individuals, there is little evidence to suggest that it need be inevitable or profound, even in subjects presenting with cognitive impairment.

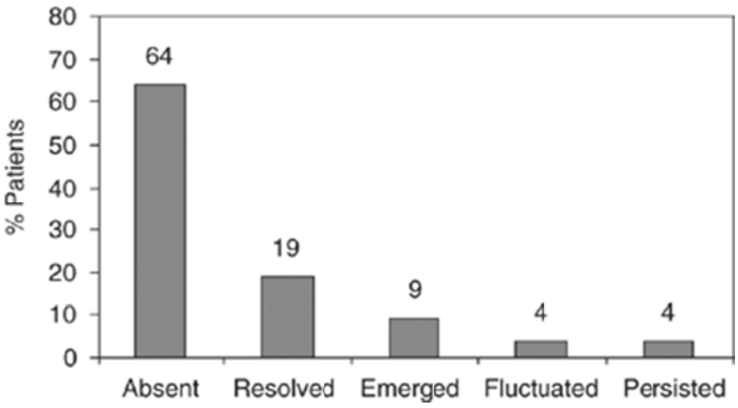

Figure 38-3. Change in cognitive function in 47 SLE patients assessed prospectively on three occasions over 5 years. Derived from Hanly JG, Cassell K, Fisk JD. Cognitive function in systemic lupus erythematosus: results of a 5-year prospective study. *Arthritis Rheum* 1997;40(8):1542–1543.

Etiology of Cognitive Impairment in SLE

Cognitive Function, Global SLE Disease Activity, and Overt NP-SLE

Chronic medical illness provides many potential generic causes of subtle cognitive dysfunction (Table 38-3). Determining whether they cause or contribute to cognitive dysfunction in SLE patients requires careful consideration on an individual basis, but the accumulated evidence suggests that such explanations alone are insufficient to account for the overall burden of cognitive impairment in SLE. The relationship between cognitive dysfunction and overall disease activity in SLE remain unclear. In one study (118), the classification of subjects into groups with inactive, mildly active, or active disease on the basis of the SLE

disease activity index (SLEDAI) scores revealed consistently poorer performance on tests of attention and memory for patients with active SLE. However, another study did not find SLEDAI scores to be significantly associated with global cognitive function (115). Additional studies have also failed to find an association between cognitive impairment and disease activity when using other methods of assessing global SLE disease activity (140 ,141).

Table 38-3: Non-SLE Causes of Cognitive Dysfunction

Causes	Examples
Direct CNS disease or injury	Ischemia Traumatic brain injury Cerebral hemorrhage Neurodegenerative disorders
Systemic illness	Hypertension Hyperthyroidism Hypothyroidism Fever
Medication	Beta blockers Antihistamines Antidepressants Antiepileptics Nonsteroidal antiinflammatory drugs
Psychological or psychiatric	Mania
Disturbance	Depression Anxiety Psychosis
Metabolic disturbance	Hyper or hypocalcemia Hyper or hyponatremia Uremia Hypoxemia
Pain	Acute or chronic
Fatigue	Acute or chronic
Sleep disturbance	Fatigue/daytime somnolence Sleep apnea

Modified from Hanly JG, Harrison MJ. Management of neuropsychiatric lupus. *Best Practice and Research Clinical Rheumatology* 2005;19(5):799–821.

Various cross-sectional studies have compared the rates of cognitive impairment between SLE patients with and without a history of clinically overt NP-SLE, although many of these predated the development of the ACR nomenclature (5). As NP-SLE events, such as stroke and antiphospholipid antibody–associated multifocal infarction, would be expected to have associated, if different, patterns of cognitive dysfunction, it is perhaps not surprising that the prevalence of cognitive dysfunction in patients with past or current NP-SLE has been found to be greater than in those with no such history (110 ,111 ,113 ,114 ,124 ,125).

Cognitive Function and Psychiatric Morbidity

Mood and psychological distress, even in the absence of a frank psychiatric diagnosis, are known to influence cognitive functioning as well as to alter performance on neuropsychological tests (108). The high prevalence of psychological disturbance in SLE has led some to hypothesize that SLE-associated cognitive dysfunction is primarily because of the psychological impact of the underlying disease. However, studies to date have both supported (115) and refuted (142) this hypothesis. Longitudinal data suggest that SLE patients who have psychiatric disturbance experience an improvement in cognition with improved psychiatric status at 1-year follow-up, although this improvement is not observed in patients with persistent psychiatric disorders. Hay et al. found that 21% of their sample of 73 SLE patients had a current psychiatric disorder and that these patients were impaired relative to SLE patients without psychiatric disorders on various verbal tests of cognition (140). Those patients whose psychiatric disorders resolved over the intervening year showed improved performance, whereas those whose psychiatric disorders persisted showed no change, and those who developed new psychiatric disorder showed decline (143). Monastero et al. (144) have also reported an association between the presence and level of depressive symptoms and neuropsychological test performance among SLE patients both with and without NP-SLE. However, while such associations suggest that psychiatric morbidity may coincide with cognitive dysfunction in SLE patients, no clear causal relationship between them has been established to date.

Cognitive Function and Medication

Most cross-sectional studies in SLE patients report no association between cognitive dysfunction and either the use (115 ,140 ,145 ,146) or dose of corticosteroids (115 ,134 ,140 ,146). Only Denburg et al., (147) in a study of a small sample of SLE patients, have suggested that brief exposure to low-dose corticosteroid has a positive effect on cognition. Hanly et al. compared those SLE patients who were receiving prednisone at three assessments during a 5-year follow-up period with those patients who were either not receiving prednisone at any assessments or were intermittently exposed to prednisone (124). A group-by-time interaction occurred only for a single neuropsychological test and no significant differences were found in pair wise comparisons between the three groups at any of the three assessments. Other medications may also have an impact on cognitive function (Table 38-3), but the potential benefits and risks must be considered in the clinical context in which they are used. Further careful study of these issues is required and the potential effects of pharmacologic treatment are best addressed through individualized assessment.

Cognitive Function and Immunologic Variables

The evidence that supports cognitive deficits being primary manifestations of SLE is derived largely from the study of autoantibodies in SLE cohorts with and without cognitive impairment. In particular, the potential role of antineuronal, lymphocytotoxic, anti-P and antiphospholipid antibodies has been examined.

Antineuronal antibodies, determined using human neuroblastoma cell lines as the source of antigen, and brain cross-reactive lymphocytotoxic antibodies have been associated with cognitive impairment in SLE patients studied at a single tertiary referral center (117), but these findings have not been confirmed by independent studies (148 ,149). Furthermore, although the identification of the fine antigenic specificity of antineuronal antibodies has so far not lead to more robust clinical-serologic associations, the possibility of specific antibody-induced brain injury in SLE remains an intriguing possibility. In this regard the recent identification of anti-NR2 antibodies and their clear pathogenic potential in animal models for inducing neuronal injury provides a new opportunity to explore this mechanism of brain injury in SLE patients. However, despite the intriguing results from animal studies, the findings from clinical studies have so far yielded conflicting evidence in support of this association (30 ,31 ,32).

Anti-P antibodies, which have been associated to a variable extent with psychosis and depression in SLE patients (33 ,34 ,36 ,37), have also been examined in for their association with cognitive deficits. Although the studies are limited in number (149), the evidence to date does not support an association.

The strongest association between cognitive impairment and autoantibodies in SLE patients has been found with antiphospholipid antibodies. For example in a study of 118 SLE patients, 33% of whom were positive for the lupus

anticoagulant (LAC) (122), there was a significantly greater proportion of individuals with cognitive impairment in LAC-positive (50%) compared to LAC- negative (25%) patients. The association between cognitive function and anticardiolipin (aCL) antibodies has been examined in a number of cross-sectional and prospective studies. In work completed at our own center, 51 SLE patients were divided into those who were persistently aCL antibody positive or negative on the basis of up to seven antibody determinations over a 5-year period (43). The relative change in performance on individual neuropsychological tests was then compared between patients who were antibody positive and negative. Those who were persistently IgG aCL antibody positive demonstrated a greater reduction in psychomotor speed compared to those who were antibody negative. In contrast, patients who were persistently IgA aCL antibody positive had significantly poorer performance in conceptual reasoning and executive ability. Similar results have been reported by Menon et al. in a 2-year prospective study of 45 SLE patients (42). These data suggest that IgG and IgA aCL may be responsible for long-term subtle deterioration in cognitive function in SLE patients.

Neuro-Imaging

Several neuro-imaging modalities have been used to visualize structural and functional abnormalities in SLE patients, particularly those with neuropsychiatric and cognitive manifestations (Table 38-4). Methods advocated for diagnostic use by the ACR Research Committee in 1999 (5) included computed tomography (CT), standard magnetic resonance imaging (MRI), and electroencephalogram (EEG). A multitude of other methods have also been employed in SLE research studies and in the clinical care of SLE patients, each of which possesses capabilities that offer unique perspectives on nervous system disease. In the management of individual patients neuro-imaging is most frequently used to support a clinical diagnosis of NP-SLE. However, there is also a growing interest in determining whether neuro-imaging can be used to distinguish between nervous system damage and disease activity that is potentially reversible and thereby amenable to therapy. Over the last decade, numerous studies have examined brain structure and function in SLE patients and the relationship to disease activity, disease course, and clinical outcomes.

Differences between Brain Structure and Function

In order to fully appreciate the brain imaging modalities that are currently available for clinical and experimental purposes, it is necessary to draw a distinction between different properties of the brain that these technologies are designed to examine. In particular, it is important to distinguish between the observed structure of brain tissue and brain function.

The distinction between structure and function can be conceptualized as a “brain vs. mind” dichotomy. Although the “brain” is a physical object that is tangible, the “mind” is a concept that can only be inferred indirectly based on observation of behavior or cognitive processes. Structure, therefore, refers to anatomy that can be observed with the naked eye, microscopically, or on an image that provides a static “picture” of the brain. Examination of structure over time can provide insight into dynamic processes of anatomical changes such as changes in the volume of the ventricles with age (150) that reflect cell loss, or to track displacement of anatomic structures in the brain as a result of a tumor. Both normal and abnormal changes in structure can sometimes be correlated with observable behavior and cognitive processes. For instance, it is widely accepted that damage to the medial temporal lobe is associated with difficulties in forming new memories (151). Such associations are thought to represent what different areas of the brain or distinct parts of the brain “do.” This is function.

Clearly, brain function is closely tied to structure but there is no precise mapping of one onto the other. For example, although the function of speech production is known to be associated with Broca's area (opercular and triangular sections of the inferior frontal gyrus), this function is complex and involves much more than simply verbal production of words. Thus, numerous other brain regions are implicated in the various cognitive processes necessary for the final outcome of speech production (152). From the standpoint of imaging and physiology, function can be conceptualized as the biological and biochemical mechanisms within the anatomical structures. Thus, brain function is often measured in terms of blood flow, brain metabolism, and biochemistry. Quantification of these physical phenomena requires more than an image, because they are not static.

Imaging Modalities: Uses and Efficacy

Modern neuro-imaging originated with the discovery of x-rays, and has evolved from the production of rudimentary two-dimensional brain images to highly sensitive and spatially detailed three-dimensional structural imaging and to quantification of functional indicators (153). The following section will briefly describe various imaging modalities that have been applied in the diagnosis and investigation of CNS abnormalities, including those occurring in SLE patients.

Computed Tomography (CT)

CT was the first neuro-imaging technique to be applied in research and in clinical practice in the 1970s and revolutionized our ability to examine brain tissue in vivo (153). This x-ray technique produces two-dimensional images of the organ of interest according to variable x-ray densities produced by differential concentrations of electrons in brain tissue. To obtain a CT image of the brain, an x-ray source and a detector are rotated around the head at ear level.

The source emits a narrow x-ray beam that is attenuated as it passes through the tissue, which absorbs the energy. The detector measures this reduction in the density of the x-ray from its origin to its destination on the other side of the head (152 ,153). The structure of the brain is reconstructed based on the strength of x-ray signals that reach the detector at different points of rotation, so the tissue types or substances in the brain are spatially mapped according to their x-ray permeability. Substances like bone that efficiently absorb x-rays allow only a fraction of the originally emitted energy to reach the detector and appear very light on the final image. CSF, which is highly permeable to x-ray energy, appears dark, whereas blood and other tissue types appear as different shades of gray (152 ,153). If x-ray opaque contrast agents are injected intravenously it is also possible to visualize blood vessels.

Table 38-4: Neuroimaging Modalities and Findings in NP-SLE

	Structural Imaging Techniques				Metabolic and Functional Imaging Techniques			
	CT	**MRI**	**MTI**	**DTI**	**MRS**	**fMRI**	**PET**	**SPECT**
Technique	Based on variable x-ray permeability of different tissues to reconstruct a 3D image of a structure.	Uses radio waves in presence of a strong magnetic field. The 3D image is reconstructed on the basis of differential tissue energy reemission rates.	Uses MRI platform. MTI measures transfer of protons between myelin and free water. Results expressed as magnetization transfer ratio histograms reflecting structural integrity of the tissue.	Based on the MRI platform. DTI produces histograms that reflect structural integrity of white matter tracks.	MRS utilizes the MRI platform and provides an MR spectrum with peaks for individual brain metabolites in circumscribed areas of the brain. MRS is an experimental technique and is not yet in clinical use.	Based on the MRI platform, this technique monitors blood flow and oxygen utilization to determine areas of brain activity associated with various tasks.	Based on the CT platform this technique detects areas of brain activity by tracking primarily glucose and oxygen metabolism.	Similar to PET, SPECT uses imaging of single photons emitted by radiologic tracers to track relative changes in regional cerebral blood flow.
Detectable Pathologies	1. Cerebral atrophy, 2. Focal white matter damage, 3. Infarcts, 4. Hemorrhage, 5. Meningeal thickening,	T1-weighted images: Same pathologies as CT. T2-weighted images: 1. Gray and white matter hyperintensities that may reflect edema and inflammation. 2. Potential to differentiate acute from chronic lesions.	1. Global brain damage and total lesion load. 2. Loss of myelin or axons in brain that may appear normal on conventional MRI.	Damage to the parenchymal structure even in absence of detectable abnormalities on conventional MRI scans.	1. Decrease in number and integrity of neurons 2. Myelin loss, 3. Inflammation 4. Abnormalities found in brain lesions or in normal appearing brain on conventional MRI.	1. Potentially useful in localization of brain function 2. May assist in assessing functional disconnectivity among brain regions.	1. Multifocal metabolic and blood flow abnormalities that may have functional correlates 2. Decreased metabolism and/or blood flow in areas that appear normal on MRI or CT may reflect damage in afferent pathways from areas with visible damage.	1. Multifocal metabolic and blood flow abnormalities that may have functional correlates 2. Decreased metabolism and/or blood flow in areas that appear normal on MRI or CT may reflect damage in afferent pathways from areas with visible damage.
Findings in SLE	Sensitive to gross abnormalities that are poorly correlated with specific neuropsychiatric manifestations. Abnormalities detected indicate structural changes, which are irreversible.	In presence of chronic cognitive deficits, MRI can detect associated focal damage. May detect gadolinium enhanced acute brain lesions indicative of active disease. White matter hyperintensities of uncertain significance Gray matter hyperintensities and edema may be indicative of active NP-SLE.	Measures of global brain damage using MTI are associated with neurologic and psychiatric symptoms.	DTI indicates loss of white matter tracks in patients with NP manifestations but it is unclear if these abnormalities are reversible.	Reduction in NAA is associated with various clinical manifestations of NPSLE including psychosis, confusional states, and cognitive impairment. Elevation of Cho is indicative of cell membrane or myelin loss and inflammation. Increase of Myo may be indicative of inflammation.	This technique is still largely experimental, and no studies with SLE patients are available at this time.	Patients with active NP-SLE experiencing psychiatric symptoms show decreased glucose metabolism in prefrontal and inferior parietal lobes bilaterally and in the anterior cingulate region.	Patients with active NP-SLE experiencing psychiatric symptoms and cognitive impairment are more likely to exhibit hypoperfusion compared to NP-SLE patients without such manifestations. Up to 50% of SLE patients may have such abnormalities in the absence of clinical findings.

Limitations of CT include its relatively rudimentary resolution. Although the contrast of the image can be adjusted to target the tissue types of interest, the results may still be difficult to interpret. In diseases that alter brain tissue density, it can be difficult to differentiate damaged tissue from the healthy tissue of similar x-ray permeability. For example, because white matter is less permeable to x-rays than gray matter because it contains more fat (e.g., myelin) and less water, demyelination serves to minimize contrast between the two on CT. Thus, diffuse, as opposed to focal, white matter demyelination can be difficult to resolve on CT images. This difficulty in distinguishing between soft tissue types because of relatively small differences in x-ray density is exacerbated by the substantial difference in x-ray permeability between soft tissue and bone. As a result, CT images tend to exhibit white artifacts in the “bony” areas of the brain (e.g., posterior fossa, temporal lobe, or any area close to the scull) that overwhelm small differences on the gray scale (152).

CT is sensitive to cerebral atrophy, which has been detected in 29% to 59% of the patients with NP-SLE (154). Other pathology, such as infarction, hemorrhage, and meningeal thickening as a consequence of inflammation may be detected as well. Although there has been some evidence that CT may also detect white matter abnormalities reflecting edema (154), these findings have not been substantiated. Also, CT may well be insensitive to the diffuse pathologies most frequently associated with NP-SLE, including chronic white matter demyelination and small infarcts (155). Overall, while CT may detect chronic damage in SLE, it is not helpful in identifying active inflammatory disease processes within the nervous system (156 ,157).

Conventional Magnetic Resonance Imaging (MRI)

Since its introduction into clinical use in the early 1980s, MRI has overtaken CT as the standard neuroimaging technique. MRI offers a number of advantages compared to CT in terms of clinical assessment and research design, because it is noninvasive and poses no health hazards even across multiple scans (as opposed to x-rays). Therefore MRI can be used repeatedly for longitudinal studies (153). Additionally, most commonly used MRI images offer better spatial resolution than those produced using CT. Although MRI still relies on the capacity of the tissue to absorb and re-emit energy in proportion to the concentration of water (hydrogen ions) in the tissue (153), the energy is delivered via radio waves in the presence of a strong magnetic field that is thousands of times greater than that of the Earth. The strength of an MRI magnet is expressed in Tesla units, a measure of magnetic flux density. Since hydrogen nuclei have an odd number of protons, they can be conceptualized as small magnets with their own local fields (153). Therefore, these nuclei align themselves with the direction of the main magnetic field when the patient is placed into the bore of the magnet. Once aligned, the nuclei absorb and emit electromagnetic energy at a resonant frequency that is specific to their surroundings (e.g., water, brain tissue) (152). The signal used to construct an MRI image is obtained by disturbing the alignment of the nuclei via application of radiofrequency (RF) pulses at various angles to the direction of the main magnetic field. MRI allows for application of RF pulses to highly circumscribed areas of the brain, allowing imaging of “slices” of tissue as thin as 1.5 mm. These pulses “flip” the nuclei into the plane transverse to the direction of the main field and rotate them in sync. Once disturbed in this manner, the nuclei re-emit the absorbed energy at different rates, producing a measurable signal (152). Nuclei in tissue that contains a high proportion of water (e.g., CSF) re-align with the dominant field, whereas tissue that is high in fat (e.g., white matter) and low in mobile water ions re-emit energy slowly. These processes of energy re-emission and re-alignment with the magnetic field generate two main time constants that form the foundation of the signal: T1, which is the time it takes the nuclei to “relax” and to return to their normal alignment within the magnetic field and T2, which measures how fast the nuclei lose alignment with each other. Both of these vary among tissue types within the healthy human brain and change in the presence of pathology (153 ,158). Focusing on one of these two time constants (i.e., weighting the image) by varying the times between applications of the RF pulses emphasizes different parameters of the tissue. A T1-weighted image is characterized by dark CSF and light white matter, whereas the opposite is true in a T2-weighted image (152). Such contrast allows visualization and detection of various pathologies. For example, white matter demyelination, which is not easily observed using CT, is readily apparent on T2 MRI images (152). A third MRI time constant, T2*, is used predominantly in functional MRI (fMRI) rather than in structural MRI (159). This relaxation type provides image effects that are similar to those produced by T2-weighted images, but while T2 decay is a result of magnetic fluctuations at a molecular level, the T2* decay is a result of large-scale inhomogeneities in the dominant applied magnetic field. Such inhomogeneities may be caused by the imperfection in the

magnet itself, or because the human head contains a variety of areas of differing magnetic susceptibility. Thus, inhomogeneities are especially profound at the boundaries between areas with different susceptibilities (e.g., air/tissue interfaces and in blood vessels), which causes very rapid T2* relaxations in such regions (159).

MRI is the preferred method of structural imaging in SLE (155). It is much more sensitive than CT for the detection of focal deficits (155) and allows visualization of large infarcts, hemorrhage, aneurysms, and transverse myelitis. Abnormalities on MRI scanning may be found in 19% (160) to 70% (161) of SLE patients. T2-weighted MRI images identify pathologic processes that cause edema and are more sensitive than T1-weighted images for the detection of abnormalities in patients with NP-SLE. Applying the technique of fluid attenuated inversion recovery (FLAIR), in order to dampen the CSF signal and highlight areas of edema, further enhances the utility of T2-weighted images (162 ,163). T2-weighted imaging also detects subcortical and periventricular white matter lesions. These lesions of increased signal intensity or brightness, referred to as “white matter hyperintensity” (WMHI) are often missed by CT, and are found in 20% to 50% of SLE patients regardless of the presence or absence of clinical NP disease. They can occur in up to 75% of SLE patients with antiphospholipid syndrome (APS) (164) but the strongest correlation is with age of the patient, overall disease severity, and disease duration (158). If the lesions are larger, occur in the corpus callosum or predominantly in a periventricular distribution and are seen on T1-weighted images, then the diagnosis of multiple sclerosis must be considered (162 ,165). Magnetic resonance angiography (MRA) permits visualization of cerebral blood flow, although it is probably not optimum for visualization of flow in small caliber vessels that are the ones primarily involved in NP-SLE.

Thus, although MRI is a sensitive technique it does not easily allow for differentiation of chronic CNS lesions from those that indicate acute changes attributed to SLE, or from CNS changes unrelated to lupus (158). This differentiation of chronic from acute disease is important in clinical decision making, because treatment often depends on determining whether the presenting symptoms are caused by active lupus, by pre-existing but currently quiescent SLE, or by non-SLE factors (155). Some guidelines have been suggested to facilitate interpretation of MRI findings in terms of disease activity or chronicity. For example, acute and reversible lesions often lack clear borders, have a filamentous pattern, and usually follow the gray-white matter junction along the sulci and gyri (154). Gray matter hyperintensity and edema in the cerebral hemispheres, the brain stem, or in the cerebellum are additional indicators of inflammatory disease (154 ,155). Determining the chronicity or acuity of a lesion can also be facilitated by intravenous gadolinium, which enhances active lesions on T1-weighted images (154) and indicates breakdown of the BBB. However, this method is not entirely reliable.

Gray matter edema may resolve over 2 to 3 weeks (160) following an acute NP event, especially in patients undergoing corticosteroid therapy (154). In a case study, Kashihara (166) examined the reversibility of MRI findings in association with treatment of NP-SLE. They suggested that laminar cortical necrosis visible on T1- and T2-weighted MRI images may be related to disease activity because of the co-occurrence with psychiatric symptoms and resolution with therapy. Their patient presented with a variety of NP symptoms including headache, fever, psychosis (olfactory hallucinations and delusions of persecution and reference), and psychomotor impairment. MRI images revealed laminar lesions bilaterally in the parietal and temporal cortex. Once the patient started steroid therapy, the clinical symptoms were alleviated within 7 months, and the lesions visible on T1- and T2-weighted images resolved in 1 and 5 years, respectively.

It is important to remember, however, that while detection of active disease is possible, it is very difficult with conventional structural imaging techniques such as CT and MRI. It is not uncommon for patients with active NP-SLE to show the same MRI results as those who are not in the active stage of the disease, and sometimes both groups of patients may exhibit normal MRI scans even when psychiatric symptoms are clearly present (154 ,155). Presentations that are likely to be accompanied by normal CT and MRI results include confusional states, affective disorders, and headaches (154). Finally, neither of these techniques can reliably differentiate SLE from other non-SLE disorders with similar behavioral and neurologic presentations, including vascular incidents unrelated to SLE, infectious meningitis, noninflammatory edema, or non-SLE related infarcts and hemorrhage.

Nonconventional MRI Imaging

Although conventional MRI is the most frequently used imaging technique, there are a multitude of other imaging modalities based on the principles of MR that provide unique information about brain structure, function, and biochemistry. These include magnetic resonance relaxometry (MRR), magnetization transfer imaging (MTI), diffusion-tensor imaging (DTI), magnetic resonance spectroscopy (MRS), and functional magnetic resonance imaging (fMRI).

Magnetic Resonance Relaxometry (MRR)

MRR is a method of quantifying T1 or T2 relaxation times in brain tissue. This has been used to identify potential structural markers of active NP-SLE associated with major NP events, such as seizures, psychosis, or coma (167). Since relaxation times of tissue may change in pathologic states because of changes in tissue density or chemical composition, relaxometry can add sensitivity to conventional MRI scans, particularly if changes in the tissue do not produce easily observable contrast changes on the conventional image.

Patropoulos et al. (167) used this technique to quantify T2 relaxation times in gray matter of NP-SLE patients in an attempt to detect edema. They included 10 patients experiencing major NP-SLE manifestations and 10 patients experiencing minor NP-SLE who were matched on measures of brain atrophy. Some patients with minor NP-SLE had a history of major symptoms that had resolved prior to the commencement of the study. They found an increase in the T2 relaxation time of the normal appearing gray matter in all patients within the major NP-SLE group compared to only 2 in the minor NPSLE group. This was interpreted as gray matter edema occurring predominantly in the major NP-SLE group. Cerebral edema may have various causes in active disease including vascular injury, release of neurotoxins from areas of inflammation, or the breakdown of the BBB (167) and MRR may yet prove to be a valid and feasible measure of the inflammatory component of active disease among patients with NP-SLE.

Magnetization Transfer Imaging (MTI)

MTI is a structural imaging modality that allows for detection of tissue damage, especially in terms of integrity of white matter tracks, that cannot be easily visualized using conventional MRI (153). The technique is based on quantification of the exchange of magnetization between macromolecule-bound protons in myelin and water protons in biologic tissues. As mentioned previously, MR derives its signal from free water protons. The bound protons are magnetized with a saturation pulse and a standard MR sequence is applied. When the pulse is terminated, there is a transfer of magnetization from the bound proton pool to the pool of free protons. The magnitude of the MR signal is measured before and after the application of the pulse and the change between the two measurement times allows for calculation of the magnetization transfer ratio (MTR) (153). The MTR is directly influenced by the amount of bound protons, which is proportional to the amount of myelin and can be assessed in regions of interest or in the whole brain (153 ,155). Therefore, a decrease in MTR usually signifies demyelination. The data obtained from three-dimensional MTI is displayed as a histogram that represents the pixel population of the entire brain image with an MTR value calculated for each pixel. As a pixel is the smallest two-dimensional component of an image, the histogram is very precise. A histogram consisting of a single, narrow, high peak represents a brain that is homogeneous in terms of MTR and has uniformly healthy tissue. On the other hand, when demyelination is present, the peak on the histogram becomes wider and lower because there is an increase in pixels with low MTR values. This indicates a reduction in healthy white matter and reflects the global lesion load in the brain (155). Such information cannot be easily obtained using conventional MRI that produces an image alone.

Studies of MTI in SLE patients conducted by Bosma et al. (168) indicated global brain damage in patients with NP-SLE. The findings on MTI significantly correlated with indicators of general neurologic functioning (Expanded Disability Status Scale) (169), psychiatric functioning (Hospital Anxiety and Depression Scale) (170) and cognitive functioning (cognitive impairment scores derived from the Wechsler Adult Intelligence Scale, Revised [WAIS-R]) (171). Furthermore, MTI abnormalities were detected in patients with a history of NP-SLE who were no longer in the active stages of the disease, and who had normal conventional MRI scans. This suggests that although psychiatric, cognitive, and neurologic symptoms of NP-SLE may resolve, the brain pathology that underlies them does not (172). Thus, MTI may assist not only in detecting pathology that cannot be seen with conventional imaging methods, but may also be useful in measuring progression of brain pathology resulting from NP-SLE, even after acute NP symptoms and signs resolve.

Diffusion Tensor Imaging (DTI)

DTI or the closely related but less sophisticated diffusion-weighted imaging (DWI) (173) provides additional information on white matter homogeneity and connectivity. DTI is based on the principle of isotropy (Brownian motion), which refers to unrestricted, chaotic movement of proton-containing molecules in free water. However, in the highly structured tissue of the brain, such as white matter and white matter tracks, it is easier for molecules to move in some directions than in others, thus creating preferential diffusion, or anisotropy. Pathologic conditions can disturb the highly structured integrity of the white matter fibers, causing loss of anisotropy and changing the diffusion behavior of the molecules (155). If RF pulses are applied at certain intervals, it is possible to obtain information about water molecule movement between pulses. This information is provided in the form of diffusion coefficients and contains vector information regarding the directions of diffusion. The level of fractional anisotropy (FA) can be calculated for individual pixels within a region of interest or for the whole brain, and the results are presented as a histogram with lower FA peaks indicating more pixels with higher diffusion values (155). Lower FA peaks indicate damage or degeneration in white matter tracks (153). DTI has been used to examine brain tissue changes in NP-SLE in terms of degeneration of parenchymal structure. Although patients with NP-SLE have been found to have more pixels with low FA values than healthy controls (174 ,175), it is unclear whether there is a difference in diffusion pattern between patients with active disease and patients who have chronic brain damage.

Thus, both MTI and DTI are capable of detecting changes in brain tissue that are not apparent on conventional MRI, but the interpretation of the results must take into account changes in the integrity of white matter that occur with age. Silver (176) collected MTI data from 41 healthy individuals for the purpose of establishing a normative database of MTR ratios for the white matter of healthy adults. They determined that ageing results in

decreased integrity of the white matter in the corpus callosum and frontal white matter and that these changes become apparent as early as 35 years of age.

Magnetic Resonance Spectroscopy (MRS)

Biochemical changes within living brain tissue can be examined by MRS (Fig. 38-4). This produces very different results from other MR techniques in that it provides information about the neurochemical composition of the tissue within a designated region of interest. Spectroscopy is most often added to an MRI scan as an extra series and adds approximately 7 minutes or more to the duration of the scan, depending on the number of regions of interest that are examined (155). It is generally sensitive to the presence of neurochemicals with fairly high concentrations (>1 mM). MRS results are displayed in form of spectra with peaks representing concentrations of various brain metabolites (158).

Proton magnetic resonance spectroscopy (1H-MRS) is the most commonly used method because, as with conventional MRI, it utilizes the signal provided by hydrogen, which is abundant in human tissue. The metabolites detected using this method typically include N-acetylaspartate (NAA). Choline compounds (Cho), Inositol (Ins), Lactate and Creatine (Cre) (155 ,158). NAA is found primarily in neurons and is represented by the highest peak on the spectral profile in healthy adult brains. This metabolite is considered to primarily indicate neuronal integrity, so a reduction in NAA indicates neuronal loss (158 ,177). The Cho peak reflects concentrations of phosphocholine and glycerophosphocholine, acetylcholine, and choline. It is associated with cell membrane breakdown and synthesis and loss of myelin. It is often elevated in patients who have suffered a stroke, brain inflammation, or acute white matter disease, all of which include membrane metabolism (177). The inositol peak represents concentrations of myoinositol and other inositol compounds and reflects membrane stabilization and turnover especially in glial cells. The concentrations of these substances may increase permanently or temporarily in conditions where membrane metabolism occurs. Lactate is undetectable in healthy brain tissue and its presence indicates anaerobic metabolism usually attributable to ischemia. The Cre peak on the spectral profile reflects concentrations of creatine and phosphocreatine. These substances are believed to be stable in most neuropsychiatric disorders, so Cre concentrations are often used as internal references to determine whether the quantities of other metabolites are elevated or diminished. However, the validity of this approach has been questioned as Cre can change in certain conditions, such as tumors (177). Although all of these metabolites can be observed at the standard clinical MRI field strength of 1.5 T, the inositol peak is only revealed when short echo time (TE) is used (35 ms or less) and is only evident as a single peak (177).

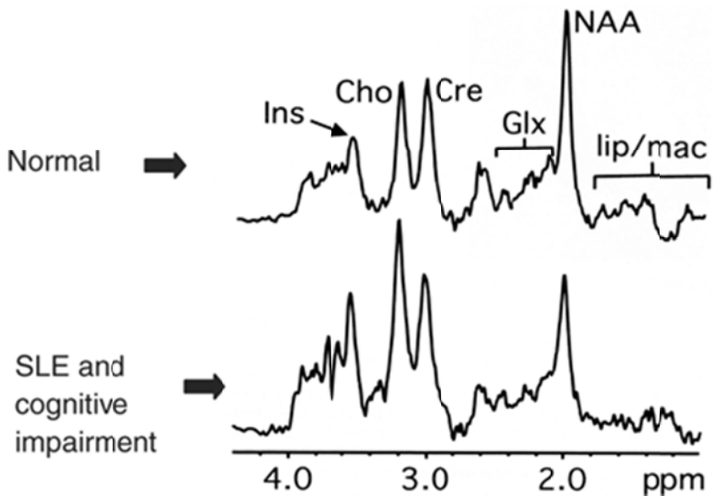

Figure 38-4. 1H magnetic resonance spectra from normal brain (upper panel) and SLE patient with neuropsychiatric SLE including cognitive impairment. From

Sibbitt WL Jr, Sibbitt RR, Brooks WM. Neuroimaging in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum* 1999;42(10):2026–2038 with permission.

Phosphorus-31 MRS (31P-MRS) is a less frequently used technique that assesses energy metabolism in the tissue as well as the concentrations of phosphorus-containing compounds involved in membrane synthesis (158). The principle measures of energy metabolism in tissue include phosphocreatine (PCr) and inorganic phosphate (158).

Some of the biochemical changes that have been noted with proton MRS in SLE and that have been linked to neurocognitive dysfunction include reduction in NAA in brain lesions seen on T2 conventional MRI images and in otherwise normal appearing gray and white matter (155 ,158). The findings are especially striking in focal lesions and likely represent areas of neuronal loss (158). Although reduction in NAA is by no means unique to SLE, it is strongly associated with various symptoms of NP-SLE, including psychosis, confusional states, and cognitive dysfunction (158). Although recovery of NAA levels has been observed (158 ,178), the circumstances governing the reversibility of decline in this neurochemical are poorly understood. It is clear, however, that reduced NAA is not characteristic only of active NP-SLE but is also seen in irreversible chronic brain injury (155).

The Cho peak has also been found to be elevated in NP-SLE in the absence of obvious structural abnormalities on conventional imaging. It has been suggested that elevation in Choline compounds can be a prognostic indicator, because it often reflects disease activity or inflammation (158). Elevation in Choline, combined with reduction in NAA has been linked to cognitive impairment in NP-SLE patients (155 ,158). Myo-inositol has also been found to be increased in normal appearing white matter of patients with NP-SLE, particularly in those with major CNS manifestations. This is felt to indicate inflammation (179). While 1H-MRS findings cannot be used to diagnose NP-SLE or to reliably differentiate it from disorders with comparable clinical presentations, MRS may be helpful in characterizing brain tissue damage particularly in the presence of otherwise normal imaging results (154).

Phosphorus MRS has been successful in identifying reduction of ATP and PCr in white matter of NP-SLE

patients (154). This is consistent with various features of NP-SLE, including cerebral ischemia, neuronal death, cell injury, or membrane turnover/degeneration (154). However, it is unclear whether these findings are unique to NP-SLE, whether they have diagnostic or prognostic utility, or whether they are correlated with specific neuropsychological or psychiatric manifestations.

There is some indication that changes observed in NP-SLE using CT and various MR techniques reflect the same pathologic processes from different perspectives. For instance, a feature of SLE that represents structural brain changes that are readily detected using CT or MRI is cerebral atrophy. Zanardi et al. (157) used CT to evaluate the relationship between the frequency and severity of cerebral atrophy and corticosteroid use, disease activity, and psychiatric manifestations in SLE patients taking steroids, non-SLE patients taking steroids, and healthy controls. Although SLE patients and non-SLE patients taking steroids were more likely to exhibit atrophy, SLE patients had more severe atrophy that was not accounted for by the corticosteroid use, disease duration, or disease activity. Nevertheless, the causes of cerebral atrophy in SLE patients remain unclear and studies are divided on the relative contributions of SLE per se and corticosteroid use (168 ,172). Cortical atrophy constitutes permanent damage that does not resolve with disease quiescence, and seizures have been reported as the most common clinical NP manifestation associated with cortical atrophy (157). Bosma et al. (168) found that brain parenchyma loses integrity and structure during active NP-SLE but that this abnormality in normal-appearing parenchyma can be detected using MTI even in absence of active disease. Damage in normal-appearing white matter can also be measured as decline in NAA on MRS and all of these measures may be used as markers of disease progression even in absence of active clinical disease (168 ,172). Loss of parenchymal uniformity using MTI and the metabolic abnormalities indicated by MRS may result from the same pathogenic mechanisms and lead to similar outcomes in patients with NP-SLE (180). The loss of parenchymal integrity and loss of NAA both indicate neuronal degeneration and axonal damage (180). Correlations between abnormal MTI findings (i.e., low MTR), abnormal findings on DWI (i.e., isotropy in diffusivity), and increased T2 on MR relaxometry support this suggestion (180).

Functional Imaging

MR imaging can also provided an assessment of brain function by means of fMRI. Activation of brain regions and networks during various types of tasks and cognitive states can be inferred by measuring physiologic changes, such as blood flow and oxygen utilization that accompany brain activity (181). The underlying assumption is that activity in a certain area of the brain causes an increase of blood flow to that area because of greater energy utilization at a molecular level. Although intuitive, it is important to appreciate that in the brain the relationship between activation and blood flow is complex. For example, some areas of the brain may be activated in response to a task and show an increase in blood flow, although involvement of other areas may manifest inhibition of synaptic activity and no increase in energy utilization. However, even inhibition can be associated with an increased demand for energy (181). Although there is a plethora of fMRI techniques that can be used to detect changes in blood flow and energy consumption, the method used most frequently is blood oxygenation level-dependent (BOLD) fMRI. In addition to being the most well developed method, it also does not require intravenous administration of contrast agents to track activation patterns. The presence of iron atoms in hemoglobin means that blood has magnetic properties, which reduces its MRI relaxation time. Blood also serves as a delivery mechanism for oxygen, and since oxygen is not very soluble in blood, it is transported bound to the iron atoms. When oxygen is bound to a hemoglobin molecule, the molecule's magnetic effect on its environment is reduced, which makes it a sensitive magnetic marker of blood oxygenation, and thereby indirectly reflects neuronal activity. As deoxyhemoglobin decreases, so does the signal from blood on the T2- and the T2*-weighted images. The opposite is true if blood oxygen levels rise. Thus, a positive change in signal on fMRI images in response to tasks (i.e., neuronal activation) is reflected by an increase in concentration of oxygenated hemoglobin (159).

Although fMRI has great potential, it has a number of limitations that have to be resolved before it can become a more efficient experimental or clinical tool. One of the problems is the need for multiple measurements and long scanning sessions. The signal changes generated by shifts in cognitive states or tasks are relatively small and in order to detect them numerous MRI acquisitions are required. The second limitation of fMRI is that, because this currently remains a new and experimental imaging procedure, there are no clear standards for interpreting associations of hemodynamic responses and neuronal activity (181). Understanding of this association is further impeded by a lack of knowledge about the exact mechanisms regulating regional blood flow. Additionally, fMRI is mainly useful for identifying activity in the gray matter where synapses are found, and not in the white matter and it has been argued that the fMRI signal reflects combinations of synaptic and dendritic charges (i.e., synaptic activity), rather than neuronal activity itself. Finally, there is no consistent relationship between excitatory synaptic activity and increase in the fMRI signal, perhaps because the contribution of inhibitory synaptic activity is variable and still poorly understood (181). Determining the exact area of activation is also problematic since the increased perfusion in the local tissue that results in the change in fMRI signal occurs on a larger spatial scale than the actual electric activation. Thus, the area of activation generally looks larger than it actually is (159). Functional MRI can be very informative of the “functional connectivity” (153) of brain

regions and disconnections can be indicative of pathology. To date, fMRI studies have not been conducted in SLE patients.

Thus, the MRI platform is a fruitful source of diagnostic and experimental information about the CNS and holds immense promise for the future of imaging in SLE. Nevertheless, it does have limitations. First, the integrity of the image is highly susceptible to movement, a particular issue in fMRI where acquisition is very time consuming (153). Additionally, MRI techniques are contraindicated for people who are claustrophobic, because the bore of the magnet is a confined space, and for individuals who have metal embedded anywhere in their bodies, as this could pose a fatal risk when exposed to high magnetic fields (153). Finally, the various abnormalities that have been detected are not restricted to a particular disease, but rather represent biochemical, functional, or structural changes, which may be the consequence of several immunopathogenic, inflammatory, or degenerative mechanisms.

When functional assessment is of interest, but fMRI is unavailable or contraindicated, positron emission tomography (PET) can be used. To some extent, this technique combines examination of brain function and biochemistry. It is based on the assumption that blood supply and glucose/oxygen metabolism within a region of the brain varies with changes in the biochemical processes that underlie the function of that anatomical region (182 ,183). Thus, as neuronal activity within an area of the brain increases, so does the blood flow to that area, and the demands for oxygen and glucose (183). One of the most valuable aspects of PET is that it is a fairly efficient method of detecting diffuse abnormalities or for localization of pathology (182). PET employs two technologies that allow for in vivo measurement and localization of neurologic processes: examination of radiological tracer kinetics and CT (182). The former provides information about the compartmental kinetics of glucose and oxygen metabolism, and blood flow, and can even assist in mapping white matter fibers and axonal projections (154 ,182 ,183). The PET scanner is designed to provide a CT image of the brain combined with concentration distributions of tracer-labeled products (182). Disadvantages of PET scanning include the exposure of patients to large doses of radiation (154 ,182), availability of radiopharmaceuticals, and cost.

Single-photon emission computed tomography (SPECT) is a more commonly available method of studying brain function. This technique was developed after PET and uses much of the same techniques (184). In contrast to the use of coincidental pairs of photons in PET scanning, SPECT uses brain images generated by CT to image single photons emitted by radiologic tracers in order to determine cerebral blood flow (185). Since photons are detected one at a time, their emission from tissue do not have an easily detectible spatial correlation, and have to be traced to their point of origin by the process of collimation (184). Collimation is key to obtaining a viable SPECT image and is “the process where electromagnetic radiation can be made into parallel beams” (185). As with PET, the data produced by SPECT is also in the form of slices that can be reconstructed in various planes over the entire 360 degrees of rotation around the patient (185). Most SPECT systems use a “gamma camera” that rotates around the subject either continuously or in discrete increments (”step-and-shoot”), where the imaging is taking place only when the system is stationary (185). SPECT also involves exposure to radioactive substances via intravenous injection or inhalation of tracers, with the latter method being more widely used (185). Some of the most commonly used tracer substances include: Iodine-123-IMP (Spectamine), Technetium-99m, Technetium-99mECD, and 99mTc-HMPAO (184).

Like other imaging techniques, SPECT also has drawbacks that must be considered (186). As with PET scanning, this technique requires exposure to radioactive substances. Since the tracers used in SPECT imaging of the CNS are most often injected into the blood stream, they circulate throughout the body before they reach the brain, and affect other organs that are not the focus of imaging (185). Furthermore, although there are over 1,000 known radioactive nucleotides, many of which can be used as tracers, SPECT radioisotopes for brain imaging must meet the following requirements: they must be able to cross the BBB; they must have a half-life sufficient to produce the images, but short enough to avoid prolonged radioactive exposure; the decay must produce photons with enough energy to escape the tissue, but not the detection system; the decay must produce more high-energy photons than low-energy ones to reduce the amount of the absorbed radiation; and the tracer must attach to the substance of interest without changing its properties (184 ,185). The substances mentioned above possess these characteristics to varying degrees, but they often underestimate regional cerebral blood flow (184). Also, the image resolution and detail produced by SPECT is inferior to other CT techniques, including both CT and PET. This is because the decay of the radioactive nuclides used to reconstruct the image is random, so only a fraction of it is detected (184 ,185). Finally, as is the case with other functional imaging modalities, SPECT does not provide a direct measure of brain activity, but rather measures concurrent physiologic changes in brain tissue that are correlated with it (184).

Despite these limitations, PET and SPECT scanning are two of the most frequently utilized procedures in studying NP-SLE. PET scans have often been found to be abnormal in NP-SLE patients, indicating multifocal metabolic and blood-flow abnormalities undetected with structural imaging methods, such as MRI and CT (154). However, just as with the other imaging techniques, the data obtained using PET have to be interpreted with caution as abnormalities in glucose absorption, oxygen use, and blood flow may not be indicative of active CNS disease. In general, nervous system damage associated with SLE, such as focal disease, may cause cell death, and the consequent decreased neuronal density may produce the same results on PET and SPECT as active NP-SLE. Furthermore, changes in blood flow and

metabolism can also occur in sites distant from those of the pathologic lesion, a phenomenon known as diaschisis (187), in which local neuronal activity is diminished in normal appearing brain as a result of loss of afferent input from a remote area of brain damage. Therefore, although PET may provide valuable functional information about NP-SLE that is not otherwise available, this technique has relatively high associated hazards and costs, and requires parallel anatomic imaging to be useful on a routine basis (154). SPECT may overcome the issue of cost and may be a valuable means of detecting various abnormalities in patients with NP-SLE. However, abnormalities observed using SPECT have not been found to differentiate patients with major NP-SLE features, such as stroke, seizures, or psychosis, from patients with milder NP-SLE features such as headaches, dizziness, and mild cognitive impairments (154). Furthermore, most such findings can be seen in SLE patients without NP-SLE, and may be chronic in some patients and reversible in others (154).

Komatsu et al. (188) used PET to compare 12 patients with active SLE, with and without psychiatric symptoms. They found that patients with active psychiatric symptoms showed decreased metabolic rates for glucose in prefrontal and inferior parietal lobes and in the anterior cingulated regions bilaterally that were not related to steroid use. These results are intriguing in that patients with active SLE but without psychiatric symptoms had normal PET scan results. In patients with psychiatric symptoms, the PET scan abnormalities and symptoms resolved concurrently with treatment. Similar results have also been reported by others using SPECT scanning (189 ,190). Huang et al. (190) examined 78 SLE patients: 48 with NP-SLE exhibiting psychiatric symptoms and 30 SLE patients without NP symptoms. They found that 90% of patients with psychiatric symptoms had regions of hypoperfusion compared to 20% of patients without such symptoms. The regions of hypoperfusion were most commonly found in the parietal lobe, and to a lesser extent in the frontal and temporal lobes, in the region of the middle cerebral artery. These results reflect the composite literature on SPECT scanning in SLE with areas of diminished uptake in 86% to 100% of patients with major NP events (e.g., stroke, seizures, psychosis), in 33% to 85% of patient with minor NP events (e.g., headache, subjective memory loss) and in 10% to 50% of SLE patients with no apparent NP disease (158 ,173).

Most functional imaging techniques suffer from similar pitfalls: lack of direct measurement of brain activity, long scan times, a lack of standards for environmental conditions during scanning, and lack of consensus on thresholds for defining brain activation. Regional and general cerebral blood flow, which is often the focus of functional imaging may be affected by a number of sensory events that are often uncontrolled (e.g., visual, auditory, or somatosensory stimulation) and by a variety of subject variables, including age, gender, anxiety, or level of arousal, time of day, blood pressure, or even ingestion of certain common substances, such as caffeine (184). Such confounding factors are difficult to control and are often ignored during imaging, possibly leading to unreliable and invalid results.

A review of the various neuro-imaging methods used in studies of NP-SLE illustrates the need for cautious interpretation of the findings. Most of the techniques that have been commonly used reveal changes that are not unique to NP-SLE and, therefore, cannot be relied upon to differentiate NP-SLE from other diseases that affect the nervous system. Additionally, sensitivity of the various imaging methods is a double-edged sword. Less sensitive modalities, like CT, cannot provide information beyond gross abnormalities, other, more sensitive techniques, such as MRI, MRS, and SPECT, may reveal unexpected abnormalities that are of dubious clinical significance. Finally, of the imaging modalities reviewed, only MRI seems to currently have the potential to distinguish active disease from chronic CNS changes, although this issue will need considerably more research to confirm. If so, MRI may be helpful in confirming favorable treatment responses.

Treatment of Psychiatric Disorders and Cognitive Impairment in SLE

Management will need to be tailored according to individual patient's needs (Table 38-5) and there remains a paucity of controlled studies to guide treatment decisions. Once a diagnosis of NP-SLE is established the first step

is to identify and treat potential aggravating factors, such as hypertension, infection, and metabolic abnormalities. Symptomatic therapy with, for example, antidepressants and antipsychotic medications should be considered, if appropriate. Immunosuppressive therapy with high-dose corticosteroids, azathioprine, and cyclophosphamide are used to varying degrees. With the exception of one study (147) there are no placebo-controlled studies examining the benefit of either oral or intravenous corticosteroids (191 ,192) in NP-SLE. Similarly, pulse intravenous cyclophosphamide therapy (193 ,194 ,195 ,196 ,197 ,198 ,199 ,200 ,201), akin to that which has been used in the treatment of lupus nephritis, has also been reported to be beneficial in NP-SLE, although only one controlled study has been performed. A recent open-label study of 13 patients with lupus psychosis reported a favorable outcome in all patients treated with oral cyclophosphamide for 6 months followed by maintenance therapy with azathioprine (202). Another study by Barile-Fabris et al. (203) compared intermittent intravenous cyclophosphamide to intravenous methylprednisone given for up to 2 years in SLE patients with predominantly neurologic disease and reported a significantly better response rate with cyclophosphamide (95%) compared to methylprednisone (54%) ($p < 0.03$). In virtually all of these studies, immunosuppressive therapy was used in conjunction with corticosteroids in addition to symptomatic therapies, such as antipsychotic medications. More targeted immunosuppressive therapies, for example B-lymphocyte depletion with anti-CD20 used alone or in combination with cyclophosphamide (204 ,205), are promising, but require further study. Anticoagulation is strongly indicated for focal disease when antiphospholipid antibodies are implicated and such therapy will usually be lifelong (206 ,207 ,208).

Table 38-5: Management of NP Events in Patients with SLE

Treatment Strategy	Examples
• Establish diagnosis of NP-SLE	CSF examination primarily to exclude infection Autoantibody profile (antiphospholipid, antiribosomal P) Neuro-imaging to assess brain structure and function Neuropsychological assessment
• Identify aggravating factors	Hypertension, infection, metabolic abnormalities
• Symptomatic therapy	Anticonvulsants, psychotropics, anxiolytics
• Immunosuppression	Corticosteroids, azathioprine, cyclophosphamide, mycophenolate mofetil B-lymphocyte depletion
• Anticoagulation	Heparin, warfarin

Modified from Hanly JG. Neuropsychiatric lupus. *Curr Rheumatol Rep* 2001;3:205–212.

The identification of a potentially reversible cause (Table 38-3) is the first step in initiating treatment for SLE patients with cognitive impairment. Simple causes of new cognitive difficulties are often identified by review of the patient's history. Recent changes in medication are among the most common. Antidepressants, anticonvulsants, and antihypertensive treatments frequently used in SLE may cause reversible cognitive problems; adjustments in drug selection and dose may result in cognitive improvement. Treatment of even mild anxiety and depression may also improve cognitive symptoms.

At present, any additional attempt to address the issue of treatment of cognitive dysfunction in SLE is at best speculative. Two approaches, pharmacologic treatment and cognitive rehabilitation, can be considered although neither have yet been systematically attempted in SLE let alone have established evidence of efficacy. Only one placebo-controlled study of pharmacologic therapy for SLE-associated cognitive dysfunction has been performed (147). Ten SLE patients who were not using corticosteroids prior to the study were enrolled in an N of one double-blind, controlled trial using 0.5mg/kg prednisone daily. Except for complaints related to cognition, these patients presumably had inactive SLE at enrollment. The authors reported improvement in cognition in 5 of the 8 subjects who completed the trial. The use of antiplatelet or anticoagulant therapy in SLE patients with antiphospholipid antibodies for the treatment of cognitive dysfunction without evidence of thromboembolic phenomena has a theoretical basis but lacks evidence for efficacy and remains controversial. Pharmacologic treatment aimed at “cognitive enhancement” has not yet been studied in SLE and has only recently been attempted in conditions such as MS (209). Such treatments may ultimately prove to be efficacious in disorders such as MS (210) and may also have potential applications in SLE. Other pharmacologic agents have been developed for the treatment of cognitive dysfunction-associated conditions, such as Alzheimer's disease and attention deficit disorder. However, the variability in the presence and persistence of cognitive deficits in SLE patients, as described earlier in the chapter, as well as the lack of biologic plausibility for efficacy remain major hurdles for the design of clinical trials. Although the actions of such medications are not disease-specific, there is currently no data to support or refute their use in the treatment of SLE-associated cognitive dysfunction.

Cognitive rehabilitation, which typically involves intensive retraining of cognitive skills, suffers from the same problems of variability in the nature, persistence, and biologic basis when considering the design and implementation of a trial of program efficacy. Although individualized cognitive rehabilitation programs may indeed prove useful for some SLE patients, demonstrating the generalized effectiveness of this approach is challenging. Cognitive rehabilitation programs have been employed in other conditions (e.g., stroke, dementia, traumatic brain injury, MS) to teach patients with cognitive dysfunction the means to functionally adapt to their impairments in order that they can maintain, if not regain, some level of independence. Until recently, no cognitive rehabilitation programs specifically intended for SLE patients have ever been developed. A novel psycho-educational group intervention targeted specifically at SLE patients with self-perceived cognitive dysfunction was designed to improve the performance of common cognitive activities they found problematic (211). Results of a pilot study of this program demonstrated that participation may result in improvement in memory self-efficacy, memory function, and ability to perform daily activities that require cognitive function (212 ,213). Although rehabilitation programs like this are not generally available, lupus patients with verified cognitive dysfunction can be referred for cognitive rehabilitation to a neuropsychologist or occupational therapist with expertise in cognitive retraining.

Summary

Neuropsychiatric manifestations of SLE are an important and challenging aspect of the disease in part because of to the diversity of NP events and their lack of specificity for lupus. Although the ACR nomenclature and case definitions have provided a much needed template for more

precise characterization of NP events in lupus cohorts, there continues to be significant variability in the reported prevalence of NP disease. This includes psychiatric disorders and cognitive impairment, which are amongst the most frequently reported NP syndromes. The primary pathogenic mechanisms contributing to NP-SLE include microangiopathy, production of autoantibodies, and inflammatory mediators. Neuroimaging is a potentially powerful tool for advancing our understanding of NP-SLE, and in the future may also facilitate the selection of the most appropriate therapies and objectively document their effectiveness.

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Chapter 39
Sjögren Syndrome

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Sjögren Syndrome

The most common eye and mouth symptoms found in SLE patients are “dryness” and may fulfill criteria for Sjögren syndrome (SS) (keratoconjunctivitis sicca [KCS]). Table 39-1 lists the current diagnostic criteria for primary SS. There has been considerable confusion about diagnostic criteria for primary SS during the past decade with the incidence of disease varying over 10-fold from the European Economic Community (EEC) criteria (2% to 5% of the general population) to the San Diego criteria (0.5% to 1% of the population). The current consensus European-American criteria for SS (Table 39-1) has incidence about 0.5%. When these symptoms occur in patients who fulfill criteria for SLE, the SS is termed 2° SS.

At the level of pathogenesis, there is a close overlap between primary SS and systemic lupus erythematosus (SLE). Indeed, if one considers SLE to be a group of diseases with each group characterized by particular autoantibodies and HLA-DR predisposition, then SS patients are very similar to the subset of SLE patients who have antibody to SS-A antibody and human leukocyte antigen (HLA)-DR3. A convenient way to think of SS is that it is “SLE with a homing receptor” for the lacrimal and salivary glands (as well as other extraglandular organs that may be affected). Thus, SS patients present with more cellular infiltrative manifestations (lacrimal and salivary glands leading to sicca symptoms, interstitial pneumonitis and nephritis, and lymphoproliferative disorders), whereas generally, our SLE patients have characteristic manifestations that appear more antibody mediated (glomerulonephritis rather than interstitial nephritis, pleurisy rather than interstitial nephritis, hemolytic anemia rather than lymphoma). However, the overlap of both cellular infiltrative and antibody-mediated mechanisms in particular SS and SLE patients often makes the diagnostic differentiation difficult or impossible (1). The main reason for labeling a patient as SS is to bring attention to their particular ocular and oral needs, as well as to their tendency toward cellular infiltrative extraglandular manifestations, including lymphoma.

History

The first descriptions of SS were reported by European clinicians between 1882 and 1925 (2). In 1892, Mikulicz observed a man with bilateral parotid and lachrymal gland enlargement that was associated with massive round-cell infiltration. In 1925, Gougerot described three patients with salivary and mucus gland atrophy and insufficiency progressing to dryness. In 1933, Henrik Sjögren reported detailed clinical and histologic findings in 19 women with xerostomia and KCS, of whom 13 had chronic arthritis. In 1953, Morgan and Castleman (3) concluded that SS and Mikulicz disease were the same entity. The distinction between primary and secondary SS was suggested by Bloch in 1960 (4). The concurrence of SS and SLE was described first by Morgan in 1954 (5), and numerous investigators in the 1950s and 1960s observed lupus erythematosus (LE) cells in patients with SS and lupuslike features. The incidence of SLE in patients with SS has been estimated to be about 20%.

Clinical Manifestations of SS

SLE patients may exhibit a wide range of symptoms involving the head and neck. These include local manifestations of their systemic autoimmune disease, infectious processes related to immune suppression, and noninfectious complications from their medications. The differential diagnosis and treatment of these problems is often difficult. Dryness can result from many other causes including drugs with anticholinergic side effects (including certain herbal supplements) and is associated with infections such as hepatitis C or retroviruses, autonomic neuropathy, depression, and fibromyalgia. In many instances, the patient may have low-titer, positive antinuclear antibodies (ANA) and have vague symptoms of dryness, fatigue, and myalgia. The differential diagnosis of these patients represents a challenge because these symptoms are so common in the population and “false-positive” ANA titers frequently are found in the normal population (6). For example, low-titer antinuclear antibodies (1:40) are found in up to 23% of normals, and the misleading “cut-off” values that come as an interpretation with these laboratory reports results in confusion of the patient and the referring physicians (6). Even at a higher titer ANA of 1:640, the actual risk for developing SLE (or SS) is less than 1 in 100 (7). Thus, it needs to be emphasized that an ANA may be used to confirm the clinical diagnosis of SLE or SS, but these tests lack the specificity to serve as a sole basis

for diagnosis (7). Further, enzyme-linked immunoabsorbent assays (ELISAs) to detect antibodies to SS-A and SS-B antigens depend heavily on the quality of the antigen used in the assay and have great variability (discussed below) (8). There is a frequent misconception that commercial kits for detection of these autoantibodies utilize cloned gene products and thus are entirely reliable; in fact, difficulties with production of cloned antigens (particularly because of the importance of their structural folding and their glycosylation) has led to difficulty in their use in the clinical laboratory and most commercial assays continue to depend on antigens prepared by affinity chromatography (8). Thus, there is lack of reproducibility for the detection of antibody to SS-A and SS-B, resulting in further confusion in diagnosis when antibody titers are used as a primary tool for diagnosis. In summary, there are several "take home lessons" for rheumatologists: (1) all patients with complaints of dryness do not suffer from a systemic autoimmune disease, but it is important that all patients with objective ocular and oral dryness deserve instruction in conservative treatment; (2) the most difficult diagnostic problem is the decision whether symptoms are from a systemic autoimmune process and thus require a more aggressive therapeutic intervention; and (3) laboratory methods can be used to support a clinical diagnosis, but should not be the sole basis for diagnosis. Also, it is important to recognize that the clinical presentation and severity of SS appears to vary in different ethnic groups and in different parts of the world. For example, in ethnic groups such as Greek or Chinese SS patients there is a higher incidence of severe interstitial nephritis (patients often present with severe hypokalemia) (9) and the types of rashes (i.e., facial erythema annulare) is more common in Japanese SS patients (10) than seen in Caucasian SS patients. Different viruses (ranging from hepatitis C, HTLV-1, and human immunodeficiency virus [HIV]) that can mimic SS have very different incidence among these groups. As the world becomes increasingly filled with international travelers, a spectrum of patients and medications not used in the United States (particular herbal and "nutritional" medications) may increasingly lead to confusion in diagnosis.

Table 39-1: Preliminary Classification Criteria Developed by the EEC

I. Primary Sjögren syndrome (SS) (if at least 4 items present)

A. Ocular symptoms (at least 1 present)
   1. Daily, persistent, troublesome dry eyes for more than 3 months
   2. Recurrent sensation of sand or gravel in the eyes
   3. Use of a tear substitute more than 3 times a day

B. Oral symptoms (at least 1 present)
   1. Daily feeling of dry mouth for at least 3 months
   2. Recurrent feeling of swollen salivary glands as an adult
   3. Drink liquids to aid in washing down dry foods

C. Objective evidence of dry eyes (at least 1 present)
   1. Schirmer I test
   2. Rose Bengal
   3. Lacrimal gland biopsy with focus score ≥1

D. Objective evidence of salivary gland involvement (at least 1 present)
   1. Salivary gland scintography
   2. Parotid sialography
   3. Unstimulated whole sialometry (≥1.5 mL per 15 minutes)

E. Laboratory abnormality (at least 1 present)
   1. anti-SS-A or anti-SS-B antibody
   2. Antinuclear antibody (ANA)
   3. IgM rheumatoid factor (anti-IgG Fc)

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Differential Diagnosis of Primary Sjögren Syndrome and Sjögren Syndrome with Systemic Lupus Erythematosus

The rheumatologist often is faced with the diagnostic difficulty of distinguishing 1° SS from 2° SS associated with SLE. These patients frequently share similar symptoms (arthralgias, myalgias, fatigue, rashes, and visceral involvement from vasculitis) as well as laboratory test findings including positive ANA and antibody to SS-A (Ro) (11). Indeed, it has been argued that SLE represents a spectrum of patients where subsets are "artificially" subdivided on the basis of their serology and the serology (i.e., antibody against SS-A) correlates better with their genotype than with their clinical features (12 ,13). If one accepts the premise that patients with SLE constitute a heterogeneous group of patients in terms of pattern of organ involvement and ANAs (13), then it reasonably can be argued that 1° SS patients represent a forme fruste of SLE where patients have only four of the necessary five criteria for diagnosis of SLE (14). The group of patients with 1° SS is genetically heterogeneous even among patients who have similar ethnic backgrounds. However, a significant proportion of Caucasian 1° SS patients have similar human leukocyte antigen (HLA) associations (HLA-DR3, and the linkage associated DQ alleles) that are found in a subset of SLE patients (15 ,16). Further, our experience with multiplex families (i.e., one member with 1° SS and another member with autoimmune disease such as RA, SLE, or scleroderma) in China (17) and in another study of a multiplex Caucasian family (18), family members (siblings, mothers, daughters) of 1° SS patients had an almost equal frequency of 1° SS or SLE. Similarly, in a large, multiplex family with multiple cases of SLE, family members with SS were noted (19). Thus, it might be postulated that certain genetic factors predispose to either 1° SS or a subset of SLE, whereas environmental (or gene recombination

events) then propel the genetically susceptible patient down the clinical pathway of either 1° SS or SLE. In this regard, the close overlap of clinical symptoms of 1° SS and a subset of SLE would be expected. We would propose that SS develops in those patients with a more lymphocyte- aggressive disease, as manifested by the infiltration of lymphocytes (predominantly $CD4^+$ T cells) into tissues normally lacking lymphoid infiltrates (i.e., lacrimal and salivary glands, as well as lung or renal parenchyma), as extension of this hypothesis is the increased frequency of lymphoma in SS patients (20). In comparison, many of the manifestations of SLE patients appear to result from pathogenetic antibodies that lead to immune-complex disease or specific antibodies against platelets or glomerular antigens. Although there is clearly a great deal of overlap in 1° SS and SLE in this regard, this is a relatively simple model for prognostically evaluating patients' symptoms (e.g., interstitial pneumonitis, interstitial nephritis, or increased lymphoma) with features of SS or SLE. Table 39-2 lists some differential points between primary SS and secondary SS with SLE.

Table 39-2: Differential Points between Primary Sjögren and Secondary Sjögren with Systemic Lupus Erythematosus

1. Sjögren syndrome (SS) is a systemic autoimmune disease characterized by objective signs of ocular and oral dryness.
2. It often is difficult to distinguish primary SS from patients with systemic lupus erythematosus (SLE) because a subset of SLE patients have secondary SS and have antibody to SS-A. Glomerulonephritis is more common in SLE and interstitial nephritis is more common in SS. Certain types of skin rashes are more common in SS patients (especially hyperglobulemic purpura) than in SLE (malar rashes), whereas both may have vasculitis rashes.
3. It often is difficult to distinguish primary SS, secondary SS, and fibromyalgia, where sicca symptoms are common and low-titer antinuclear antibody (ANA) are relatively common (up to 20% of normal adults at titer 1:40, and 5% at titer 1:160); minor salivary gland biopsies and antibodies against SS-A help distinguish SS from fibromyalgia.
4. The lip biopsy must be read by individuals experienced in their evaluation, because only focal lymphocytic infiltrates should be quantitated and nonfocal infiltrates are nonspecific. An increased sensitivity and specificity of diagnosis may be achieved by evaluating several serial sections of minor salivary gland biopsies (183), in a method analogous to examination of serial sections of temporal artery biopsies in patients with giant cell arteritis (183).
5. Hepatitis C patients often have sicca symptoms and a subset have a positive ANA.
6. Symptoms of ocular irritation may result from blepharitis in addition to aqueous tear deficiency and symptoms of oral discomfort may be from superimposed oral candida infections.

Presently, the label “Sjögren syndrome” alerts the rheumatologist to the particular ocular and oral needs of the patient with sicca symptoms as well as to their particular problems of lymphoproliferative disorders. Thus, it is important for rheumatologists not to get bogged down in currently fashionable debates over classification criteria. The key point is that diseases are best classified by etiology and that the etiology of SLE and SS remains unknown; thus, we are left with classifying clusters of symptoms/signs and the key point is how to determine prognosis and treatment. The pattern of rashes in 1° SS patients differs somewhat from those in most SLE patients. Malar rashes are more common in SLE, because of the presence of malar rash serving as criteria for SLE. However, the malar rash of SLE must be distinguished from rosacea, which can contribute to blepharitis and ocular symptoms mimicking 1° SS (21). Patients with 1° SS have a relatively higher incidence of hyperglobulinemic purpura based on retrospective studies (22), and the purpura may be associated with a type II mixed cryoglobulin (23) containing a monoclonal rheumatoid factor with a particular idiotype (24). In Japanese patients, particular types of rashes such as erythema annulare (particularly with location on the face) are more common in SS than SLE patients) (10 ,25). In the past, a psoriaform skin rash termed “subacute” lupus when associated with a negative ANA (done using mouse kidney substrate) and a positive anti-SS-A antibody (26). These patients had a high frequency of SS-like symptoms. In recent years, a different substrate for detection of ANAs (Hep 2 cells) has been used and patients with “subacute lupus” now are shown to have a positive ANA (27) and are frequently diagnosed as SS (28 ,29 ,30). A wide range of additional skin lesions is found in both SLE and SS, ranging from leukocytoclastic vasculitis to the purpura associated with low platelets (31). Mouth (intra-oral) lesions occur in both SS and SLE. However, the characteristic SLE lesion is an oral ulcer. The most common mouth lesion in SS patients is from oral candida (32). These lesions are recognized by the presence of angular cheilitis and erythematous patches (often resembling telangiectasias) on the hard palate (33). The use of corticosteroids and antibiotics predispose to oral candida in a patient with decreased salivary flow. Also, oral lesions in patients on methotrexate may not be a result of drug allergy, but to oral candida.

Sjögren Syndrome: Pathogenesis of Symptoms

It is important to recognize that SS patients complain about their dry, painful eyes, and mouth, whereas rheumatologists talk about the patient's lacrimal and salivary glands, their autoantibodies, and acute phase reactants. The patient is describing ocular symptoms because of increased friction as the eyelid (particularly the upper lid) passes over the orbital globe. When the tear film is inadequate and the “viscosity” of movement between the eyelid and globe is inadequate, the lid adheres to the surface layers of the globe and can

actually pull epithelial cells away from the surface layer of the conjunctiva and cornea. It is these epithelial defects that are viewed clinically as “keratoconjunctivitis sicca” and corneal abrasions. As a result of the “insult” to the epithelial surface, an inflammatory response (release of cytokines and influx of inflammatory cells) occurs. As a result of the lack of adequate tear film, the “wounding” process continues and the normal healing process is impaired because an adequate tear film is required to provide “nutritive” and anti-inflammatory substances. Thus, in SS patients, exposure to certain environmental stresses can lead to KCS lesions that are very slow to heal. Similarly, the tongue and buccal mucosal surfaces require lubrication for the tongue to move around the mouth and for the actions of speaking and swallowing. However, there are important differences in the oral symptoms and the ocular symptoms. The eye is a “clean” environment (i.e., not colonized) while the mouth has high levels of resident aerobic and anaerobic organisms. A dry mouth is not necessarily a painful mouth. Common problems in the mouth involve changes in the microbial flora, especially with the emergence of chronic candidiasis (discussed below) or periodontal disease as a result of particular organisms. Further, there are important differences in the types of neural innervation, mucins, cytokines, and enzymes in the secretions of the mouth and the eye. Figure 39-1A shows normal lacrimal and salivary flow regulated through feedback mechanisms. The mucosal surfaces of the eye and mouth are heavily innervated by unmyelinated fibers that carry afferent signals to the lacrimatory or salvatory nuclei located in the medulla. These medullary nuclei, which are part of the autonomic nervous system, are influenced by higher cortical inputs including taste, smell, anxiety, medications, and depression. The efferent neurons innervate both glandular cells and local blood vessels. The blood vessels provide not only water for tears and saliva, but also growth factors including hormones (e.g., insulin) and matrix proteins (e.g., fibronectin and vitronectin) of the lacrimal and salivary glands. In response to neural stimulation through muscarinic M3 receptors and vasoactive intestinal peptide (VIP) receptors, glandular acinar and ductal cells secrete water, proteins, and mucopolysaccharides (mucins). This complex mixture forms a hydrated gel that lubricates the ocular surface (i.e., tears) and the oral mucosa (i.e., saliva). In the simplest model of SS (Fig. 39-1B), the lacrimal or salivary gland is incapable of adequate response to neural signals as a consequence of local immune infiltrates and their derived cytokines. The actual processes in SS or autonomic neuropathy are more complicated than indicated in these schematic diagrams, which are designed primarily to emphasize that salivation or lacrimation are part of a regulatory circuit involving the central nervous system (CNS) (34). The stages in pathogenesis of SS include: (1) the change of small endothelial vessels to high endothelial venules that express adhesive molecules and release chemokines, such as regulated on activation, T cell expressed and secreted (RANTES) and lymphotactin; (2) the migration

of CD4+ T cells into the gland in the center of the lobule of the lacrimal or salivary gland where they form a cluster (or foci) of lymphocytes; (3) release of cytokines including interferon-γ and tumor necrosis factor (TNF)-α by the T cells and interleukin 1 (IL-1) by the epithelial cells; (4) upregulation of HLA-DR, DQ, and invariant chain (Ii) by the epithelial cells and perhaps costimulatory molecules such as B-7; (5) upregulation of perforin and granzyme that may lead to damage of ductal cells; (6) increased expression of Fas on CD4+ T cells and Fas-ligand on the epithelial cells, that may lead to increased apoptotic death of epithelial cells; (7) increased production of bcl-2, and bcl-x by the lymphocytes that helps prevent their own apoptosis; and (8) clonal expansion of B cell clones and increased risk of karyotypic translocation [t 14:18] associated with transformation to a non-Hodgkin lymphoma (35). As a result of mutual stimulation of T cells, B cells, and epithelial cells, cytokines such as IL-1, IL-6, and TNF-α are released and lead to increases of acute phase reactants including ESR (fibrinogen) and C-reactive protein that are measured in clinical assays. Table 39-3 , summarizes several of the pathogenetic steps. Figure 39-2A shows the lymphocytic infiltrates in a minor salivary gland biopsy, in comparison to a normal gland (Fig. 39-2B). The infiltration of the lymphocytes (shown to be CD4+ T cells by immunohistology) under the basement membrane and in direct contact with the epithelial cells is shown in frames C and D. The changes in the high endothelial cells containing red blood cells (frames E and F) and adherent T cells that are migrating through the vessel wall are seen under low-power electron microscopy.

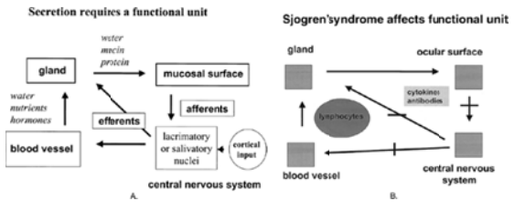

Figure 39-1. A, "Circuit" that controls normal tear flow or salivation and interruption of the circuit in patients with Sjögren syndrome. The stimulation of the ocular or oral mucosal surface leads to afferent nerve signals that reach the lacrimatory or salivatory nuclei in the medulla. Efferent neural signals stimulate both blood vessels and glandular epithelial cells. The medullary signal may be affected by cortical inputs that reflect stimuli such as taste, smell, anxiety, or depression. The efferent neural signal to the gland is mediated by acetylcholine. The gland contains receptors for acetylcholine of the muscarinic class, particularly M3 receptors (shown by arrow). **B**, In Sjögren syndrome, lymphocytic infiltrates in the gland secrete cytokines that inhibit the release of neurotransmitter and the response of receptors that initiate glandular secretion.

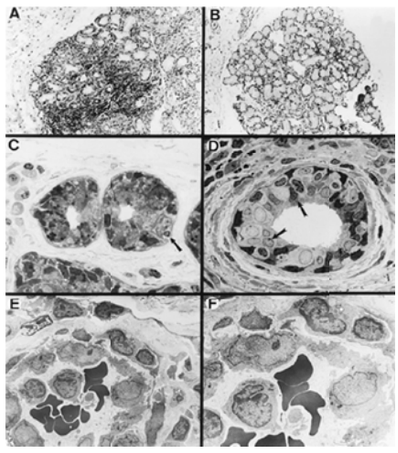

Figure 39-2. Minor salivary gland biopsy from patients with Sjögren syndrome **(A)** and from a patient with fibromyalgia (a histologically normal biopsy) in **(B)**. Higher power views of the Sjögren biopsy are shown in frames **(C)** and **(D)**, with low power electron microscopic views in frames **(E)** and **(F)**.

Table 39-3: Pathogenetic Features of Sjögren Syndrome

1. Sjögren syndrome is a systemic autoimmune disease. The target organs (lacrimal or salivary glands) are accessible to biopsy, making this disease well suited for clinical-pathologic correlations.
2. Biopsies of lacrimal or salivary glands indicate that only about 50% of the acinar or ductal structures are destroyed. Thus, the severe dryness results from dysfunction of the residual glands. This may be the result of release of cytokines, metalloproteinases, or autoantibodies that interfere with glandular function or their response to neurotransmitters.
3. The predominant infiltrate in the glands is CD4+ T cells (memory phenotype) that release Th1-like cytokines. However, the lymphomas that develop with increased frequency are because of B cell non-Hodgkin lymphomas.
4. The epithelial cells are not merely innocent targets in the pathogenesis. In animal models, intrinsic defects in glandular development may play a role in generating tissue-specific autoantigens. Early in the disease, chemokines are secreted and later in the disease, the epithelial cells express class II histocompatibility antigens and secrete proinflammatory cytokines.

A new way to look at both SS and SLE is that the immune system is hyperreactive to different "danger" signals that usually work by triggering the innate immune system (i.e., activation of dendritic cells to liberate type I interferon) (36). Using methods of immunohistology and gene profiling, numerous IFN-α–producing cells were detected in salivary gland biopsy specimens, despite low IFN-α levels in the serum (37). Autoantibodies to RNA-binding proteins, combined with material released by necrotic or late apoptotic cells, were potent inducers of IFN-α production in plasmacytoid dendritic cells (PDCs). It appears that RNA-containing immune complexes (such as anti-SS antibody) triggering PDCs by means immune complexes that interact with Toll 7 receptor and Fc-γ receptor IIa (37).

Thus, a unified theory of SS pathogenesis might include the ability of multiple pathways (including viral injury) to initiate the production of IFN in the salivary/lacrimal glands as a result of activation of "danger" pathway (36 ,38), and the continued IFN-α synthesis is perpetuated by RNA-containing immune complexes (particularly SS-A antigen/anti–SS-A antibody) (37). These locally produced immune complexes contain dsRNA (hYRNA) that activate local PDCs to prolong IFN-α production at the tissue level. The SS patient is predisposed to make anti-SS antibody because of HLA-DR3, (and linked DQ loci) and perhaps complement C4 deficiency. This IFN-α promotes the

autoimmune process by a vicious circle-like mechanism, with increased autoantibody production and formation of more endogenous IFN-α inducers.

Evaluation of Symptoms in the Dry-Eye Patient

SS patients usually describe a burning or “foreign” body sensation in their eyes. The symptoms often are worse at the end of the day and are relieved by the use of over-the-counter (OTC) artificial tears (which most patients have tried prior to seeing a rheumatologist). Symptoms of itching are poorly correlated with objective findings of keratoconjunctivitis (39). Patients may be relatively symptom free until their condition is precipitated by the use of medications with anticholinergic side effects (such as OTC cold remedies or prescription medications) or environmental stress to the tear film (such as airline travel or exposure to dry winds). Often identification and alteration of the offending medication may be all that is required. Contact lenses, especially the soft gas-permeable type, can contribute to corneal abrasions because adequate tear film may not be available to wash out foreign substances trapped under the lens. Thus, if contact lenses are worn, they should be taken out at night and the patient needs to be cautioned about the risk of corneal abrasions. One study has suggested that SS patients should be offered photorefractive surgery (i.e., excimer laser keratectomy) if they do not tolerate contact lenses (40); however, caution about the use of invasive procedures involving the cornea needs to be exercised until long-term follow-up of SS patients with refractive surgery (essentially a cosmetic procedure) is available.

When evaluating the patient with a complaint of dry eyes, it is important to determine if the objective signs of dry eyes are commensurate with the patient's symptoms. Methods to measure the integrity of the corneal surface and tear film include rose bengal, fluorescein staining, lissamine green staining, or the tear breakup time are described below. For example, the absence of a significantly abnormal exam using rose bengal (a test that is performed easily by the rheumatologist) should suggest a search for additional causes to explain the patient's ocular complaints. These may range from eye strain (poor refraction), blepharitis (irritation and low-grade infection of the Meibomian glands in the lids), blepharospasm (uncontrolled blinking from an increased local neural reflex circuit), or symptoms resulting from anxiety or depression (41). Unstimulated tear flow is referred to as basal flow. This tear secretion derives from the minor tear glands located predominantly in the upper lid. They probably are stimulated by a local reflex arc involving neural receptors in the lid and probably do not involve neural loops that regulate the major salivary glands. This decrease in basal secretion correlates fairly well with symptoms of dryness but not closely with KCS. Decrease in basal tear flow is very common in the general population, especially the elderly, and does not signify an autoimmune condition. A decrease in basal tear flow also may be the initial symptom in SS patients. These symptoms of eye discomfort occur even though the patient can generate tears when they cry (i.e., supratentorial stimulation) or are exposed to certain stimuli such as onions (nasal lacrimal reflexes). Tear volume usually is measured by the Schirmer test; however, this test is performed routinely in several distinct ways and the results are often quite different. It is important for the rheumatologist to note the methods of quantitation of tear flow when evaluating patients clinically (or reports by their ophthalmologist) or reviewing the published literature. Many ophthalmologists (and clinical studies) measure the Schirmer I test with topical anesthetic (Ophthaine) and reflects the basal secretory rate of the minor lacrimal glands in the eyelids (i.e., not the major lacrimal glands). This test is sensitive but not specific and may be diminished for many reasons. Most commonly, rheumatologists perform the Shirmer I test in the absence of anesthetic. This value reflects both the minor glands and the stimulation of the major glands. However, the extent of stimulation of the major lacrimal glands is variable. This test is convenient, has less sensitivity, and has more specificity. In addition to the Schirmer I test (either with or without anesthesia), the Schirmer II test provides a rapid way to measure the maximal output of minor plus major lacrimal glands. The Schirmer II test is performed by gently inserting the cotton end of a cotton swab into the nose, where it stimulates the nasolacrimal gland reflex (42). The volume of tearing is measured by Schirmer paper strips without anesthesia. The stimulated tearing reflex involves a neural loop in which afferent fibers from the ocular surface travel to the midbrain (lacrimatory nucleus) where they stimulate efferent adrenergic and cholinergic nerves that travel back to the lacrimal gland (cholinergic) and its blood vessels (adrenergic) (43). A diminished Schirmer II test has good specificity for SS, but lacks sensitivity to early stages of SS. The presence of an increased tearing on the Schirmer II test has been a good predictor of response to oral pilocarpine as a way to stimulate secretions, in the author's experience. A wide range of tear volume flow (i.e., values on the Schirmer I test with anesthesia) occurs in SS patients as well as in normals, and is poorly correlated with signs and symptoms of KCS. Similarly, the volume of saliva produced as basal secretion or after stimulation correlates poorly with symptoms of dry mouth and objective signs of severe periodontal problems (44). This suggests that the qualitative content of tears and saliva (i.e., specific glycoproteins and mucins) plays an important role in the maintenance of ocular and oral mucosal integrity and that decreases in tear volume are not the sole cause of problems. An important implication of these findings is that the next generation of artificial tears, artificial salivas, and toothpastes may contain bio-engineered products that provide these important functions lacking in the currently available products. In

addition to the volume of tears, the quality of the tear film is assessed by simple procedures such as rose bengal. This material is readily available from pharmacies that carry ocular supplies and a single drop is administered into the lower eyelid. After rinsing the rose bengal out with a preservative-free tear, the residual staining of the conjunctiva and cornea can be determined with an ophthalmoscope. Although this evaluation by the rheumatologist does not replace the more accurate evaluation of KCS by slit lamp performed by ophthalmologist, the test does provide a rapid method for assessing the significance of patient's complaints. The rose bengal should not be left in the patient's eye (before rinsing out with an artificial tear) for a prolonged period, as this may lead to local irritation. In comparison, lissamine green is reported to cause less irritation, but requires a slit lamp for adequate quantitation. The results obtained with both rose bengal and lissamine green methods are very dependent on the methods for performing the test and the “training” of the observer. In research studies (or in the evaluation of published studies by the rheumatologist), it is important to recognize limitations of the different methods and the variable between different observers.

Evaluation of Symptoms of Dry Mouth

The principal oral symptom of SS is mouth dryness with a broad range of severity. Not all patients complain of dryness specifically; many describe difficulty in swallowing food, problems in wearing dentures, changes in their sense of taste, increased incidence of dental caries, chronic burning symptoms, intolerance to acidic or spicy foods, and the inability to eat dry food or speak continuously for more than a few minutes. Nutrition may be compromised and patterns of sleep disturbed. On examination of the mouth, the SS patient lacks the normal salivary pooling under the tongue and may have rapidly progressive caries. The mouth frequently exhibits petechial (i.e., small, red, nonpalpable) lesions on the hard palate and/or a lichen planus-like appearance (fine white, lacy strands) on the buccal mucosa. Also, these lesions only may be detected in the recesses of the buccal mucosa on careful examination. These petechial and lichen planus-like lesions result from chronic oral candidiasis infection in the SS patient; it is uncommon for an SS patient's mouth to exhibit the plaquelike appearance (thrush) found in severely immunocompromised patients. Another manifestation of oral candidiasis in the SS patient is angular cheilitis, a condition that must be treated at the same time as the buccal mucosal candidiasis. Progressive periodontal disease should be suspected based on the increased need for dental restorations and the presence of cavities at the gum line. The loss of teeth and requirement for dentures at any age, but particularly in the younger patient, may have significant emotional and economic consequences. Patients with dentures may change their social patterns of interpersonal interactions. For example, their social life frequently involves eating meals with friends and the patient may feel uncomfortable about not being able to eat the same foods. Further, the patient's diet may be shifted over to preprocessed foods that often are higher in sugars and thus further accelerates the rate of their periodontal problems.

Systemic Manifestations of Sjögren Syndrome

Patent's with 1° SS as well as those with 2° SS associated with SLE may have a wide variety of systemic manifestations. Table 39-4 lists most of their manifestations, which are also covered in other chapters. However, several of these manifestations (i.e., CNS, lymphoma) have been reported more commonly in 1° SS and will be discussed more fully below.

Table 39-4: Extraglandular Manifestations in Patients with Sjögren Syndrome

Respiratory	Chronic bronchitis secondary to dryness of upper and lower airway with mucus plugging Lymphocytic interstitial pneumonitis Pseudolymphoma with nodular infiltrates Lymphoma Pleural effusions Pulmonary hypertension
Gastrointestinal	Dysphagia associated with xerostomia Atrophic gastritis Liver disease including biliary cirrhosis and sclerosing cholangitis
Skin and Mucous membranes	Candida—oral and vaginal Vaginal dryness Hyperglobulinemic purpura Raynaud phenomenon Vasculitis
Endocrine, neurologic, and muscular.	Thyroiditis Peripheral neuropathy involvement of hands and/or feet and/or feet Mononeuritis multiplex Myositis
Hematologic	Neutropenia, anemia, thrombocytopenia Pseudolymphoma Lymphadenopathy Lymphoma and myeloma
Renal	Tubular-interstitial nephritis (TIN) Glomerulonephritis, in absence of antibodies to DNA Mixed cryoglobulinemia Amyloidosis Obstructive nephropathy as a result of enlarged periaortic lymph nodes Lymphoma Renal-artery vasculitis

CNS Symptoms of Sjögren Syndrome

Similar to SLE (45), a wide variety of CNS manifestations has been reported in SS patients (46 ,47). These range from vasculitic lesions of the CNS, thrombotic lesions associated with anticoagulants, affective disorders (48), autonomic neuropathy (49), myelopathy (50), cranial nerve neuropathy (51), dystonias (45), and autoimmune hearing loss (52 ,53). One of the main diagnostic problems for the clinician is that many patients with poorly defined neurologic diseases and a positive ANA (and perhaps a positive anti-SS-A antibody) are defined as having SS (or a forme fruste SS) even when they lack objective signs of KCS (54). It is again worth emphasizing that a relatively high normal proportion of the normal population have a false-positive ANA (6), the variation in results among assays for anti-SS-A antibody (8) and that positive ANA have been found in increased frequency in patients with other hematologic disorders that appear distinct from either SLE or SS. Thus, rheumatologists often are faced with a patient with predominantly neurologic or affective disorder problems and a positive ANA for the question of whether immune suppressive therapy is indicated for SS. This problem is certainly most commonly encountered in the patient with symptoms of fibromyalgia where sicca symptoms are a prominent complaint. During litigation regarding the relationship of silicone breast implants and autoimmune disease (55), a slightly higher association (relative risk about 1.5) was found for SS than for SLE or scleroderma (relative risk about 1.0) (56). However, minor salivary-gland biopsies did not support the diagnosis of SS (57 ,58) in those patients studied and suggested that the cause of their dryness could be because of a mild autonomic neuropathy, perhaps associated with their fibromyalgia (59). Several earlier studies suggested a relatively high frequency of demyelination in the CNS in primary SS patients (60 ,61). Although patients with sicca SS may develop symptoms and signs similar to multiple sclerosis patients, several issues remain unclear. First, the markedly increased frequency of demyelinating features was reported during a relatively short period at a single medical center. Longer-term follow-up studies in the same medical center did not confirm the earlier reports of a markedly increased frequency (62). The most likely explanation for the earlier reports derive from the later improved sensitivity of magnetic resonance imaging (MRI) brain scans where it was possible to better distinguish vascular lacunae from regions of demyelination and the improvement of assays for detection of antinuclear antibodies. Second, it was recognized that multiple sclerosis patients may develop dryness as a result of an associated autonomic neuropathy at the level of the CNS, rather than as a consequence of an autoimmune process involving the lacrimal or salivary glands (63). Depression can present in many clinical forms including difficulty concentrating, poor appetite, or as a sleep disorder in both SLE and SS (64). The precise role of inflammation and hormone imbalances

associated with SS as a contributing factor to depression remains unclear, but certainly depression is caused in part by chemical alterations in the brain (65 ,66). Stress, poor sleep, and chronic illness can all contribute to depression. When antidepressant medications are used to help regulate sleep patterns and treat fatigue, drugs with relatively less anticholinergic side effects (such as selective serotonin reuptake inhibitor [SSRI] agents that interfere with serotonin uptake) are preferred over tricyclic antidepressants (often given for fibromyalgia).

Systemic Medications and SS Patients

The overall approach to systemic therapy in the patient with SS is similar to that in the SLE patient. Disease manifestations are subdivided into nonvisceral (arthralgias, myalgias, skin, fatigue) and visceral (lung, heart, kidney, brain, peripheral nervous system). Nonvisceral manifestations generally are treated with salicylates, nonsteroidal agents, and often hydroxychloroquine. Particular attention to difficulty in swallowing pills in the SS patient is necessary, because the decreased salivary content can lead to pills becoming stuck in the midesophagus with resultant erosions of the mucosa. Little improvement with salivary or lacrimal flow rates has been noted with nonsteroidal anti-inflammatory drugs (NSAIDs), although some increase in tearing and salivation may occur after systemic corticosteroids. In terms of NSAIDs for the SS patients, indomethacin is the only agent readily available as a suppository for the patient with difficulty swallowing tablets. Flurbiprofen has been shown in a pilot study to decrease periodontal inflammation and resultant gum disease (67). Among the slow-acting drugs, antimalarials (hydroxychloroquine) have proven useful in decreasing the arthralgias, myalgias, and lymphadenopathy in SS patients (68 ,69), similar to its benefit in some SLE patients (70). We have used hydroxychloroquine (6 to 8 mg/kg/day) in SS patients where there is elevation of erythrocyte sedimentation rate (ESR) and polyclonal hyperglobulinemia, because these laboratory abnormalities suggest that symptoms of arthralgia and myalgia may have an inflammatory cause. In a European study (71), hydroxychloroquine improved ESR but did not increase tear-flow volumes. Comparison of drug benefit in SS patients in European and United States studies is strongly influenced by the very different inclusion criteria for diagnosis of SS (described above). When taken at the proper dose (6 to 8 mg/kg/day), hydroxychloroquine has a very good safety record, although there remains a remote possibility (probably less than 1/1,000) (72) of significant build-up in the eye. For this reason, periodic eye checks (generally every 6 to 12 months) are recommended so that the medicine can be discontinued if there is any significant build-up. The general safety of hydroxychloroquine at these doses has been recently confirmed, particularly in comparison to other available therapies (73 ,74). However, toxicity still must be considered in rare patients and may be associated with abnormalities in electroretinograms (75). In patients with cognitive features associated with SS, the use of atabrine has been advocated (70). However, the patients should be screened for G6PD deficiency prior to this drug and a “yellowing” of the skin is common. This skin change can be partially ameliorated using oral vitamin A (solatene). These agents are not readily available from most pharmacies but can be obtained from compounding pharmacies. For visceral involvement including vasculitic skin lesions, pneumonitis, neuropathy, and nephritis, corticosteroids are used in a manner similar to SLE patients. As in other autoimmune disorders, a key question is how to taper the corticosteroids because these agents have additional problems of accelerating their periodontal problems. Drugs such as hydroxychloroquine, azathioprine, and methotrexate are used to help taper the corticosteroids. In one study, methotrexate appeared most useful (76). It seemed likely that several of the newer agents approved for rheumatoid arthritis (leflunomide and TNF antagonist, etanercept) will prove useful in selected SS patients. Although an initial report suggested benefit of TNF inhibitors (infliximab) (77), subsequent studies by the same group of investigators in a large multicenter trial failed to demonstrate benefit (78). Similarly, etanercept therapy was not statistically beneficial in SS patients including their sense of fatigue and fibromyalgia like symptoms (79 ,80). In early open labeled studies, rituximab may be useful in SS patients with extraglandibular manifestations (81). In some SS patients, cyclosporin may be used (82), but the tendency toward interstitial nephritis in many SS patients limits the usefulness of the drug. For life-threatening illness, cyclophosphamide occasionally is required. However, the increased frequency of lymphoma in SS patients (83) requires caution in the use of cyclophosphamide and has suggested its use as a pulse therapy rather than daily administration.

Topical Therapy for the Dry-Eye Patient

The mainstay of treatment for the dry-eye patient is the regular use of artificial tears (84). When evaluating a particular artificial-tear preparation (Table 39-5), the patient must carefully determine whether: (1) the tear gives benefit but does not last long enough, or (2) the tear burns immediately upon installation. Artificial tears can be considered to have at least two distinct components: the moisturizing component and the preservative. If the tear is helpful, but does not last long enough, then a more viscous tear (such as a higher concentration of hydroxyethylcellulose) or a different vehicle to concentrate the moisturizing element (such as a polymer-like dextran) is indicated. If the tear burns soon after installation, then an irritant reaction to a preservative in the tear must be considered (85). These reactions were much more frequent in the past when benzalkonium chloride and thimerosal commonly were used in artificial tears. However, it should be remembered that these preservatives still are widely used in other ophthalmologic preparations (particularly topical antibiotics) and may contribute to ocular irritation. Irritation of the eyelids in some patients with blepharitis may be related to the preservatives present in some ocular lubricants (used at night), as well as the use of excessive amounts of lubricant at night that plug the Meibomian glands. It is important for the patient to identify environmental factors and medications that contribute to dry-eye symptoms. For example, symptoms of dry eyes will be exacerbated by low-humidity environments such as airplanes, highly air-conditioned offices or department stores, and outdoor areas with strong dry winds. The increased use of tears before the onset of symptoms will be symptomatically helpful and prevent corneal abrasions. The use of cool-mist humidifiers at night (or even in the office) and wrap-around sunglasses when outdoors will help retard tear evaporation. Among patients who wear glasses, the optometrist can add moisture shields to the frames. In patients who like or need to be outdoors, ski goggles can provide a local “moisture chamber.” If the particular artificial tear seems helpful, but benefit does not last long enough, punctal occlusion may be performed on a temporary or permanent basis (86). The puncta are the small openings at the medial aspects of the lids. Blockage of the puncta can be done with silicone plugs or by electrocautery (86). Previously, a trial of “temporary” punctal occlusion with collagen plugs (i.e., temporary occlusion that lasts several days) was advocated. However, clinical experience has indicated that adequate occlusion with collagen plugs only is achieved in a minority of patients and this procedure is no longer advocated as an adequate trial. In some patients, a prior punctal occlusion may reopen and this can be determined easily by instilling fluorescein in the patient's eye. If the puncta has reopened, the fluorescein will drain into the

nasopharynx and the patient will experience the characteristic taste of fluorescein. This simple test will indicate the need (or absence) for repeat punctal occlusion. Topical cyclosporin has been used to improve the keratoconjunctivitis in two multicenter trials of patients with SS, leading to its Food and Drug Administration (FDA) approval (87). High levels of cyclosporin are achieved in the tear film, but there is little absorption into the systemic circulation. The beneficial effects of cyclosporin are as a result of its ability to serve as an anti-inflammatory agent. However, additional benefits may result from stimulation of prolactin receptors on the cell surface of corneal cell and lacrimal gland acinar cells (88). Adjuncts to therapy have included acetylcysteine 10% drops to break up mucous strands, but these drops have the smell of rotten eggs and are thus objectionable to most patients (89). Vitamin A and related preparations have a theoretical role in the treatment of dry eye, because vitamin A-deficient patients have increased keratinization of the corneal surface (90); however, more recent studies have not supported the initial enthusiasm for this form of treatment (84).

Table 39-5: Therapeutic Principles

1. Therapy includes topical replacement of lubrication (artificial tears and saliva), as well as preservation of tears by punctual occlusion.
2. Local inflammation of the ocular surface may be treated by use of anti-inflammatory substances such as topical cyclosporin.
3. The normal tear film or saliva lubricants contain mucins as well as saliva. Although current therapies can replace aqueous secretions, they are still deficient in replacement of the mucin components.
4. New oral medications help stimulate muscarinic M3 secretions to lead to increased water content of secretions.
5. The overall treatment program for Sjögren syndrome is similar to systemic lupus erythematosus with the use of corticosteroids (that can be used for short intervals), nonsteroidal agents, and slow-acting antirheumatic drugs (hydroxychloroquine, methotrexate, and perhaps newer agents such as leflunomide and tumor necrosis factor inhibitors) for chronic management of extraglandular manifestations, and the use of cytotoxic agents (i.e., cyclophosphamide) for life-threatening vasculitis.

Oral Therapy for the Dry-Eye Patient

Pilocarpine, an agonist for muscarinic M3 receptor, has been used as an oral preparation to stimulate the salivary and lacrimal-gland secretion. Initial studies in 1986 indicated the benefit of pilocarpine in SS patients (91). The effects on salivation were more marked than effects on lacrimal stimulation. Also of note, the relationship between subjective symptoms and objective measurements of saliva flow showed a weak correlation. These results were interpreted to indicate that pilocarpine stimulated “water” flow, whereas symptoms correlated with mucin secretion, which was not adequately quantitated in the studies. During recent years, a series of additional studies on oral pilocarpine have been presented (92 ,93 ,94) and led to approval to market for symptoms of dry mouth. The available dosage is 5 mg up to four times daily. The most common side effect is increased sweating or gastrointestinal intolerance, which generally is controlled by decreasing the dose. A recent clinical study using a different oral-muscarinic agonist (cevimeline, Snow Brand SNI-2077) was presented in abstract form (95). In comparison to pilocarpine: (1) cevimeline has a longer half life (4 hours) than pilocarpine (1.5 hours); and (2) cevimeline has a higher specificity for muscarinic M3 receptor (salivary and lacrimal gland) in comparison to muscarinic M2, (cardiac tissues) by about 10-fold. This ratio of binding to M3 receptor (i.e., efficacy) and M2 receptor (i.e., toxicity) may prove important. In the clinical studies, an objective improvement in tear film as measured by exfoliative cytology was reported with cevimeline, which the same investigators had not seen in their prior studies with pilocarpine in the same population of study patients (95). In SS patients and normal controls treated with cevimeline, the secretion rates of amylase and IgA showed significant increases in both groups (96). The secretion rate of lysozyme significantly increased only in the control group, whereas the secretion rate of secretary component C (SCC) significantly increased only in the SS group. Cevimeline augments not only the salivary flow rate but also the secretion rate of some digestive and/or defense factors from infections. It may be beneficial for SS patients to continue taking cevimeline to prevent oral infections, and other serious sequelae. Systemic sialagogues have been used to increase salivation. Three agents have been studied in controlled trials. Bromhexine, a mucolytic agent, was not found to increase salivary flow rate but patients described subjective benefit (97). Anetholethrithione (Sialer) showed significant effects on saliva output in one study of SS patients with mild secretory hypofunction (98); however, a later study failed to find a significant response in patients with severe hypofunction (99). A series of controlled studies have indicated that pilocarpine exerts stimulatory properties in SS and postradiation therapy (91 ,100). The drug acts primarily as a muscarinic-cholinergic agonist with mild ß-adrenergic activity. In these studies, pilocarpine 5 mg three times a day increased salivary flow for several hours, in comparison with placebo (101). Side effects were common, including sweating, flushing, and increased urination (101). Further studies will be required to compare this treatment with other agents containing iodide (SSKI or organidin) that are occasionally helpful in some SS patients. A dry mouth is not necessarily a painful mouth. It is common for an SS patient to develop a low-grade oral yeast infection (33). Predisposing factors include recent use of antibiotics and/or corticosteroids. Treatment of this problem is particularly difficult in the patient with dentures, because continued excoriation of the mucosal surface occurs. Many topical antifungal drugs (including Mycelex troches) are available, but these oral preparations suffer from a low content of antifungal agent and a high concentration of glucose (to improve the taste) and thus contribute to dental decay if used chronically (33). The reason for inclusion of dextrose (rather than aspartame as a “sweetener”) was because of concern about long-term effects of aspartate by the FDA at the time when the drug was first introduced; attempts by the author to have Mycelex reformulated without dextrose by the manufacturer have not been successful. Nystatin and clotrimazole (available as vaginal suppositories that can be sucked) are both helpful, but must be sucked for about 20 minutes twice daily for at least 6 weeks to prevent recurrence of oral candidiasis (102). Patients with very dry mouth will require periodic sips of water to help dissolve the troches. For angular cheilitis, topical antifungal creams are used two to three times per day for several weeks. This must be done concurrently with the treatment of intraoral candida treatment, because the angular cheilitis serves to reinfect the buccal mucosa (and vice versa). To permit drug access to all intraoral mucosal sites, patients must remove their dentures while antifungal tablets are dissolving. The dentures

also must be treated to remove traces of candida, and the method of disinfectant must be discussed with the dentist. However, it usually is sufficient to soak the complete denture overnight in benzalkonium chloride (for example, a1/700, dilution of the surgical-scrub solution, Zephiran). The dentures must be carefully cleaned with a toothbrush and nystatin powder must be applied to the fitting surfaces of the upper denture before reinserting the denture. In extreme cases, a short course of oral antifungal therapy (such as ketoconazole or fluconazole) may be required to control oral candidiasis. The use of topical fluorides may help protect dental enamel. In some patients, a neutral fluoride drop may be applied by toothbrush or by their oral hygienist. In other patients, direct contact of the dental surfaces and the fluoride gel can be achieved by using dental plates at night to apply the fluoride. These plates are made specifically for each patient by their periodontist. Additionally, varnishes may contain fluoride, and a new generation of varnishes additionally release antibiotics that may retard periodontal disease, oral candidiasis, and caries (87). The use of correct technique of toothbrushing to massage the gums and remove debris is important, because this normal function of saliva is diminished in SS patients. In some patients, a rotating toothbrush (such as Oratec [Oratec Products, Inc.]) is useful, together with regular oral hygiene from a technician experienced in dry-mouth care. A variety of saliva substitutes are available. They differ in their flavoring agents and preservatives. MouthKote (Parnell Pharmaceuticals, Inc.) and Salivart (Xenex Laboratories, Inc.) sprays contain mucins, which are glycoproteins that help lubricate the mouth and thus provide relief for a longer time than simply rinsing with water (103). They can be obtained by calling 1-800-457-4276. After administration of these sprays, parotid flow rates are increased for 7 to 8 minutes in SS patients; however, the sense of “oral well-being” may last for several hours. Also, the use of oral balance gel at night may prove helpful and may be obtained by phone (. Electrical (vibrating) stimulation was used to stimulate saliva in some patients with mildly decreased flow rates (104), although the cost of the apparatus has precluded wide usage. A single-blinded, controlled trial was conducted to test the efficacy of low-dose oral human IFN-α to improve salivary function in patients with SS (105). Fifty-six outpatients with primary and four patients with secondary SS were assigned randomly into treatment groups of either IFN-a or sucralfate (control). The IFN-α (150 IU) or sucralfate (250 mg) was given orally three times a day for 6 months. After 6 months of treatment, 15 of 30 (50%) IFN-α-treated patients had saliva production increases at least 100% above baseline, whereas only 1 of 30 (3.3%) sucralfate patients had a comparable increase ($p = 0.001$). Serial labial salivary- gland biopsies of nine IFN-α responder patients showed that lymphocytic infiltration was significantly decreased ($p = 0.02$) and the proportion of intact salivary-gland tissue was significantly increased ($p = 0.004$) after the IFN-α treatment. This agent is currently in double-blind trials.

Special Therapeutic Considerations in the Dry-Eye Patient

Anesthesia and Surgery

SS patients have particular problems during the preoperative, perioperative, and postoperative periods. The normal preoperative instruction is no fluids by mouth after dinner or midnight on the day prior to surgery. In the absence of normal saliva flow, these patients have great discomfort that can be reduced by the use of artificial salivas. Because the main concern is aspiration of stomach contents during anesthesia, these patients can safely use oral mouth sprays such as MouthKote (described above) without increased risk. These also are useful in postoperative patients including those who are not able to take food by mouth. Operating rooms and postoperative recovery areas are maintained at extremely low humidity, particularly as nonhumidified oxygen blows over a face mask. Therefore, SS patients have increased risk of developing corneal abrasions during surgery and in the postoperative setting. The decreased blink reflex of the patient during anesthesia further contributes to this problem. The administration of ocular lubricants prior to surgery and in the postoperative recovery suite will reduce the chance of this complication. The patient is advised to take their Refresh PM (Allergan) or GenTeal (CIBA) ointment with them to the hospital. This will allow the ointment to be readily available for the anesthesiologist. It is increasingly common for hospitals to tell the patient to not bring their medications with them to the hospital (because all medications must be identified by the nurse for medical legal reasons) but specialty medications such as ocular or oral lubricants are not readily available in most hospitals. The patients should bring their medications in labeled containers (including patient name and name of medication) to minimize the work in “logging in” their own medications. The patient may expect a certain resistance from the nurse when they present their own medications (more work for the nurse), but they should persist, explaining that their rheumatologist wanted them to have their ocular and oral lubricants available. (We have yet to see a first-morning case delayed while the anesthesiologist waited for the pharmacy to send them a tube of lubricant, so better for the patient to bring their own. Indeed, we often tape the chart shut with a tube of lubricant attached to make sure it is “seen” by the anesthesiologist). Upper airway dryness of the SS patient may lead to mucus plug inspissation during the postoperative period, followed by obstructive pneumonias. The use of humidified oxygen and avoidance of medications that excessively dry the upper airways (i.e., used by anesthesia to control secretions) will help prevent this problem. Also, adequate hydration and respiratory therapy to keep airways clear is important. This problem has become more common because current practice is for one anesthesiologist to take the history and a different one to perform the procedure. Therefore, we advocate that the patient take the “special

instructions at time of surgery" page in this chapter with them at the time of surgery. An additional problem for the anesthesiologist is the poor state of teeth in the SS patient. Thus, a higher risk of damage to teeth during intubation must be considered. Not only can this lead to loss of the teeth and their subsequent aspiration, but these patients have great expense in preparing dentures to their remaining teeth that will be greatly affected by any further tooth loss. Finally, assessment of the "fluid status" of the SS patient in the postoperative period may be relatively difficult. Normal clinical clues such as the moisture in the ocular and oral membranes may be quite misleading. Further, some SS patients have a tendency for interstitial nephritis, which prevents adequate urine concentration and fluid balance. This problem may be exacerbated by antibiotics such as aminoglycosides.

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Chapter 40
Ocular, Aural, and Oral Manifestations

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SLE and the Eye

Systemic lupus erythematosus (SLE) may affect any organ or tissue in the body and the eye is no exception. Although the human eye measures less than 3 cm from cornea to retina, the eye contains a diverse array of structures, and almost any of these can be the target of inflammation. The manifestations of SLE in the eye are therefore varied and range from dry eye, infiltrative keratitis, scleritis, episcleritis, retinal vasculitis, optic neuropathy, and orbital inflammation.

The commonest of these manifestations is dry eye in the form of keratoconjunctivitis sicca as a result of secondary Sjogren syndrome. Chapter 39 discusses this topic in detail. Of the other ocular pathologies, retinal vasculopathy in the form of cotton wool spots is the next most common, with ominous systemic implications. Optic neuropathy, although rare, is associated with poor visual prognosis. Less common manifestations will also be briefly covered in this section, in addition to the known side effects of a common medication used in SLE—anti-malarials.

Retinal Vascular Disease

Retinal vascular lesions are the commonest intra-ocular manifestations of SLE. The most common of these are localized retinal infarctions at the level of the retinal nerve fiber layer. These are visible on ophthalmoscopy as cotton wool spots (Fig. 40-1) and are often asymptomatic if they are located in the periphery of the retina. Occasionally, these cotton wool spots may be associated with intraretinal hemorrhages and may result in a “Roth spot” (Fig. 40-2).

The published prevalence of retinal cotton wool spots in patients with SLE varies from 3% to 29% (1 ,2 ,3). However, the largest prospective study over 15 years of serial observation of 550 patients with SLE found that these lesions were present in 7% of their population (4).

Severe, occlusive retinal vasculopathy is far less common but is usually visually devastating, with one series reporting a final acuity of worse than 20/200 (i.e., legal blindness) in 55% of eyes affected by this type disease (2). The manifestations seen in this type of vaso-occlusive disease include central retinal artery occlusions (Fig. 40-3), multifocal retinal arteriolar occlusions (Fig. 40-4), capillary bed occlusion resulting in widespread retinal ischemia with secondary retinal and optic disc vitreal heme that may lead to vitreal hemorrhage (Fig. 40-5) or tractional retinal detachment, and central and branch retinal vein occlusions (Fig. 40-6) (2 ,4 ,5 ,6 ,7 ,8 ,9). Patients presenting with any of these occlusions usually complain of a sudden, painless loss of vision and/or a loss of visual field that classically respects the horizontal meridian. Fortunately, this subtype of retinal vasculopathy is rare with an incidence of less than 1% in the above prospective series of 550 patients (4). Other smaller series have reported an incidence of 2% to 8% (1 ,10).

Although retinal vascular involvement is most commonly asymptomatic in SLE patients, its presence is associated with active SLE in 88% of patients and lupus cerebritis in 73% (2 ,4). A strong correlation between the presence of retinal vascular involvement and lupus anticoagulant or antiphospholipid antibodies has also been found in several studies in addition to a decreased survival time (2 ,4 ,5 ,10 ,11). The presence of lupus retinal vascular disease is therefore a marker of poor prognosis for survival.

Choroidal Vascular Disease

Retinal vessels are readily seen with an ophthalmoscope and therefore, retinal vascular disease is relatively easy to assess. In contrast, the choroidal vasculature is deep to the retina and usually obscured by the retinal pigment epithelium. Abnormalities of choroidal vessels in SLE are rarely reported but also much more difficult to appreciate than retinal vascular disease. A choroidal vasculopathy usually results in multiple foci of serous retinal detachments that eventually resolve with residual scarring of the retinal pigment epithelium and some degree of permanent visual impairment (5 ,12 ,13).

Optic Neuropathy

Central nervous system (CNS) involvement occurs in up to 39% of patients in SLE (14 ,15). Optic nerve involvement is

far less common and is estimated to occur in up to 1% of all SLE patients (4 ,16).

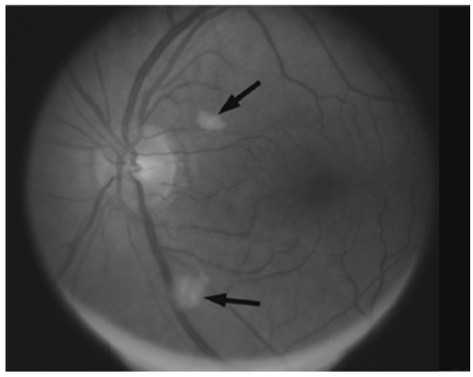

Figure 40-1. (See color plate.) Color fundus photograph of cotton wool spots (arrows).

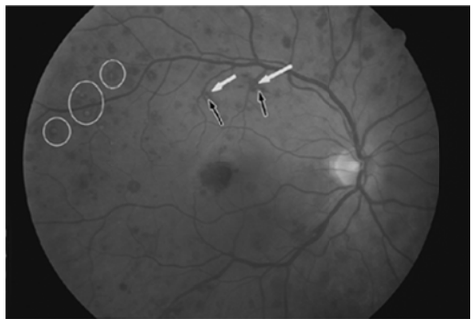

Figure 40-2. (See color plate.) Roth spots circled, with central white spot that may either represent fibrin or retinal infarct (black arrow) surrounded by intraretinal hemorrhage (white arrow).

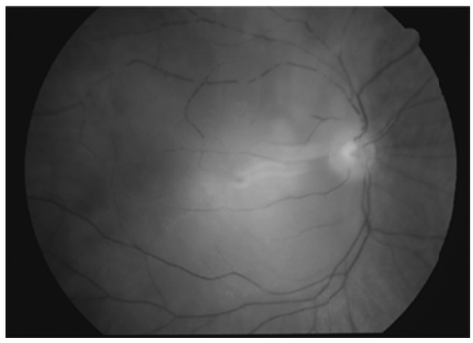

Figure 40-3. (See color plate.) Central retinal artery occlusion of the right eye. Note the ischemic, pale, edematous retina.

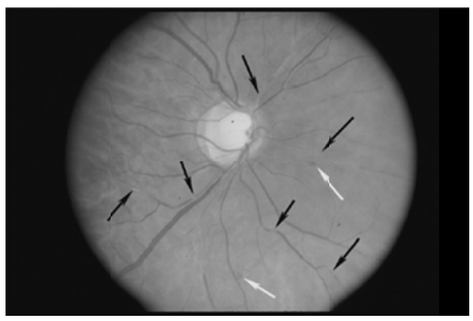

Figure 40-4. (See color plate.) Retinal vasculitis in SLE manifest by multiple arteriolar occlusions (black arrows) and intraretinal hemorrhages (white arrows).

The pattern of optic nerve involvement in SLE is varied. In some cases, the patients present with symptoms and signs consistent with an acute optic neuritis (16 ,17) characterized by the acute onset of retrobulbar pain aggravated by ocular movement, an afferent pupillary nerve defect, visual field loss or scotomata and either a swollen (Fig. 40-7) or normal appearing optic disc.

In others, the presentation may be more insidious. The patient instead may present with painless loss of vision that may be gradual in its onset (18) with an afferent pupillary defect and an arcuate or altitudinal visual field defect present on examination.

In both patterns of presentation, the pathogenesis is thought to be the same: microvascular occlusion leads to demyelination of the optic nerve in milder cases and optic nerve infarction in severe cases (16).

The visual prognosis in individuals with SLE optic neuropathy is poor, as it is notoriously difficult to treat. The standard treatment is corticosteroid either orally or pulsed.

However recovery (if any) is slow, with final visual acuities of 20/200 or worse in several series (16).

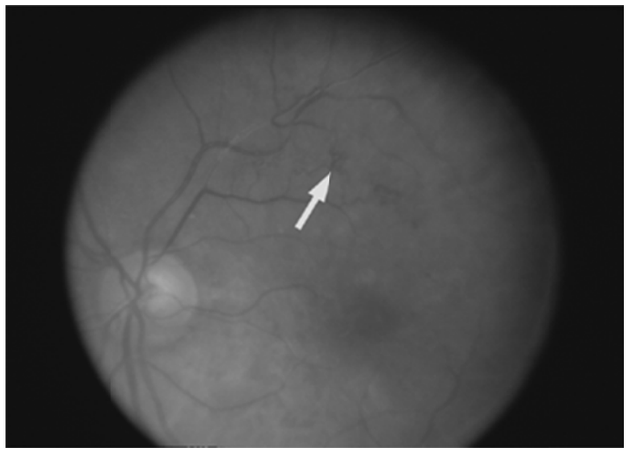

Figure 40-5. (See color plate.) Neovascularization of the retina (white arrow).

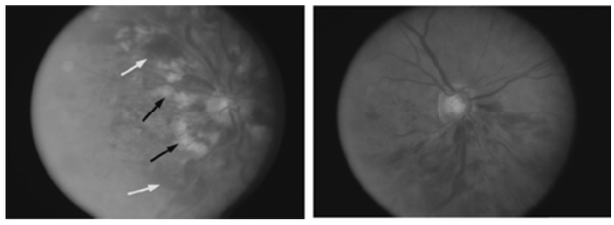

Figure 40-6. (See color plate.) **A**, Central retinal vein occlusion with widespread intra-retinal hemorrhages (white arrows), dilated tortuous retinal veins and cotton wool spots (black arrows). Note that cotton wool spots are not a consistent finding in retinal vein occlusions. **B**, Branch retinal vein occlusion. Note the sectorial distribution of the hemorrhages that distinguishes this from a central vein occlusion.

More recently, some success has been reported with the use of intravenous cyclophosphamide in addition to steroid treatment. Rosenbaum et al. (17) reported a marked improvement in visual acuity and visual function in three patients treated with this regimen with a later series of 10 patients reported similar results with 50% of patients regaining normal visual acuity after treatment (19).

It should also be mentioned that SLE optic neuropathy is a rare cause of optic nerve disease in comparison to other causes of optic neuritis, such as multiple sclerosis (MS).

Certainly the distinction between SLE optic neuropathy with CNS involvement and multiple sclerosis can be difficult, as both may result in the same signs and symptoms and the two conditions have even been described to co-exist (20). However, the response of lupus optic neuropathy to treatment is slow in comparison to the rapid response of MS related optic neuritis (17).

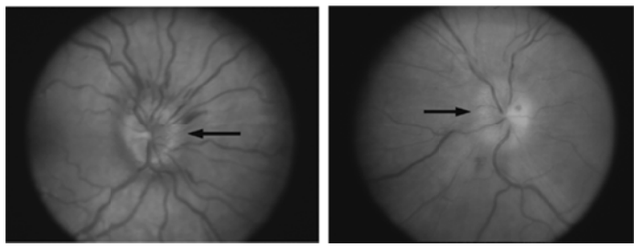

Figure 40-7. (See color plate.) Swollen optic disc. Note the blurred margins (black arrow) that distinguishes this from other causes of a prominent disc such as disc drusen.

Episcleritis and Scleritis

Both scleritis and episcleritis may occur in SLE (21 ,22) and are a result of small vessel vasculitis affecting the tissues of the ocular coat—namely the sclera and episclera.

Episcleritis is a benign, non–vision-threatening disease that results in ocular injection and mild to moderate periocular discomfort. It usually runs a benign course and often resolves spontaneously after period of a few weeks. It responds well to topical steroid drops or oral nonsteroidal anti-inflammatory medications. Although it may occur in SLE, the vast majority of patients presenting with episcleritis do not have an underlying systemic inflammatory disease.

Scleritis is a deeper and more severe inflammation that runs a more chronic course. It can result in visually debilitating complications if left untreated. These complications include severe scleral thinning (scleromalacia) resulting in prolapse of intra-ocular structures, corneal

scarring, glaucoma, and serous retinal detachments. Clinically, scleritis is often characterized by severe periocular pain, deep ocular injection, and marked tenderness of the affected area to palpation. Less commonly, scleritis may instead present with severe pain and blurred vision without ocular injection if the posterior sclera is involved. More importantly, the development of scleritis is thought to be a serious development in SLE, as it has been described to parallel the degree of disease activity elsewhere (23 ,24 ,25).

Although those presenting with scleritis are more likely to have an underlying systemic inflammatory disease than those presenting with episcleritis, the vast majority of patients with scleritis have rheumatoid arthritis (26 ,27), with other vasculitic diseases such as Wegener granulomatosis coming a distant second. In comparison, SLE is an extremely rare cause of scleritis.

Corneal Disease/Keratitis

As mentioned previously, the commonest ocular manifestation in SLE is keratoconjunctivitis sicca (KCS), which results in a poor tear film and secondary corneal changes such as small dry spots or epithelial erosions (superficial punctuate keratopathy) (1). However, other corneal disease may rarely occur, with a few cases of ulcerative keratitis and deep keratitis or inflammation of the deeper corneal layers (stroma) with secondary impairment of vision being described (28 ,29). These presentations are thought to be related to vasculitis affecting the surrounding limbal vessels. Ulcerative keratitis can also occur in association with scleritis.

Uveitis

Although described, uveitis, or intra-ocular inflammation, is an extremely rare association with SLE (23).

Orbital Inflammation

As SLE may affect any tissue of the body, orbital tissues such as the lacrimal gland (most commonly resulting in sicca), extraocular muscles and other orbital tissues may also be involved, leading to symptoms of pain, proptosis, lid swelling, and diplopia. Such an orbitopathy from SLE alone is exceedingly rare, with only a handful of case studies being reported (22).

Chloroquine and Hydroxychloroquine Toxicity

Chloroquine or hydroxychloroquine toxicity is classically characterized by the development of a bilateral bull's eye maculopathy that is visible on fundoscopy (30) (Fig. 40-8). At this stage, observant patients may complain of a paracentral scotoma, whereas others may be asymptomatic. However, should drug exposure continue, further irreversible damage to the retina occurs that is manifest by retinal pigment atrophy resulting in widespread retinal pigmentary changes and retinal vascular attenuation. By this stage patients have severe visual field loss, decreased visual acuity and impaired night vision (31 ,32).

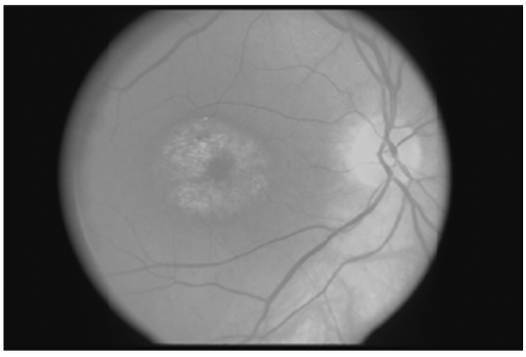

Figure 40-8. (See color plate.) Bull's eye maculopathy.

The first sign of toxicity is thought to be decreased visual function in the paracentral visual field that is detectable prior to the development of clinically visible bull's eye maculopathy. Cessation of the drug at this early stage is thought to reverse these changes. However, once signs of maculopathy are visible clinically, these changes have been shown to be irreversible (32).

The risk of retinal toxicity with chloroquine is higher than for hydroxychloroquine and occurs at lower doses (>3 mg/kg/day) (32 ,33). The risk of retinal toxicity for hydroxychloroquine is far lower with only 20 cases being reported, with the majority occurring in individuals on high doses (>6.5 mg/kg/day) (32).

However, hydroxychloroquine toxicity has occurred in individuals at lower doses (i.e., <6.5 mg/kg/day), particularly if treatment is continued for more than 5 years (34). Other risk factors have also been identified, such as co-existing retinal, renal, and liver disease, obese body habitus, and age older than 60 years (32). Recent research has also suggested that carriers of an ABCR gene polymorphism, which has been linked to Stargardt disease, a form of hereditary maculopathy, may also be prone to developing either chloroquine or hydroxychloroquine toxicity at low doses despite a normal ophthalmic examination prior to treatment (35). Therefore it would appear that the development of hydroxychloroquine (and chloroquine) toxicity may not purely be related to the dose, but also a variety of genetic and environmental factors.

Several recommendations for regular ophthalmic screening of patients treated with chloroquine and hydroxychloroquine have been proposed to detect patients prior to developing irreversible, severe vision loss. However, no “gold standard” for screening has yet been established with major drawbacks being the high cost of most screening recommendations given the relative rarity of this complication

(36) and the variability in the susceptibility of individual patients (as discussed above).

The American Academy of Ophthalmology currently recommends that a baseline ophthalmic examination be done prior to commencing treatment that at a minimum includes a dilated fundal examination and an Amsler grid or Humphrey 10–2 visual field examination that assesses the paracentral visual field (32). This examination would therefore also identify those with pre-existing retinal disease that would place the individual at higher risk.

Those at high risk were identified as those with:

- Doses > 6.5 mg/kg/day
- Treatment duration more than 5 years
- Obese habitus
- Co-existing retinal, renal, or liver disease
- Age greater than 60 years

A repeat examination for high risk individuals is recommended on a yearly basis. Those not in the high risk category are screened at the same rate that is recommended in the general population (i.e., approximately 2 to 4 yearly in those younger than 65 years of age and 1 to 2 yearly in those 65 years or older).

Another investigation that has been proven useful in the diagnosis of early hydroxychloroquine toxicity includes multifocal electroretinography (mfERG). This is an electrophysiologic test that assesses the electrical function of various parts of the retina when it is stimulated by specialized light stimuli. Several studies have now shown that mfERG is more sensitive than standard examinations (e.g., visual field testing, visual acuity, clinical examination) in cases of suspected toxicity (37 ,38). However the role of mfERG in widespread screening is still to be determined.

More recently, a potentially more cost effective method of screening using a modified Amsler grid has been proposed (39). This test could be easily administered in a nonophthalmic clinic, such that only those with an abnormal test are referred for a full ophthalmic examination. This screening tool is currently still under development and its true sensitivity is still unknown. However, it certainly may prove to be a far more cost effective screening method than the current recommendations that exist today.

Antiphospholipid Antibody Retinopathy

Retinal vascular disease associated with antiphospholipid antibodies is characterized by diffuse retinal vascular occlusion, often in association with symptoms of a rheumatologic disease (40).

However, the vast majority (>90%) of cases of occlusive retinal vascular diseases (e.g., retinal arterial or venous occlusions) occur in the elderly who have other systemic vascular risk factors, such as atherosclerosis or hypertension (41 ,42). Therefore, primary and secondary antiphospholipid antibody disease accounts for a very small proportion of these presentations.

Despite the rarity of this condition, several studies have shown that up to 24% of patients with occlusive retinal vascular disease and no cardiovascular risk factors have high titers of antiphospholipid antibodies. This is significantly higher than in control populations in which these antibodies were present in less than 9% (40 ,43 ,44 ,45).

Therefore, young patients (i.e., younger than 50 years of age) who present with diffuse occlusive retinal vasculopathy should be investigated for the presence of these antibodies, because of the possible ocular and systemic complications that may require prophylactic therapy.

Oral Manifestations

Painless oral ulcers are included in the 1982 classification criteria of SLE and thus were found to be more specific for the recognition of lupus than such common manifestations as alopecia, Raynaud phenomenon, sicca, or fever. Oral ulcers are frequently present during an acute flare of systemic lupus and could occur on any part of the oral mucosa (46). Lesions on the hard palate may range from patches of erythema to frank ulceration and mucosal hemorrhage. Inclusion of these nonspecific erythematous patches resulted in an estimated frequency as high as 45% based on a Swedish series of 51 patients with lupus (47). Pathology of oral lesions includes epithelial acanthosis or hyperplasia, disturbed epithelial maturation, and liquefactive degeneration of basal epithelial cells (47). Immunofluorescent testing of skin and mucosal biopsy tissue frequently shows subepithelial immunoglobulin and complement deposition (the lupus band). Although its presence is not limited to clinically involved tissue, a lupus band is associated with systemic lupus as opposed to rarely being associated with chronic cutaneous lupus (48).

Extensive and diffuse inflammation of the oral cavity, oral mucositis, is a relatively frequent occurrence in lupus and could be a result of immune mediated mucosal inflammation (49). A drug reaction such as seen with methotrexate could have a similar appearance and may require the addition of high doses of folic acid supplementation. There is a curious syndrome of isolated and extensive oral mucositis without any systemic disease, negative ANA using Hep 2 cells but positive ANA using stratified epithelial cells. The recurrent oral lesions are responsive to hydroxychloroquine and probably share the same pathophysiology as the mucositis seen in patients with multisystem disease (50). Oral candidiasis and herpes simplex viral infection should always be suspected, actively sought, and treated especially in patients on steroids and other immunosuppressives (49 ,51). The only finding of oral candidiasis, especially in patients with xerostomia, may be a burning tongue with a characteristic smooth and red surface. Recurrent aphthous stomatitis and cutaneous lesions with discoid lupus features have been reported in patients suffering from X-linked chronic granulomatous disease. The presence of severe recurrent and chronic infections in childhood is a clue to this rare disorder (52).

Discoid lesions could occur on mucosal surfaces and are frequently painful (47 ,49). The lesions frequently involve the labial and buccal mucosae. Lesions have an irregular outline with slightly elevated patches and striated surface, the latter sometimes becoming eroded and transformed into indurated, whitish, raised, scar-like tissue. Biopsy of oral mucosal discoid lesions reveals a similar appearance to that seen in skin and include hyperkeratosis, normal or decreased thickness of the stratum granulosum, irregular acanthosis and atrophic stratum spinosum, focal liquefaction of stratum basale, single cell keratinization, lymphocytic epithelial inflammation, homogeneous thickening in a band like distribution at the basement membrane, and perivascular lymphocytic infiltration with tissue edema.

There is an association between lichen planus of the oral cavity and lupus (53 ,54 ,55). Oral lichen planus lesions usually occur on the buccal mucosa, lips, and tongue with lesions rarely found on the palate or gingiva. The lesions may be asymptomatic or may present with burning or itching. In many cases oral lichen planus lesions occur without concomitant skin lesions. The lesions may vary from a coalescence of small pearly gray, hyperkeratotic nodules to a lace-like pattern of hyperkeratotic streaks on an erythematous background with erythema most pronounced at the border. Bullous lichen planus is a rare variant of lichen planus and patients present with ulceration of the tongue and cheeks and less commonly of gingivae and lips. Bullous lesions are rarely preceded by vesiculation. The ulcers are smooth and adjacent mucosa is atrophic and erythematous with linear white striae. A biopsy is frequently required to make this diagnosis and needs to be taken from nonulcerated erythematous areas. The histopathology demonstrates dense subepithelial lymphocytic infiltration with liquefaction of the basal epithelial layers (49). Bullous SLE is a chronic, wide spread, nonscarring, subepidermal, blistering eruption associated with circulating antibodies to type VII collagen (56). Oral blistering lesions may be found in association with cutaneous disease and can be confused with oral bullous lichen planus.

Xerostomia as a result of secondary Sjögren syndrome is often associated with periodontal disease and poor dentition and is discussed in Chapter 39 .

Therapy of oral mucosal lesions is empiric and includes treatment of the underlying systemic disease with corticosteroids, antimalarials, or immunosuppressives. Intra-oral topical or intralesional steroids may be required for limited oral disease. Oral swish and swallow of antifungal agents for oral candidiasis or tetracycline for aphthous ulceration may be helpful. Avoidance of spicy foods and frequent sips of liquid with meticulous dental hygiene are important ancillary therapies that are often overlooked.

Scalp and Hair Involvement

Patients with lupus may lose hair for a variety of reasons and several patterns of hair loss have been described. Diffuse hair loss is generally nonspecific and has a broad differential including stress associated with acute multisystem disease (57) side effects from immunosuppressives, or hypothyroidism (58). Patients with severe acute systemic lupus are prone to developing diffuse hair loss from telogen effluvium, a condition in which an abnormally high percentage of normal hairs from all areas of the scalp enter telogen, the resting phase of hair growth. A lag of 3 to 4 months between the acute flare of lupus and diffuse hair loss is common because of the time taken for hair to cycle from anagen through catagen and finally telogen phase. Initially the patient complains of increased hair shedding followed by diffuse hair thinning. Hair thinning occurs on all parts of the scalp as well as pubic and axillary hair. Since this is a noninflammatory condition, the scalp has a totally normal appearance. The root tip of the extracted hairs has a clubbed structure of normal telogen hair with a normal shaft. Since there is no structural damage to hair follicles, the hair loss reverses with resolution of the systemic illness, withdrawal of immunosuppressives, or correction of hypothyroidism (59).

Patchy alopecia in lupus is usually associated with loss of hair follicles in association with discoid lesions of the scalp (49 ,59 ,60). Biopsy reveals marked erythema, atrophy, telangiectasia, and follicular hyperkeratosis. This may require therapy with intralesional steroids, antimalarials, steroids, or retinoids.

Other causes of scarring alopecia should be considered and include trauma, burns, lichen planus, acute severe fungal infections, such as tinea capitis, viral infections, such as herpes zoster, or bacterial infections (60). Damaging hair grooming or hair coloring practices may result in nonscarring hair loss from traction or fragile damaged hair (hair breakage) respectively and are reversible causes of alopecia in patients with lupus (59).

A peculiar abnormality of individual hairs has been termed “lupus hair” and is characterized by hair fragility and increased waviness especially in a frontal distribution (61). Lupus has been found in association with porphyria cutanea tarda and the latter disorder is a known cause of temporal hirsutism. Vitiligo of the scalp with concomitant patches of white hair is associated with patients suffering from lupus and is also found among their first-degree relatives (62). A deep scalp biopsy with transverse and longitudinal sections of the tissue block has been reported to provide useful information in patients with scalp and hair abnormalities (63). Transverse sections demonstrate the perifollicular and follicular interface changes of lupus as well as anagen/telogen ratio. Vertical sections demonstrate changes of lupus at the dermo-epidermal junction and papillary dermis as well as the subcutaneous tissues changes of lupus panniculitis. A good history and careful examination of the scalp and hair will frequently negate the need for a scalp biopsy (59 ,60).

Nasal Septal Disease

The nasal septum is an easily overlooked tissue bed where inflamed blood vessels are easily visualized utilizing an otoscope. Unfortunately the finding of nasal septal inflammation

in lupus is nonspecific and confounded by nonlupus causes, such as environmental dryness and airborne irritants, allergic rhinitis, upper respiratory tract infections, excoriations from self-inflicted trauma and sicca as part of secondary Sjögren syndrome. In association with active lupus, nasal septal inflammation may progress to deep ulceration and rapid destruction of septal cartilage with nasal septal perforation (64 ,65 ,66 ,67 ,68 ,69 ,70 ,71 ,72). Nasal symptoms of nasal inflammation include stuffiness, hyposmia, epistaxis, or a high-pitched whistling sound, but nasal disease may be totally asymptomatic. The largest study to date is from the University of Toronto Lupus clinic, where 40 (4.6%) of their 885 patients studied had a nasal septal perforation (65). This group found an association with involvement of other mucosal beds as well as with active multisystem disease. They postulate vascular inflammation as an etiology.

Overt or occult nasal cocaine use should always be sought in patients with destructive nasal septal disease. A strong association with Raynaud phenomenon was found with nasal septal perforation in a variety of disparate rheumatic diseases including rheumatoid arthritis, psoriatic arthritis, progressive systemic sclerosis, SLE, and mixed connective-tissue disease (MCTD) (68). Other causes of nasal septal perforation include Wegener granulomatosis, sarcoidosis, Behcet disease, lymphoma, tuberculosis, syphilis, midline lethal granuloma (probably a form of lymphoma), and cryoglobulinemia.

Nasal biopsies, although frequently obtained, provide limited useful information unless the biopsy specimen includes inflamed blood vessels (64 ,66). Treatment consists of control of the systemic disease, active, nasal irrigation and humidification. Closure of the defect, although seemingly an attractive option, may be associated with poor healing of the tissue flap as a result of ongoing vasculitis. A silastic button may avoid this complication (67).

Relapsing Polychondritis

Patients with lupus may have a clinical syndrome of inflammation of cartilage including nasal bridge, external ear, and upper airways akin to that seen in relapsing polychondritis. Ultimately lupus will involve the skin, other organs, and characteristic autoantibodies, distinguishing it from relapsing polychondritis will be detectable. In a series of 62 patients with relapsing polychondritis 22 patients had a total of 27 associated diseases including rheumatoid arthritis (7), myeloproliferative syndrome (4), SLE, or systemic vasculitis (three each), ulcerative colitis, autoimmune thyroiditis, ankylosing spondylitis, or autoimmune hemolytic anemia (two each), Crohn disease, pulmonary fibrosis, or insulin-dependent diabetes mellitus (one each) (73). A case report and review of the 16 patients reported in the literature with relapsing polychondritis and SLE provided fairly convincing evidence that this a true association rather than two diseases occurring together by coincidence (74).

Laryngeal Involvement

Hoarseness may occur in patients with lupus and may be caused by laryngeal and pharyngeal edema of obscure origin or inflammatory vocal cord nodules (75 ,76 ,77). The incidence of laryngeal involvement in lupus ranges from 0.3% to 30% (78 ,79). The cricoarytenoid joint is a synovial joint and is much less commonly involved in lupus than in rheumatoid arthritis. Involvement of this joint may lead to vocal cord edema, immobility, and stridor. Several case reports have been published with acute and life-threatening involvement of the cricoarytenoid joint in patients with lupus, requiring treatment with high-dose steroids and in a particularly severe case a tracheotomy (80 ,81 ,82 ,83). Gastroesophageal reflux disease with hoarseness may occur in association with lupus-related motility disorder of the distal esophagus. Oral candidiasis may spread into larynx and esophagus resulting in odynophagia and hoarseness.

Angioedema

Angioedema refers to a group of disorders characterized by the sudden onset of one or more localized swellings up to several centimeters in diameter affecting the skin and/or mucous membranes without pain, itching or erythema, and fading without scarring within 24 to 72 hours (84). Attacks of angioedema are caused by the absence of C1-esterase inhibitor and spontaneous and uncontrolled activation of C1, which cleaves and inactivates C4, resulting in a cascade of events and subsequent release of factors affecting vascular permeability (85 ,86 ,87). Cutaneous swelling overlying joints may be confused with frank arthritis. Abdominal pain because of visceral edema may be confused with serositis. Respiratory or gastrointestinal tract edema occurs only in the inherited form.

Angioedema is divided into hereditary and acquired categories with the acquired group caused by cold, exercise, sun, autoantibodies to C1-esterase inhibitor (88), and excessive consumption of C1-esterase inhibitor in lymphoproliferative disorders (84). Only 1 (0.53%) of 193 patients with lupus was found to have a high titer IgG antibody to C1-esterase, whereas localized angioedema or urticarial vasculitis was found in 8 patients (4%) (89). Three patients with severe lupus and acquired angioedema, reduced levels of C1 esterase inhibitor, and no demonstrable antibodies to C1 esterase inhibitor were reported with normalization of C1-esterase inhibitor levels following steroid treatment (90). This association has been questioned (91). A case of acquired C1-esterase inhibitor and lupus was reported with resolution following treatment with hydroxychloroquine (92). A patient with hypocomplementemic urticarial vasculitis, angioedema, and SLE was resistant to steroids and intravenous immunoglobulin (IVIG), but responded to a course of four infusions of rituximab (93).

The association of lupus and hereditary angioedema has been known for decades. The first cases were reported in

identical male twins who had discoid lupus erythematosus and hereditary angioedema. They had low levels of C1-esterase inhibitor and C4 and their mother had lupus with a spontaneous mutation in C1-esterase inhibitor (94). A daughter of one of the twins developed full-blown lupus, angioedema, and reduced C1-esterase levels and function (95). Hereditary angioedema is an autosomal dominant disorder with deficiency of C1-esterase inhibitor and is divided in type I with quantitative deficiency in C1-esterase inhibitor and type II with reduced or normal levels of C1-esterase inhibitor levels, but a nonfunctional enzyme (84). A characteristic finding is reduced C4 in patients with C1-esterase inhibitor, which in turn may predispose patients to the development of lupus, likely a result of disordered immune complex clearance. The National Institutes of Health (NIH) series of 157 patients with hereditary angioedema included 1 patient with full-blown lupus and a second patient with hydralazine induced lupus (96). The Cleveland Clinic experience of 220 patients with hereditary angioedema described a lupus-like illness in 4 patients (1.8%) (97). Treatment of hereditary angioedema include infusions of C1-esterase inhibitor (98 ,99 ,100) and plasmapheresis (101).

In addition to the classic C1-esterase inhibitor deficiency, other inherited complement deficiencies have been associated with angioedema and lupus. A low C4 level as a result of C4 null allele has been associated with discoid lupus or urticaria/angioedema syndrome (102). Heterozygous C2 deficiency was found in a patient with angioedema, myasthenia gravis, and SLE and responded to treatment with cimetidine and steroids (103).

Cervical Lymphadenopathy

Cervical lymphadenopathy in association with diffuse lymphadenopathy may occur in patients with lupus. Biopsy demonstrates nonspecific reactive lymphoid tissue. Lymphadenitis, characterized by prominent necrosis with typical hematoxylin bodies, may occur in lupus, and in severe cases may require immunosuppressive agents (104 ,105). Kikuchi-Fujimoto disease most commonly occurs in young females and is usually a self-limiting illness characterized by a fever, constitutional symptoms, and cervical as well as diffuse lymphadenopathy with histiocytic necrotizing lymphadenitis, but no hematoxylin bodies on biopsy (106 ,107 ,108). Painful cervical lymphadenopathy is a hallmark of Kikuchi-Fujimoto disease and has been reported in association with lupus (109 ,110 ,111 ,112). Although rare, death can occur from multisystem disease in lupus with associated Kikuchi-Fujimoto disease and may warrant aggressive immunosuppression (113). Rosai-Dorfman disease, a rare proliferative syndrome of histiocytes, can present with enlarged major salivary glands and painless cervical lymphadenopathy and one case has been reported in association with lupus (114). Infections with atypical organisms such as mycobacteria other than tuberculosis (TB) and cat-scratch disease may occur in patients with lupus, especially if on immunosuppressive agents. Lymphoma in association with Sjögren syndrome should always be considered in asymmetrical or rapidly enlarging painless lymphadenopathy. Spontaneous jugular vein thrombosis may mimic cervical adenopathy and has been reported in a variety of associated disorders including discoid lupus and antiphospholipid antibody syndrome (115).

Thyroid Disease

Autoimmune thyroid disease, characterized by antibodies directed against thyroid antigens including thyroglobulin and microsomes is relatively common in lupus. Antithyroglobulin and antimicrosomal antibodies are also found in lupus patients even in the absence of thyroid disease. In a group of 300 lupus patients, 7.3% were found to have thyroid disease (116). Hypothyroidism was found in 5.7%, significantly more than the background rate of 1% in the community. Hyperthyroidism was found in 1.7%, which is similar to the community background rate. Fourteen percent of the total cohort had thyroid autoantibodies, whereas these antibodies were present in 15 of the 22 patients with overt thyroid disease. The NIH experience of 332 hospitalized lupus patients provided very similar figures with 7.5% prevalence of thyroid disease with the majority (6.6%) being hypothyroid (117). This ill group of patients not surprisingly demonstrated features of the sick euthyroid syndrome. Multiple abnormalities were found in the pituitary-thyroid and pituitary-gonadal axes among 11 newly diagnosed lupus patients (118). These patients had normal T4 and TSH but an exaggerated TSH response to TRH indicating latent hypothyroidism. Another group provided data in support of the hypothesis of slow universal progression of the autoimmune process—progression from mild thyroiditis to clinical disease over time (119).

Interestingly, 58 of 168 (35%) patients with autoimmune thyroid disease (Hashimoto thyroiditis and Grave disease) were ANA positive compared to 7 of 75 (9%) healthy controls. Five of these patients had Sjögren syndrome and 2 had lupus (92). Two patients with lupus, acutely painful neck swelling and fever were reported from Hong Kong with suppurative thyroiditis caused by *Staphylococcus aureus* and tuberculosis, respectively, indicating the need for a needle aspiration and bacteriology to distinguish infectious causes thyroiditis from acute autoimmune thyroiditis (120). Antithyroid medications, such as methimazole, used for the treatment of diffuse goiter or frank hyperthyroidism have been implicated in the development of drug-induced lupus (121 ,122).

Temporomandibular Joint

The temporomandibular joint is less frequently involved in lupus than in juvenile chronic arthritis, rheumatoid arthritis, or osteoarthritis and may present with aural pain, clicking, and difficulty with jaw opening (123 ,124 ,125). Because there is a high rate of fibromyalgia in patients with lupus, temporomandibular joint syndrome could be expected in

this subset of patients with lupus. The fibromyalgia-associated joint dysfunction may be a result of bruxism or masticatory muscle myofascial pain dysfunction in distinction to structural temporomandibular joint defects seen in temporomandibular degeneration/dysfunction and other inflammatory conditions.

Ear Involvement and Lupus

SLE often has a significant impact on the inner ear to cause hearing loss, tinnitus, and vertigo (126 ,127 ,128 ,129 ,130 ,131 ,132). Ear involvement can be unilateral or bilateral, the latter often asymmetric. Although often reported as predominantly affecting mid to high frequencies, low frequencies are affected as well. Onset of hearing loss can be sudden (hours to days), rapidly progressing (days to weeks), or slowly progressing (weeks to months).

Reported prevalence rates of ear manifestations in SLE vary (15% to 57%). This is attributable to small studies (less than 20 patients) and variables such as inclusion criteria for lupus, definitions of hearing loss (self-reporting versus comprehensive audiometric testing), and age of patients (autoimmune hearing loss masked by age-related hearing loss). Detection and diagnosis also are confounded by status of systemic disease, noise-induced hearing loss, and ototoxic drugs, such as antimalarials, nonsteroidal anti-inflammatories, aminoglycoside antibiotics, and diuretics (133 ,134).

Often inner ear dysfunction (hearing loss or vertigo) is the first manifestation of autoimmune disease, prior to any other systemic symptoms (135 ,136). Ear problems also commonly occur during a period of lupus remission or inactivity (126). Thus, many patients are initially seen by otolaryngologists and not rheumatologists, which slows the correlation with autoimmune disease. Nevertheless, ear problems are generally responsive to glucocorticoid and cytotoxic drug treatment (131 ,134 ,137 ,138), as well as plasma exchange (139 ,140) and anticoagulants (141). Permanent hearing loss because of autoimmune disease has been restored with cochlear implants (142), offering some hope to patients who no longer respond to medical management.

It is interesting that other common autoimmune diseases have a similar prevalence of hearing loss, vertigo, and/or tinnitus, including rheumatoid arthritis (143 ,144 ,145 ,146 ,147 ,148), Wegener granulomatosis (149), Cogan syndrome (150), relapsing polychondritis (151), Behçet disease (152), and progressive systemic sclerosis (153). Ear manifestations in these other autoimmune diseases imply that a similar immune process is responsible for ear pathology.

Mechanisms of Immune-Mediated Inner Ear Disease

Ear manifestations of systemic autoimmune disease are part of the general classification of immune-mediated inner ear disease. Ear dysfunction, with or without other organ involvement, often is associated with circulating antibodies or immune complexes, but how they impact the inner ear is unknown (134 ,154 ,155). Hearing loss in SLE has been inconsistently correlated with circulating autoantibodies to deoxyribonucleic acid (DNA), cardiolipin, phospholipids, and endothelial cells (126 ,127 ,129 ,156). However, some patients with sudden hearing loss and MéniÜre disease also have the same elevated antibodies, but without any other autoimmune disease symptoms (157 ,158 ,159 ,160). These patients may represent those in whom the ear is the first organ impacted by disease.

It has been proposed that circulating antibodies or immune complexes interfere with vasculature of the inner ear, compromising its function. Endothelial cell tight junctions of inner ear vessels create the blood-labyrinth barrier (similar to the blood-brain barrier) and its breakdown causes loss of inner ear potentials and hearing loss. In fact, temporal bones from autoimmune disease patients commonly show vasculitis, vascular necrosis, connective tissue proliferation, and new bone formation, all presumably secondary to such vascular breakdown (161 ,162 ,163 ,164).

Some insight into potential mechanisms of immune-mediated inner ear disease has come from studies of mouse models for SLE. All commonly studied autoimmune mice (MRL/lpr, C3H/lpr, New Zealand Black, Palmerston North) have been shown to have inner ear disease and hearing loss (165). In fact, these mice have elevated antibodies against most of the antigens reported for human autoimmune hearing loss. The predominant area affected is the stria vascularis, a vascularized epithelium responsible for ion homeostasis of inner ear fluids. The blood labyrinth barrier normally protects this region, but considerable deposition of immunoglobulin and other inflammatory factors is seen on these vessels with progression of systemic disease. As a result, the tight junctions leak and endocochlear potentials decline. Hearing loss in the mice can be restored with glucocorticoid treatments.

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Chapter 41

Hematologic and Lymphoid Abnormalities in Systemic Lupus Erythematosus

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Lymphadenopathy

Lymphadenopathy, a common clinical manifestation of systemic lupus erythematosus (SLE), can be generalized or regional in distribution, especially in the cervical and axillary regions. Hilar adenopathy rarely is seen in SLE (1). In their series of 520 patients, Dubois and Tuffanelli (2) observed adenopathy in 59%, with axillary adenopathy in 42% and cervical adenopathy in 24%. Cervical adenopathy was an initial manifestation in 2% and generalized adenopathy in 1% of patients. The nodes usually were nontender and discrete, and they varied in size from shotty to 3 to 4 cm in diameter. The glandular enlargement was so pronounced in some patients that malignant lymphoma was suspected. Lymphadenopathy as the presenting manifestation of SLE has been reported by other authors (3 ,4).

Lymphadenopathy was more common in children than in adults and most marked among African-American patients. Meislin and Rothfield (5) reported similar findings, with lymphadenopathy or hepatosplenomegaly in 69% of children as compared to 35% in adults with SLE. Among 698 adult patients with SLE collected from six large series in the literature, the frequency of regional and generalized lymphadenopathy ranged from 30% to 78%, with a mean prevalence of 50% (6 ,7 ,8 ,9 ,10 ,11).

Shapira et al. (12) observed SLE patients with lymphadenopathy to have more constitutional symptoms, lupus skin rash, hepatomegaly, splenomegaly, higher anti–double-stranded DNA (dsDNA) antibodies, and hypocomplementemia than those without lymphadenopathy.

Histopathology of the Lymph Node

The characteristic finding in the lymph nodes in SLE is a diffuse, reactive hyperplasia (13 ,14 ,15). Varying degrees of coagulative necrosis and lymphoid follicular hyperplasia are seen. Hyperplastic germinal centers with plasmacytosis and varying number of immunoblasts in the interfollicular areas are found. In the necrotic areas and within the sinuses are occasional extracellular amorphous bodies, 5 to 12 μm in diameter, that stain intensely with hematoxylin. These hematoxylin bodies contain aggregates of deoxyribonucleic acid (DNA), immunoglobulins, and polysaccharides (13), and when present, they are considered to be characteristic of lupus lymphadenitis (16). Cells resembling Reed-Sternberg cells also have been described in patients with SLE (17).

Necrosis of small blood vessels located in the necrotic areas of the lymph nodes has been reported. Deposits of immunoglobulin and C3 were found in the walls of venules and arterioles suggesting that vasculitis because of deposition of immune complexes may be important in the pathogenesis of the lymph node necrosis in SLE (18).

Medeiros et al. (19) examined the immunohistologic features of the lymph node in SLE and found both follicular and paracortical hyperplasia, with paracortical foci of necrosis. Two predominant cell populations within and surrounding the necrotic areas were identified: $CD11b^+CD15^+$ histiocytes, and $CD8^+CD3^+$ lymphocytes. The interfollicular regions in the nonnecrotic areas were populated by T cells, and the lymphoid follicles were composed of polytypic B cells. $CD4^+/CD3^+$ T lymphocytes outnumbered $CD8^+/CD3^+$ T cells by a 3:1 ratio. The immunohistologic characteristics bear similarities to those of the necrotizing lymphadenitis of Kikuchi and Fujimoto.

Three histologic patterns of reactive follicular hyperplasia have been described by Kojima et al. (20) in SLE. These are histologic findings of multicentric Castleman disease, T-zone dysplasia with hyperplastic follicles, and nonspecific follicular hyperplasia. Reactive hyperplasia with numerous enlarged coalescing (giant) follicles with distortion of the architecture has also been reported in SLE (21). These observations emphasize the variety of histopathologic findings in lupus adenopathy.

Deftos et al. (22) reported an SLE patient with lymphadenopathy and hypercalcemia. There was no malignant transformation; however, there was

an abundant expression of parathyroid hormone–related protein in the lymph nodes. This finding suggests that the hypercalcemia was a result of an increased production of the hormone.

Kikuchi-Fujimoto Disease and SLE

Kikuchi-Fujimoto disease (KFD) or histiocytic necrotizing lymphadenitis is a self-limited lupus-like illness of unknown cause in young women that is characterized by cervical adenopathy, fever, weight loss, and a prodrome of upper respiratory tract infection. Other than a mild leukopenia in 50% of patients, laboratory investigations generally are unremarkable. The disease may be confused clinically with SLE and histologically with malignant lymphoma (23). Diagnosis is based on the characteristic histologic changes in the lymph nodes with paracortical necrosis, mononuclear infiltrate, and absence of neutrophils and plasma cells (24). These morphologic changes may be indistinguishable from lupus lymphadenitis (25) and especially when the histology is atypical. The presence of hematoxylin bodies, prominent plasma cells, and deposition of DNA in the blood vessel wall in lupus lymphadenitis help differentiate it from Kikuchi disease (24 ,26). Before a diagnosis of nodal KFD is made, serologic tests are necessary to exclude SLE.

Fatal cases of KFD are uncommon (27). Extranodal involvement is rare and has been documented in the skin, bone marrow, and myocardium. The skin rash in KFD is noncharacteristic and appears as single or multiple erythematous papules or plaques on the face, extremities, and trunk lasting for weeks to months and can mimic SLE. Spies et al. (28) described a constellation of histopathologic features in the cutaneous lesions of KFD, including the presence of cells that stain with the CD68 marker for histiocytes.

Coexistent KFD and SLE have been reported in a few patients (29 ,30 ,31 ,32 ,33). The disease can occur along the course of SLE, simultaneously with the onset of SLE, or can precede the development of full-blown SLE (29 ,33 ,34). Two of 108 patients with KFD who were examined retrospectively developed SLE (24). Two of 61 KFD patients from China developed SLE 1 month and 5 years later (35). The concurrent onset of mixed connective tissue disease and KFD also has been reported (36).

Castleman Disease and SLE

Castleman disease or angiofollicular lymph node hyperplasia is a rare lymphoproliferative disorder of unknown etiology characterized by lymphadenopathy with or without constitutional symptoms and clinically resembles malignant lymphoma. There are three histologic variants (hyaline vascular, plasma-cell, and mixed) and two clinical types (localized and multicentric) (37). The multicentric type can present with clinical features that may mimic systemic connective tissue disease including rheumatoid arthritis (RA), Sjögren syndrome, and SLE (38 ,39 ,40). Castleman disease should be considered in a patient with lupus-like presentation and with persistent lymphadenopathy despite corticosteroid therapy. Castleman disease can also develop along the course of a connective tissue disease (41).

Kojima et al. (42) found histopathologic features of Castleman disease in the lymph nodes of 5 of 19 (26%) of SLE patients presenting with peripheral lymphoadenopathy. The significance of these findings at this time is not clear.

Recent studies have provided evidence to support the role of human herpesvirus 8 or Kaposi sarcoma-associated herpesvirus in the pathogenesis of Castleman disease (43).

The Spleen in SLE

Splenomegaly is not an uncommon finding in patients with SLE. In large series, the frequency of splenic enlargement ranges from 9% to as high as 46% (2 ,7 ,10 ,44 ,45 ,46 ,47). Dubois and Tuffanelli (2) found splenic enlargement in 9% of their 520 patients. When present, splenomegaly often is associated with hepatomegaly. In some patients, the spleen is so large that it can extend to the iliac crest (48).

The characteristic histopathologic picture of the spleen in SLE is periarterial fibrosis or onionskin lesion. First described in 1924 by Libman and Sacks (49), this lesion is defined as the presence of at least 3 to as many as 20 separated layers of the normally densely packed periarterial collagen of the penicillary or follicular arteries, producing the appearance of concentric rings. Larson (46) found the lesion in 40 of 51 SLE spleens (78%) that were examined at autopsy. Calcified fibrous nodules that are continuous to the onionskin lesions have been described (51).

Although considered to be highly characteristic of SLE, periarterial fibrosis may be seen in a few other diseases. Kaiser (52) examined the specificity of the splenic lesion in 18 patients with SLE and 1,679 control cases at autopsy. Of these, 15 patients with SLE (83%) and 53 of the control subjects (3.2%) with various diagnoses were positive. In addition to SLE, the only group of patients in whom the lesion was found to be significantly more prevalent than in the rest of the controls was composed of those with essential thrombocytopenic purpura, with a frequency of 4 in 13 (3%).

Isolated infarction of the spleen that is associated with circulating lupus anticoagulant and thrombosis may occur (54). Spontaneous rupture of the spleen and the splenic artery in the absence of vasculitis also has been reported (55 ,56).

Functional Asplenia

Functional asplenia is a condition that is characterized by failure of the splenic uptake of radiolabeled sulfur colloid and the presence of Howell-Jolly bodies, Pappenheimer bodies, spherocytes, and poikilocytes in the peripheral blood smear. Functional asplenia is associated with a number of diseases, including sickle cell anemia, and it

predisposes to infections, especially by pneumococci or other encapsulated organisms. In 1980, Dillon et al. (57) described its occurrence in a patient with lupus, and since then, several other cases have been reported (58 ,59). Most patients with SLE and functional asplenia who develop pneumococcal or salmonella bacterial sepsis die (58).

The condition is relatively uncommon in SLE. Of 70 patients who were screened by peripheral blood smear, 5 showed changes that were suggestive of functional hyposplenia; however, only 3 patients had no splenic uptake of the radiolabeled sulfur colloid, yielding a frequency of 4.3% (57). In another study, functional asplenia was found in 2 of 44 patients with SLE (4.6%) who were studied by determining the presence of vacuolated red blood cells using phase-contrast microscopy, a method that is more reliable than examination of the peripheral blood smear for assessing splenic function (60 ,61).

The mechanism of functional hyposplenia in SLE is unclear. In those patients who died, the spleen showed atrophy without evidence of vasculitis (58). Functional hyposplenia also can be transient and reversible (58). It does not seem to be related to disease activity in SLE, and it may manifest clinically as an overwhelming infection in a patient who is in disease remission. Other mechanisms that have been proposed include thromboses of the splenic vessels, circulating serum factors, and reticuloendothelial blockade (59 ,62). The rare occurrence of congenital asplenism and SLE has been reported as well (63).

Because of the apparent high risk for pneumococcal infection, the peripheral blood smear of patients with SLE should be screened routinely for Howell-Jolly bodies. Polyvalent pneumococcal vaccine should be considered in patients with functional asplenia, because despite the splenic dysfunction, they can still mount an antibody response (58 ,64). In general, the antipneumococcal antibody titer is lower in patients with SLE than in vaccinated healthy subjects (65), and only half of the SLE patients develop a fourfold antibody response (66). Nevertheless, Uthman et al. (67) showed that the 23-valent pneumococcal vaccine is protective in SLE with autosplenectomy.

The Thymus Gland in SLE

Structure of the Thymus

A central lymphoid organ, the thymus gland is critical in the development and differentiation of T cells and the induction of autoimmunity. Lymphocytes originate in the bone marrow and must migrate to the thymus to acquire immunocompetence. Microscopically, the thymus gland is composed of several lobules; each lobule consists of a lighter-staining medulla, which is populated predominantly by epithelial cells, and a darker-staining cortex, which is populated by lymphocytes. Hassall corpuscles in the thymic medulla are mature epithelial cells that form concentric layers and become keratinized. Myoid cells with cross-striations are located adjacent to the Hassall corpuscles.

At puberty, when the thymus has reached its maximum size, the organ begins to undergo gradual physiologic involution, which is characterized by the loss of cortical thymocytes, spindling of epithelial cells, and an increase in adipose tissues. During periods of acute stress, cortical lymphocytes are rapidly depleted (i.e., stress involution) (68).

The size of the thymus in SLE, as assessed by pneumomediastinography, was found to be small, even in patients who had not received corticosteroids (69). Serial measurements in patients receiving prednisone showed a significant reduction in thymic size following steroid therapy. Most information on the histology of the thymus in SLE comes from early studies in 13 patients with SLE, which were conducted before thymic functions were fully understood (68 ,70). Changes that were associated with stress involution included a pronounced depletion of lymphocytes, resulting in cortical atrophy and disorganization of the medulla. Aggregates of epithelial cells and cystic Hassall corpuscles were seen in the medulla. These abnormalities were not specific for SLE and also were seen in patients with long-standing terminal illness. An increased number of plasma cells, Russell bodies, and germinal centers in the thymus, similar to those found in patients with myasthenia gravis (MG), was present, suggesting an immunologic reaction within the thymus (71).

The activity of thymic hormone decreases in patients with SLE, especially in those with clinically active disease (72 ,73). This decreased activity is a result of its low serum concentration rather than the presence of a circulating inhibitor.

Thymectomy has been performed in a small number of patients with SLE. No significant clinical improvement was noted, however, and the titer of the antinuclear antibodies (ANAs) remained unchanged (68 ,74).

Myasthenia Gravis and SLE

MG is a neuromuscular disorder that is characterized by a fluctuating weakness of the skeletal, bulbar, and respiratory muscles. Like SLE, MG has a predilection for young adults, with a female predominance. In MG, however, neuromuscular fatigue and an inability to sustain repeated muscular contractions are present. The basic defect is the reduction of available acetylcholine receptors (AChRs) at the neuromuscular junction because of an autoimmune process. Immunoglobulin G (IgG) antibodies to AChRs are present in 85% of patients.

The association between MG and SLE has fascinated investigators for some time. In 1963, Alarcon-Segovia et al. (75) reported the appearance of SLE in two patients several years after thymectomy for the treatment of MG, and they collected nine other patients from the literature. Since then, several reports of coexistent SLE and MG have appeared, although it is questionable whether some of these reported cases had definite SLE (76). Of 20 cases reviewed by Killian and Hoffman in 1980 (77), only 10 fulfilled the 1973 American Rheumatism Association (ARA) criteria for the

classification of SLE. Ciaccio et al. (78) reported 2 patients with co-existent SLE and MG and found an additional 42 in the literature. Most of the 24 patients who were reported before 1972 failed to meet at least four of the 1982 ARA criteria, whereas 12 of the 20 patients reported after 1972 fulfilled these criteria.

Of the 44 reported patients with co-existent MG and SLE, MG preceded SLE in 32 (72%), and in the remaining 12, MG followed SLE. In 13 patients, SLE developed following thymectomy for the treatment of MG (78). Polyarthritis and serositis were the most common presenting features of SLE. Malar skin rash was present in 20%, discoid lupus erythematosus (LE) lesions in 11%, and photosensitivity in 7%. The disease that develops later tends to dominate the clinical picture and prognosis.

A study of the immunologic effects of thymectomy in MG showed that long-term thymectomized MG patients had mild T cell lymphopenia, expansion of some Vß T cells, polyclonal increase in serum immunoglobulins, and high levels of autoantibodies, including anti-dsDNA and anticardiolipin antibodies. In contrast, these immunologic abnormalities were not seen in nonthymectomized and recently thymectomized MG patients. Two long-term thymectomized patients developed SLE and undifferentiated connective tissue diseases (80). Thus, thymectomy is effective in the treatment of MG, but the clinical data suggest that it may lead to the development of systemic autoimmunity in genetically predisposed subjects.

A patient with widespread cutaneous and mucosal eruption following MG and a thymoma with features of cutaneous LE and pemphigus erythematosus has been described (81). A pair of monozygotic twins who were concordant for MG underwent thymectomy, and one twin developed frank, severe SLE 18 years later. The other twin remained relatively well after thymectomy except for mild symptoms, leukopenia, and positive ANA (82). Primary antiphospholipid syndrome developed 2 years after thymectomy in patients with MG (83).

Both SLE and MG are characterized by the presence of autoantibodies. ANAs are found in 30% of patients with MG, and IgG anticardiolipin antibodies are seen in 25%, especially those with thymic abnormalities (84). Additionally, antibodies to organ-specific antigens, such as skeletal muscle, thyroid, and thymic cells, are abundant (85). The most characteristic serologic abnormality in MG is the presence of IgG antibodies to AChRs. No cross-reactivity between anti-DNA and AChRs antibody has been noted (86). SLE and MG differ in regard to certain cell-mediated immune functions. Whereas patients with SLE have decreased cellular immunity, as measured by skin testing, migratory inhibition factor production by mononuclear cells, and peripheral blood lymphocyte response to mitogens, patients with MG have a normal lymphocyte response to mitogens (87). Enumeration of lymphocyte subsets have yielded normal to abnormal numbers (85). Diaz-Jouanen (87) suggested that SLE and MG represent opposite extremes in the spectrum of abnormalities and modulations of the immune response by the thymus that may lead to autoimmunity.

The coexistence of autoimmune disorders in 721 patients with MG has been examined (88). Sixty-six other autoimmune diseases were found in 60 subjects (8.3%), including 3 patients with SLE and 5 patients with RA. Oosterhuis and de Hass (89) found a 1% prevalence of SLE in a large series of patients with MG. Of their 142 patients with MG, 2 had SLE and 7 RA, suggesting that RA and not SLE was more frequently associated with MG. Killian and Hoffman (77) found 5 cases of SLE among 1,604 cases of MG that were collated from five large series. Taking the prevalence rate of SLE as between 1/2,000 and 1/25,000 and that of MG as 1/20,000, they concluded that the 5 patients with SLE in the population of 1,604 patients with MG occurred other than by chance. These numbers would be even more significant if the patients with SLE who did not fulfill the ARA criteria were included in the computation. A population-based study of MG in Norway showed a prevalence rate of 9.6 per 100,000 inhabitants; SLE was found in 8.3 of the patients with MG, which was 200 times higher than the prevalence of SLE (0.039%) in the general Scandinavian population (90).

Transient neonatal MG is a syndrome in infants that is mediated by the transplacental transfer of maternal IgG antibodies to AChRs, causing a weak suck, hypotonia, and difficulty in swallowing and respiration. A case of co-existent neonatal LE and transient MG in an infant who was born of a lupus mother with acetylcholine antibodies but without myasthenic symptoms has been reported (91).

Thymomas are uncommon neoplasms arising from thymic epithelial cells with variable morphology. They predominantly occur in adults, with no gender predilection (92). Of patients with thymoma, 40% have parathymic syndromes (93), the most common being MG, pure red cell aplasia, and adult-onset acquired hypogammaglobulinemia. Thymomas are found in 15% of patients with MG and also have been described in those with connective tissue diseases, including SLE. Of 598 patients with thymomas who were collected from the literature, 8 had SLE (94). In some cases, thymoma and SLE have been associated with another disorder, such as progressive multifocal leukoencephalopathy (95), vacuolar myopathy (96), or red cell aplasia (97). The effect of removal of the thymoma on the clinical course of SLE is variable (98 ,99). Larsson (100) described a 62-year-old woman with SLE and coexistent thymoma; the tumor enlarged while the SLE improved. In contrast, a thymectomy in another patient temporarily reduced the clinical and laboratory manifestations of SLE (79).

In summary, SLE and MG occasionally may co-exist, with the MG preceding SLE in 75% of patients. Thymectomy for MG appears to be a precipitating factor for development of SLE in some patients. Table 41-1 summarizes the

lymphoid and thymic abnormalities that are seen in patients with SLE.

Table 41-1: Lymphoid and Thymic Abnormalities in Systemic Lupus Erythematosus (SLE)

Lymphadenopathy is seen in 30% to 78% of patients with SLE; the nodes are discrete and nontender; prominent enlargement of lymph nodes may develop particularly in children and blacks.
The spleen is palpable in 9% to 46% of patients; an enlarged spleen is present in 67% of autopsy cases; the onionskin lesion in the splenic arterioles is a characteristic finding; hyposplenism is noted in <5%.
Germinal centers consisting of focal collections of lymphocytes are noted in the thymic medulla of patients with SLE; myasthenia gravis and SLE occasionally co-exist.

Hematologic Changes in SLE

Hematologic abnormalities are exceedingly common in SLE and often are presenting manifestations of the disease. Sometimes, their features may mimic those of primary blood dyscrasias, and the nature of the underlying disorder can be completely overlooked unless SLE is considered in the differential diagnosis and specific diagnostic studies performed.

Anemia

Prevalence

Most patients with SLE develop anemia at some time during the course of their disease. Michael et al. (101) reported that 87 of 111 patients with SLE (78%) had a hemoglobin level of lower than 12 g/dL at diagnosis. Subsequently, 15 of 24 patients who had normal hemoglobin level on presentation developed anemia. In general, anemia was moderate, but it was severe in some patients. The anemia usually was normochromic and normocytic, and it appeared to depend partly on the severity and duration of the illness. Three of their patients had autoimmune hemolytic anemia. The experience of other investigators has been similar to that of Michael et al. Hemoglobin of below 11 g/dL was present in 51% of the 520 patients of Dubois and Tuffanelli (2), in 73% of the 150 patients of Estes and Christian (7), and in 98% of the 275 patients of Haserick (44).

Classification

Anemia in SLE can be classified into two broad categories according to putative mechanisms: nonimmune and immune. The nonimmune-mediated group includes anemia of chronic disease, iron deficiency anemia, sideroblastic anemia, anemia of renal disease, drug-induced anemia, and anemia secondary to another disorder (e.g., sickle cell anemia). Immune-mediated anemias in SLE include autoimmune hemolytic anemia, drug-induced hemolytic anemia, aplastic anemia, pure red cell aplasia, and pernicious anemia.

A prospective study of 132 SLE patients with anemia (defined as hemoglobin of 12 g/dL or less in women and 13.5 g/dL or less in men) found the most common causes to be anemia of chronic disease (37%), iron deficiency anemia (36%), and autoimmune hemolytic anemia (14%). Other causes (13%) included pernicious anemia, chronic renal failure, cyclophosphamide-induced myelotoxicity, and miscellaneous conditions (102). It is not uncommon to see combination of two or more factors in the etiology of anemia in individual patients.

Anemia of Chronic Disease

The most common type of anemia in SLE is anemia of chronic disease. The red cells on the peripheral blood smear are normochromic and normocytic. The serum iron concentration is reduced, and the total iron-binding capacity is unchanged or slightly low. A decrease in the iron saturation of transferrin is present. The bone marrow examination usually is normal, with adequate iron stores (101). The anemia develops slowly unless it becomes complicated by other factors, such as blood loss. The reticulocyte count is low for the degree of anemia (103).

Iron Metabolism

Iron metabolism was investigated by Burger et al. (104) in 11 patients with SLE using 59Fe. Iron use was decreased in 7 of these patients. Radioactivity over various organs differed from normal, with increased levels of radioactivity over the spleen and liver. The increased amount of absorbed iron did not appear to serve the purpose of hemoglobin synthesis, but was stored instead. Plasma iron turnover, on the other hand, was elevated in most patients. The life span of erythrocytes was reduced in the absence of hemolysis. It was concluded that the anemia of chronic disease in patients with SLE may be attributed to insufficient bone marrow activity, shortened red-cell life span, and, possibly, poor uptake of iron.

Whittingham et al. (105) found low mean serum iron levels in SLE. Following the administration of prednisolone, a two- to fourfold rise in the serum iron level occurred. This increase in the serum iron concentration was not sustained.

Pathogenesis

Recent investigations on the pathogenesis of the anemia of chronic disease indicate that multiple factors are involved, including a modest shortening of red cell survival,

impaired erythropoiesis, and disturbances in the storage and mobilization of iron stores in the reticuloendothelial system. There is blunting in the production of erythropoietin in response to the anemia and the responsiveness of erythroid progenitors to erythropoein is reduced. Inflammatory cytokines including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, IL-10 are believed to be implicated in these abnormalities (106 ,107 ,108).

Tzioufas et al. (109) found serum antibodies to erythropoietin in 15% of SLE patients, and the presence of these antibodies was associated with severe anemia and active disease. Whether these antibodies can directly inhibit erythropoiesis in SLE or not remains to be investigated. Schett et al. (110) found decreased serum level of erythropoietin in SLE patients when related to the degree of anemia indicative an inadequate response. Although anti-erythropoetin antibodies were detected in 40% of patients their presence was not associated with the severity of the anemia.

Hepcidin, a hormone synthesized by liver during inflammation, regulates iron metabolism and is implicated in the anemia of chronic disease. Hepcidin inhibits iron intestinal absorption and the release of stored iron in macrophages resulting in hypoferremia and decreased delivery of iron to maturing red cells in the bone marrow. Recent studies have shown that IL-6 induces the synthesis of hepcidin (111). Serum levels of hepcidin have not been measured in SLE, however, it has been reported that elevated levels of IL-6 correlate with anemia, suggesting that possible linkage of lupus anemia with hepcidin (112).

Iron Deficiency Anemia

Iron deficiency anemia is most commonly a result of menorrhagia and to GI blood loss secondary to the chronic use of nonsteroidal antiinflammatory drugs (NSAIDs) and corticosteroids.

Differentiating between anemia as a result of iron deficiency and anemia of chronic disease may be difficult in some patients. In general, a low serum ferritin level is associated with depletion of body iron stores. However, in inflammatory conditions such as RA, ferritin is an acute-phase reactant and the serum level may become elevated. Thus, serum ferritin may be normal or elevated in an iron-deficient anemic RA patient. In SLE, the serum ferritin level is positively correlated with disease activity and high anti-dsDNA titer (113). Measurement of the serum level of soluble transferrin receptor and the ratio of the transferrin receptor to serum ferritin have been reported to be useful in diagnosing iron deficiency in the presence of chronic inflammation and anemia of chronic disease (108 ,114).

Hemoglobinopathies

Sickle cell anemia and SLE share common clinical manifestations, including arthralgias, chest pain, pleural effusion, cardiomegaly, nephropathy, strokes, and seizures. Additionally, my group has found that patients with sickle cell hemoglinopathies have an increased prevalence of autoantibodies, including ANAs (50). The co-existence of SLE and sickle cell anemia has been reported (50 ,53 ,115 ,116 ,117 ,118 ,119), and in some of these patients, the recognition of SLE was delayed because of similarities in the clinical features of these conditions (120 ,121). Wilson et al. (122) postulated that abnormalities in the alternative pathway of complement in sickle cell hemoglobinopathy may predispose patients to immune complex disorders, including SLE, but no evidence has been found to show that SLE is more prevalent in those with sickle cell hemoglobinopathies. The risk for infectious complications is increased in both conditions. Hydroxyurea has been reported to reduce the severity and frequency of vaso-occlusive crises in a patient with sickle cell anemia and SLE (123).

In northeast Italy, where there is a high prevalence of ß-thalassemia, Castellino et al. (124) reported that the ß-thalassemia trait was seen less frequently among SLE patients than in the general population. However, SLE patients with the ß-thalassemic trait tended to have Sjögren syndrome and more severe course.

Sideroblastic Anemia

A few cases of sideroblastic refractory anemia in SLE have been reported, including one terminating in erythroleukemia (125). Another patient who had refractory anemia with excess of blasts, which is a myelodysplastic syndrome, developed SLE (126). These cases probably are incidental and do not represent a true association with SLE.

Immune-Mediated Anemias

The inhibition of erythropoiesis by cellular and serum factors may be important in the pathogenesis of chronic anemia in patients with SLE. The number of colony-forming unit-erythroids (CFU-Es), which are the late erythropoietic precursors, has been found to be significantly reduced in the bone marrow of anemic patients with SLE (127). Moreover, the formation of CFU-E was inhibited in vitro by autologous and allogeneic T lymphocytes from untreated subjects, but not by those from steroid-treated patients with SLE (127). The activity of monocytes to stimulate bone marrow fibroblasts to produce a hemopoietic growth factor was found to be diminished in SLE (128). Bone marrow stromal cells play an important role in hemopoiesis, and the diminished production of growth factor may be another cause of hemocytopenia in SLE.

Circulating inhibitors of erythropoiesis also have been described (129 ,130 ,131 ,132 ,133 ,134). Sera from patients with SLE and anemia of chronic disease suppressed CFU-E formation (132). Dainiak et al. (130) characterized the serum inhibitor as having the physical properties of an immunoglobulin, and its presence was associated with disease activity. The inhibitor was removed by plasma exchange and by steroid therapy.

Pure Red Cell Aplasia

Pure red cell aplasia (PRCA) is an autoimmune hematologic condition that is characterized by severe normochromic normocytic anemia, reticulocytopenia, absence of red cell precursors, and normal white cell and megakaryocyte lines in the bone marrow. Different pathogenetic mechanisms have been proposed for PRCA, including humoral suppression of erythropoiesis by serum antibodies to erythroblasts, to CFU-Es, or to erythropoietin, and T cell mediated suppression of erythropoiesis.

PRCA is associated with a number of autoimmune conditions, and rare cases of co-existent PRCA and SLE (and procainamide-induced LE) have been reported (135 ,136 ,137 ,138). In a review of 24 cases in the literature, Habib et al. (139) found that SLE was diagnosed either before or concomitantly with PRCA and the clinical features of these patients were not different from the general SLE patient population, with the exception of less frequency of pleuritis.

The pathogenesis of PRCA in SLE probably involves different immune mechanisms and may vary from one patient to another. Elevated serum titer of antibodies to erythropoietin has been reported in PRCA and SLE, and the antibody titer decreased with response to therapy (140). Serum and IgG fraction from SLE patients with PRCA inhibited the growth of red cell precursors (129 ,141). T cell suppression of erythropoeisis has been described in a SLE patient without evidence of a humoral inhibitor (142).

Several therapeutic modalities have been used in the treatment of PRCA, including corticosteroids, immunosuppressive agents, plasmapheresis, and lymphapheresis. PRCA coexistent with SLE responded to corticosteroid therapy, however many of the patients remained steroid-dependent (139). Good response to recombinant erythropoietin (143), high-dose intravenous (IV) γ globulin (140), plasmapheresis (144), and cyclosporine alone or in combination with mycophenolate (142 ,145) have been reported in SLE patients with PRCA.

Aplastic Anemia

Aplastic anemia is an uncommon hematologic condition characterized by pancytopenia, low reticulocyte count, and a hypocellular bone marrow. It rarely occurs in SLE secondary to the use of nitrogen mustard derivatives, azathioprine, antimalarials, or other agents, such as chloramphenicol (146) and dapsone (147). Wang et al. (148) reported severe pancytopenia and bone marrow hypoplasia among Chinese patients receiving chlorambucil for lupus membranous nephropathy. In a few published cases, the aplastic anemia was considered to be caused primarily by the underlying disease; however, the role of drugs cannot be completely excluded (131 ,149). Rarely, pancytopenia and aplastic anemia can be the initial manifestation of SLE antedating the other clinical features of the disease (150). Aplastic anemia has been reported in an infant with neonatal lupus erythematosus (LE) (151).

The presence of circulating antibodies to precursor bone marrow cells suggests that some cases of aplastic anemia may be the result of an autoimmune process. Brooks et al. (152) identified a complement-dependent IgG antibody that suppressed the growth of allogeneic granulocyte-macrophage progenitor cells in vitro. Bailey et al. (153) found a noncomplement IgG antibody that inhibited in vitro granulocyte-macrophage progenitor cells and erythroblast-forming units in a patient with SLE and aplastic anemia. The IgG antibody disappeared on recovery of the patient. Other investigators have suggested T cell–mediated suppression of hematopoiesis in aplastic anemia (154). Erythroid progenitor burst-forming units colonies were cultured from the patient's bone marrow. Removal of $CD8^+$ lymphocytes led to a marked increase in immature erythroid progenitors, suggesting that suppressor lymphocytes inhibited the development of erythroid progenitors (151).

Various regimens have been used successfully for the treatment of aplastic anemia that is associated with SLE. These include androgens, cyclophosphamide, cyclosporine, antithymocyte globulin, and plasmapheresis in conjunction with systemic corticosteroids (153 ,154 ,155 ,156 ,157).

Bone Marrow Findings and Function

Michael et al. (101) examined the bone marrow aspirates of 32 patients with SLE and found them to be normal in most. Plasma cells were increased in 13 patients. One patient had a hypoplastic marrow, and two patients with autoimmune hemolytic anemia had a hypercellular marrow. Similar findings have been reported by others (158).

Careful studies by Burkhardt (159) of bone marrow biopsy specimens in 21 patients with SLE have shown significant alterations in the blood vessels, cellular elements, and intercellular substance. Compared with those of control subjects, an increased frequency of the following changes in SLE was found:

- Subintimal swelling of the arteries and arterioles, with evidence of the deposition of proteins in the vessel wall, was seen.
- Endothelial swelling and dissociation were found in the sinusoids.
- The ground substance was edematous, with fibrinoid and sclerosing changes.
- A proliferation of histiocytes bearing cytoplasmic inclusions of iron-positive protein material was noted.
- Diffuse plasma cell proliferation occurred, with the formation of Russell bodies.
- There was a reduction of granulopoiesis with predominantly immature forms, and necrobioses with opal nuclei were observed.

Feng et al. (160) examined the bone marrow of 23 SLE patients presenting with pancytopenia. The most common abnormalities, which were seen in 9 patients (39%), were hypoplasia and dyserythropoiesis. The latter refers to nuclear budding and immaturity in the presence of full

cytoplasmic hemoglobin. Lymphocytosis was found in 5 patients (22%). Other abnormal findings included gelatinous transformation, plasmacytosis, and marrow hyperplasia. Pereira et al. (161) found global hypocellularity (48%), increased reticulin proliferation (76%), and necrosis (19%) to be the most common abnormalities in the bone marrow in 21 SLE patients with peripheral cytopenias. An interesting finding is the presence of storage and hemophagocytic histiocytes in the bone marrow of SLE patients during episodes of hemocytopenia (162).

These morphologic changes are not specific for SLE; and may be seen in patients with RA and other diseases (159). Nevertheless, these findings, as well as the presence of autoantibodies and cellular factors that impair bone marrow precursor cells, suggest that the bone marrow is a major target organ in SLE.

The number of $CD34^+$ primitive hematopoetic stems cells in the bone marrow were found to be decreased in active SLE and the reduction may be a result of increased apoptosis (163). Recent data suggest that autoreactive T-lymphocytes in the bone marrow of SLE patients may damage stem and progenitor cells and impair the capacity of the bone marrow stroma to support hematopoeisis. Intensive immunosuppression and autologous stem cell transplantation in one SLE patient presumably to eliminate autoreactive lymphocytes restored some of the abnormalities of hematopoesis (164).

Sera from leukopenic SLE patients containing anti-dsDNA antibodies induced apoptosis of allogeneic normal $CD34^+$ bone marrow cells in vitro. The nature of the serum factor and its significane on bone marrow function in SLE are unclear (165).

Autoimmune Myelofibrosis

Myelofibrosis is characterized by excessive deposition of collagen and extracellular matrix proteins in the bone marrow stroma, hypercellularity, and neoangiogenesis. These changes may be associated with a variety of conditions including myeloproliferative disorders, hematologic and nonhematologic malignancies, endocrine disorders, and other conditions. Myelofibrosis occurring in the setting of SLE has been described in a few cases (129 ,166 ,167 ,168 ,169 ,170 ,171). Conversely, SLE is a rare etiology of myelofibrosis.

Paquette et al. (172) introduced the term “autoimmune myelofibrosis” in their report of eight patients with myelofibrosis and SLE. Myelofibrosis developed either before or concurrent with the diagnosis of lupus in five patients, while in the remaining three, myelofibrosis developed after the onset of SLE. Five patients fulfilled four or more American College of Rheumatology (ACR) criteria for SLE, and three patients met three criteria. All had positive ANAs, anti-DNA antibodies, and/or positive LE cell test. The patients presented with pancytopenia, and bone marrow biopsy showed fibrosis with increased amounts of fibrillar reticulin, collagen, and fibroblasts. Half of the patients had splenomegaly.

Steroid therapy improved the peripheral blood cytopenias in half of the patients with SLE and even reversed the myelofibrosis in a few (173 ,174 ,175). Compared to patients with idiopathic myelofibrosis and myeloid metaplasia, patients with SLE and this condition tended to be younger, had a lower frequency of splenomegaly, and were responsive to steroid and immunosuppressive therapy. High-dose γ globulin has also been used successfully in the treatment of myelofibrosis in SLE (176).

Gelatinous Transformation of the Bone Marrow

Gelatinous transformation of the bone marrow is characterized by atrophy of fat cells, loss of hematopoetic cells, and accumulation of extracellular mucopolysaccharides abundant in hyaluronic acid. It has been reported in a wide spectrum of chronic diseases including SLE and is associated with cachexia and severe illness. Ng and associates (177) reported 3 of 30 SLE patients with pancytopenia had gelatinous transformation of the bone marrow. Two of these patients were cachectic, one of whom had concomitant tuberucolosis.

Hemophagocytic Syndrome

Hemophagocytic syndrome (HPS) is severe acute febrile illness characterized by pancytopenia, hepatosplenomegaly, lymphadenopathy, liver dysfunction, and pathologic finding of macrophage activation with phagocytosis of mature and precursor blood cells in the bone marrow and other tissues. Elevated serum ferritin and triglycerides may be present. HPS may be a primary condition or a reactive event in infections, malignancy and autoimmune diseases, including adult-onset Still disease and SLE.

Wong et al. (178) described six patients with SLE who presented with fever and severe pancytopenia related to HPS. A study of 40 consecutive cases of reactive hemophagocytic syndrome in Hong Kong identified two patients with SLE (179). A review of 14 SLE cases in France showed that HPS occurred frequently with the onset of SLE although some cases were triggered by an infection (180). Relapses of HPS occurred in four patients during lupus disease flares. HPS associated with Epstein-Barr virus (EBV) activation during immunosuppressive therapy for lupus nephritis has been reported (181).

The pathogenesis of HPS in SLE is not known but its occurrence during disease flares suggests that it may be related to increased IL-18 production (182).

Various modalities have been used for the treatment of HPS in SLE including corticosteroids, IV γ globulin and plasmapheresis (179 ,183 ,184). Concomitant infection should be treated aggressively.

Pernicious Anemia and SLE

Pernicious anemia often is associated with other autoimmune disorders, such as Sjögren syndrome and Hashimoto

thyroiditis, yet only a few cases of co-existent SLE and pernicious anemia have been reported (185 ,186 ,187 ,188). The treatment of pernicious anemia when accompanied by SLE requires the administration of both vitamin B12 and systemic corticosteroids. Replacement therapy with vitamin B12 usually is not sufficient to reverse the hematologic abnormalities in patients with lupus and pernicious anemia (189).

Molad et al. (190) found that 18.6% of 43 female patients with SLE had abnormally low serum cobalamin levels, but none had pernicious anemia. As a group, SLE patients had lower mean levels of cobalamin than control subjects did. Low serum levels of transcobalamin II and unsaturated vitamin B12 capacity correlated with lupus disease activity (191).

Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AIHA) is not an uncommon cause of anemia in patients with SLE. Approximately 7% to 15% of those in large series developed AIHA (47 ,192 ,193 ,194). Among a group of 186 patients with SLE who were studied by Alger et al. (192), 17 (7%) had Coombs-positive hemolytic anemia. Of these 17 patients, 6 had Evans syndrome (i.e., the concurrence of AIHA and immune thrombocytopenic purpura). In a prospective study of 126 SLE patients, 16 (12.7%) developed AIHA, and 3 had Evans syndrome (195).

AIHA may be the initial manifestation of SLE, occurring in 2% to 6% of patients (29 ,196). In five of ten patients who were studied by Videbaek (197), AIHA was the initial and dominant clinical feature of the disease for several months, and years, before other manifestations of SLE appeared. AIHA developed during the course of SLE in the other patients.

Classification

AIHA can be classified into two major types with respect to the anti-erythrocyte antibody and the optimal temperature of antibody reactivity with antigens on the red cell surface. The warm type of AIHA is mediated by IgG antibodies that are capable of reacting with antigens optimally at 37°C. Cold agglutinin AIHA is mediated by IgM complement-fixing antibody, which binds optimally to red cell antigens at 4°C. (Chapter 27 discusses the immunology of anti-erythrocyte antibodies in SLE.)

Warm AIHA

Warm AIHA is the predominant type in patients with SLE. Red blood cells that are coated by the warm IgG antibodies are removed from the circulation, primarily by sequestration in the spleen. The antibody-coated red blood cells undergo membrane alteration in vivo, resulting in the formation of spherocytes. Matsumoto et al. (198) examined the fine structure of the spleen in AIHA during SLE and found that erythrocytes coated with IgG and complement were phagocytosed completely by splenic macrophages and, to a lesser extent, by sinus endothelial cells. In contrast, in the liver, only evidence of occasional phagocytosis of sensitized erythrocytes by the Kupffer cells was found, confirming that the spleen is the major site of red blood cell destruction.

The symptoms and clinical findings in AIHA are variable. Symptoms that are referable to the anemic state, such as weakness, dizziness, and fever, are common. Evidence of hemolysis, including jaundice and dark urine, may be found. AIHA in the setting of SLE develops gradually in most patients, but occasionally it may present as a rapidly progressive hemolytic crisis (196 ,197).

With a significant degree of hemolysis, anisocytosis and macrocytosis often are noted in the peripheral blood smear. Nucleated red blood cells are seen in patients with marked hemolysis. Occasionally, polychromatophilic red cells, stippled cells, and Howell-Jolly bodies are found. The bone marrow is hyperplastic, frequently with a shift to the left in the myeloid series (196). Reticulocyte counts are elevated in association with a significant degree of hemolysis after hemorrhage and during systemic corticosteroid therapy.

The serum haptoglobin level usually is reduced during active phases of hemolysis (199 ,200 ,201). In the presence of infection, cancer, or steroid treatment, the serum haptoglobin level rises; consequently, if hemolysis coexists with these factors, the serum level may be normal (199 ,201). This also is observed when bone marrow function is impaired.

AIHA with Positive ANA

Tan and Chaplin (202) found low-titer ANA in 39% patients with idiopathic AIHA who did not have an underlying systemic connective tissue disease or lymphoproliferative disorder. No correlation was found among ANA and various clinical or serologic parameters. Favre et al. (203) described three patients with AIHA and one with immune thrombocytopenic purpura with positive ANA and anti-dsDNA antibodies that did not develop the full clinical picture of SLE after long-term follow-up. None had evidence of clinical renal disease, but on kidney biopsy, all had mild mesangial proliferation of focal glomerulonephritis. Miescher et al. (204) enlarged their series to ten patients. All had AIHA and/or autoimmune thrombocytopenia with positive ANA and low-titer or negative anti-dsDNA antibodies. Two of their patients had mild arthralgias; however, none developed clinical features of SLE. The authors suggested that these patients represent a transitional form between SLE and AIHA and autoimmune thrombocytopenia.

SLE Subset with Hematocytopenia

Alger et al. (192) compared the features of 31 patients with SLE who presented with AIHA and/or immune thrombocytopenia to a group of 62 patients with SLE but without

hematologic manifestations. The former group, which was younger and included a greater percentage of females, had a lower prevalence of fever, polyarthritis, serositis, cutaneous vasculitis, nephropathy, and central nervous system (CNS) disease, and had fewer complement abnormalities. It was suggested that patients with SLE who develop AIHA and/or immune thrombocytopenia constitute two related subsets of lupus patients with a relatively better prognosis. The high frequency of antiphospholipid antibodies in SLE patients with hemocytopenia further supports this concept (205 ,206). In contrast, Nossent and Swaak (146) reported that hemolytic anemia, neutropenia, or lymphopenia did not influence the survival rate of patients with lupus. Moreover, late-onset thrombocytopenia was associated with a decreased probability of survival.

Kokori et al. (207) reported that the recurrence rate of hemolytic anemia in a group of SLE patients with AIHA was low among treated patients (4 per 100 persons-years). They also found an association between AIHA and IgG anticardiolipin antibodies and thrombosis.

Combined Warm and Cold AIHA

Sokol et al. (208) found that 7% of 865 patients with AIHA who were referred to a blood transfusion center had warm IgG and cold IgM anti-red cell antibodies, and that both antibodies contributed to the hemolysis. Of the patients in this group, 20% had SLE. The high frequency of SLE was confirmed by Shulman et al. (209), who found that 5 of 12 patients (42%) with combined warm and cold AIHA had SLE.

IgG and C3d usually are present on the patient's red blood cells. In the serum, both IgG warm antibodies and high-thermal-amplitude cold IgM auto-agglutinins are detectable. The IgM antibodies are reactive over a broad temperature range, from 0°C to 30°C or higher (209). Patients with this type of AIHA have severe hemolysis but generally are responsive to corticosteroid therapy (209).

Treatment

Medical Therapy

Systemic corticosteroids, 1 to 1.5 mg/kg of prednisone daily or its equivalent, are efficacious, and remain the mainstay of the treatment for AIHA in patients with SLE. It is preferable to administer steroids parenterally to the symptomatic and acutely ill patient and later switch to an oral preparation when the patient has stabilized and improved. The dose is maintained for at least 4 to 6 weeks and gradually tapered, provided that a continued and/or sustained response occurs. No controlled trials have been reported on the use of corticosteroids in the treatment of AIHA in SLE, but our clinical experience (and that of others) indicates that approximately 75% of patients respond satisfactorily to steroid therapy (210). The response rate is similar to the 76% reported in patients with idiopathic warm AIHA (210 ,211). All 16 patients with SLE and AIHA who were followed prospectively responded to steroid therapy (120).

The mechanism of action of corticosteroids in AIHA is multifactorial, including an effect on tissue macrophages, altering of antibody activity, and a decrease in antibody production (211). In steroid-responsive patients who were studied by Priofsky (210), the clinical response was evident within a week. Stabilization of the hematocrit occurred within 30 to 90 days after the initiation of therapy.

In patients with severe and rapidly progressive hemolytic anemia, pulse methylprednisolone should be tried, 1 g intravenous for 3 consecutive days, followed by the conventional steroid dose (212). The reticulocyte count can be used to monitor the response to treatment and to detect any relapse as the steroid dose is tapered. A drop in the reticulocyte count is associated with a relapse in the hemolytic process.

Various treatment measures have been tried in patients who fail to respond to systemic corticosteroids alone or in those who continue to require a moderate to high dose of prednisone to control hemolysis. Most of the experiences have come from uncontrolled clinical studies. Priofsky (210) used azathioprine, 2 to 2.5 mg/kg, in conjunction with prednisone, 10 to 20 mg/day, in patients with AIHA who failed to respond to full therapeutic doses of prednisone. Danazol in conjunction with high-dose corticosteroids has been reported to be useful in the treatment of warm AIHA, including that associated with SLE (213). A combination of prednisone and danazol has been reported to be effective as a first line as well as for refractory AIHA including SLE (214). Plasmapheresis (215), high-dose IV γ globulin (216), and vinca-laden platelets (217) have been used with some success in a small number of patients with idiopathic AIHA refractory to conventional therapy. Intravenous IgG has been shown to be effective in 40% of 73 patients with warm AIHA; thus, it is not recommended as a standard therapy but may be useful as an adjunctive therapy for selected patients, such as those with toxicity to other treatments (218). Mycophenolate mofetil may be an effective second-line agent in the treatment of AIHA in SLE (219).

Rituximab, a chimeric monoclonal antibody to CD20 that depletes B cells and is used for the treatment of B cell lymphomas has been tried in autoimmune diseases including SLE. Our preliminary experience and those of others in severe SLE including patients with AIHA and immune thrombocytopenia indicate that Rituximab is an effective agent (220 ,221 ,222). Controlled trials are needed to establish its therapeutic use and safety in SLE.

Splenectomy

Splenectomy is used to treat patients with idiopathic warm AIHA who require a high maintenance dose of prednisone (20 mg/day or more), patients who have frequent relapses, or those with serious side effects to steroid therapy. In general,

splenectomy as a treatment is less effective for warm AIHA than for immune thrombocytopenia. The response rate to splenectomy in those with idiopathic warm AIHA is 50% to 60% (223), with reduction in the steroid maintenance dose or amelioration of the hemolysis.

It was suggested by early workers that splenectomy for idiopathic immune thrombocytopenia or idiopathic AIHA somehow unmasks occult or latent lupus, thus causing a dissemination of the disease. Best and Darling (224) reviewed the pertinent evidence both for and against this controversial issue, and they concluded that splenectomy does not lead to the dissemination of SLE, but on the contrary, has a beneficial effect for many patients.

Early studies on a small number of cases suggested that splenectomy is not efficacious in the treatment of warm AIHA associated with SLE (2 ,197). More recent studies have shown that splenectomy is of some value in the treatment of selected cases, but that this benefit may not be long-lasting. Of seven patients with SLE and warm AIHA (five had concomitant immune thrombopenia) who underwent splenectomy, six showed a sustained increase in hematocrit of greater than 20% (225). Rivero et al. (226) compared the clinical course of 15 patients with SLE and AIHA and/or immune thrombocytopenia who underwent splenectomy and 15 SLE patients who were treated medically for the hemocytopenia. Splenectomy produced short-term benefit, but at follow-up, no clear-cut difference in the clinical course of the two groups was seen. The splenectomy group had a significantly higher frequency of cutaneous vasculitis and serious infections after surgery. More of the splenectomized patients eventually required immunosuppressive therapy than did those in the medically treated group at follow-up.

Patients with SLE and AIHA who fail to respond to systemic corticosteroids alone should be given immunosuppressive agents next. The role of other measures in these patients, including IV γ globulin, plasmapheresis, mycophenolate, cyclosporine, and other agents, remains to be defined. Splenectomy probably should be reserved for the patient with acute fulminant AIHA who fails to respond to aggressive medical treatment (226). Laraparoscopic splenectomy has been shown to be a relatively safe procedure for most patients including those with SLE (227). Polyvalent pneumococcal vaccine should be given to splenectomized patients, such as those with functional asplenia.

Blood Groups and Transfusions in SLE

The distribution of ABO and Rh blood types of 138 patients with SLE who were studied by Dubois and Tuffanelli (2) was normal; 86 of the patients were Rh positive. Similar results were obtained by Leonhardt (228), who found no differences in the major blood groups of 54 patients with SLE, 221 of their relatives, and 5,668 healthy blood donors.

The prevalence of adverse reactions to blood transfusion has been low. In 82 patients (16%) studied by Dubois who received one or more blood transfusions, only 3 developed reactions, 1 developed urticaria, and 2 developed fever. Of the patients of Harvey et al. (10), 39 (35%) received one or more blood transfusions. Over 200 blood transfusions were administered, and the frequency of untoward reactions was low. Occasionally, a brief febrile response or urticaria occurred, but these patients subsequently were transfused without recurrence of the reaction. In contrast, Michael et al. (101) commented that in their experience, transfusion reactions appeared to be more common in patients with SLE than in the general hospital population, but no statistical data were presented.

Blood transfusions should be avoided whenever possible, not only because of the risks but also because of the observation that patients with SLE develop iso-antibodies against red cell antigens. Callender et al. (229 ,230) reported a patient with SLE who, in response to multiple blood transfusions, developed iso-agglutinins to five different red cell antigens that usually are ignored by most individuals. Multiple types of iso-agglutinins were found in the serum of another transfused patient with probable SLE (231). Another patient with SLE developed hemolytic anemia following multiple blood transfusions, with the appearance of three atypical hemagglutinins (232). A hemoglobinuric transfusion reaction resulting from Rh isosensitization has been described as well (233).

The antibody response of patients with SLE to blood group antigens has been examined following experimental immunization. The intravenous injection of 1 mL of incompatible whole blood led to the appearance of an unusually high isoagglutinin titer in a patient with SLE (234). Zingale et al. (235) immunized 15 SLE patients and matched controls with incompatible blood group substances. Patients with SLE developed higher titers of isohemagglutinin antibodies than the controls, as well as the transient appearance of a false-positive test for syphilis, circulating anticoagulants, antithyroglobulin, and antikidney antibodies. In contrast, the antibody production to various exogenous antigens following immunization in SLE has been either normal or depressed (236 ,237).

The few indications for blood transfusions in patients with SLE include those with acute massive bleeding, symptomatic patients with severe anemia, or those with concomitant severe heart disease or cerebrovascular ischemia. The response of patients with SLE and autoimmune hemolytic anemia to systemic corticosteroids generally is prompt and favorable, so that blood transfusion may not be necessary. Circulating anti-erythrocyte antibodies make blood cross-matching difficult (238).

Recombinant Erythropoietin Therapy

The anemia of chronic renal failure can be treated effectively with recombinant human erythropoietin, provided that sufficient iron stores are available (239). Used mainly for patients with end-stage kidney disease who are on maintenance hemodialysis, it improves quality of life and avoids

blood transfusion. Epoetin therapy is also efficacious in the chronic anemia of cancer, HIV infection and in surgical patients. It is well tolerated except for a mild “flu-like” symptoms, and development or aggravation of hypertension. Thrombosis and seizures have been reported (240). Recently, rare cases of pure red aplasia associated with neutralizing anti-erythropoetin antibodies have been described (241).

Whether patients with SLE who are on hemodialysis with anemia respond differently to erythropoietin therapy than patients without SLE has not been specifically investigated. My group has treated SLE patients on chronic hemodialysis before renal transplantation, and with good results.

Lim et al. (242) have shown that erythropoietin also is effective in ameliorating the anemia in a group of predialysis patients with chronic renal failure as a result of SLE and other conditions. Patients with SLE and end-stage renal disease who are on hemodialysis may become resistant to erythropoietin therapy if the underlying disease becomes active (243). Erythropoietin also has been found to be useful in a pregnant SLE patient with nephritis (244) as well as in patients with RA and anemia of chronic disease (245).

In vitro studies have shown that recombinant erythropoietin can increase the production of immunoglobulins by B cells, cause proliferation of bone marrow progenitor cells, and increase the expression of erythrocyte complement receptor type 1 (246). Theoretically, recombinant erythropoietin can be beneficial to patients with lupus by increasing clearance of immune complexes or be harmful by stimulating autoimmune responses. A longitudinal study of five patients with SLE but without renal failure who self-administered recombinant erythropoietin, however, showed no significant changes in anti-dsDNA, antiphospholipid antibody titers, C3, and renal function. One patient with anticardiolipin antibodies who was on estrogen replacement therapy developed thrombophlebitis that related temporally to erythropoietin therapy (247). Further studies are necessary to examine the possible relationship between erythropoietin therapy and thrombotic episodes in SLE.

Thrombocytopenia and Platelet Disorders

Frequency and Significance

Thrombocytopenia, which is defined as a platelet count of less than 150,000 cells/mL, is not an uncommon finding in SLE. The prevalence among seven large series in the literature has ranged from 7% to 52%, with a mean cumulative percentage of 14.5% (2 ,7 ,10 ,11 ,46 ,101 ,248) (see Chapter 30 , Table 30-1).

The degree of thrombocytopenia is variable, but profound thrombocytopenia is uncommon. Mild thrombocytopenia often appears during an exacerbation of SLE without causing bleeding tendency. Platelet counts were available for 86 patients in a series of studies by Harvey et al. (10), and in 23 they were definitely depressed. Of the 12 who had platelet counts of less than 50,000/mL, patients were included in whom thrombocytopenia purpura was a predominant feature of their disease. Also, 7 had counts of between 50,000 and 100,000/mL, and 4 had counts of between 100,000 and 150,000/mL. None of these patients had abnormal bleeding. In a series by Larson (46) of 196 patients, 15 (8%) had platelet counts of below 100,000/mL on at least two occasions. Nine patients had purpuric lesions at the time that platelet counts were depressed, but six patients did not. An additional group of 9 patients had purpuric skin rash as a prominent feature of their illness, but the platelet count in these patients was never found to be below 100,000/mL. In 112 patients with SLE who were studied for hemostatic functions, Gladman et al. (248) found 18 patients with a platelet count of less than 150,000/mL. In this group, 10 patients had counts of below 100,000/mL, yet only 1 patient developed petechiae.

In association with thrombocytopenia, the usual coexistent laboratory abnormalities, such as a prolonged bleeding time and diminished clot retraction when the platelet count is below 50,000/mL, are noted. A positive tourniquet test may be present without any platelet deficit, however, and simply may reflect vascular fragility resulting from SLE or prolonged steroid therapy (249). Coexistent hemostatic defects often are observed in patients with SLE and low platelet counts (248). A correlation between the presence of anticardiolipin and other antiphospholipid antibodies and thrombocytopenia in SLE, as well as in chronic immune thrombocytopenic purpura, has been recognized (see Chapters 25 and 52).

The clinical significance of thrombocytopenia in SLE has been examined. A prospective study of 19 patients with platelet counts of less than 100,000/mL revealed two distinct clinical groups: patients who were thrombocytopenic only during severe multisystem disease flares and patients with a chronic low platelet count and intermittent mild flares in other systems. None of the patients in either group developed severe bleeding, and whether acute or chronic, thrombocytopenia itself did not determine the subsequent course and prognosis of the patient (250). In two large studies on survivorship, the presence of thrombocytopenia appeared to be a significant risk factor for a worse prognosis in SLE (251 ,252). When the significance of thrombocytopenia was examined in a highly selective subset of patients with SLE and biopsy-proven nephritis, Clark et al. (253) found that thrombocytopenia was a useful index of disease activity.

Immune Thrombocytopenic Purpura

A special relationship exists between SLE and autoimmune thrombocytopenic purpura (also referred to as idiopathic or immune thrombocytopenic purpura; ITP), both of which primarily afflict young females. Some patients with ITP who initially are considered to be idiopathic later may develop a classic clinical picture of SLE. Further, a thrombocytopenic purpura, clinically indistinguishable from ITP, may occur along the course of SLE.

From 3% to 15% of patients with ITP go on to develop SLE (254). In a group of 62 adults with chronic ITP who were studied by Difino et al. (255), 3 patients (4.8%) developed SLE. Perez et al. (256) found that 6 of 18 patients with ITP (33%) tested positive for ANA at presentation, and that four of them developed classic SLE, within a mean duration of 2.3 years. A retrospective study of 117 patients with ITP showed a positive ANA in 24 patients, and 4 of them developed SLE (257). Patients with high-titer ANA tested positive for anti-Ro/SSA and anti-La/SSA antibody. Thus, patients with ITP and high-titer ANA and precipitating antibodies to Ro/SSA may develop later SLE (258). It is noteworthy that anti-Ro/SSA antibody is associated with thrombocytopenia in SLE (259). Firkin et al. (260) coined the term lupoid thrombocytopenia to refer to a group of patients with chronic ITP who were ANA positive but did not have other clinical or laboratory findings of SLE. None had anti-DNA, anti-Sm, or anti-Ro/SSA. The value of using this label is questionable, however, because the ANA-positive patients were similar in every other respect to those in the ANA-negative group.

In 1956, Dameshek and Reeves (257) emphasized the high frequency of SLE that occurs following splenectomy for apparent ITP. In a series of 51 consecutive patients, 8 subsequently developed definite SLE, with 2 others being probable and 6 possible. Thus, at least 31% of the patients in this group eventually developed clinical manifestations of SLE, and 15 had definite SLE (261). A study of 115 ITP patients who underwent splenectomy revealed that 14 (12%) patients subsequently developed SLE. The high percentage may in part be a result of a referral bias in a tertiary care center (262).

Other investigators have since disputed this claim and found that splenectomy in ITP does not lead to the development of SLE (224 ,263 ,264 ,265). In 1960, Doan et al. (264) reviewed their experience with 381 cases of thrombocytopenic purpura over a 28-year period and found that SLE caused the syndrome in 2% of patients. After splenectomy, the prevalence of new SLE cases was 1.2%. Splenectomy did not precipitate or disseminate the symptoms or signs of SLE in any patient, and the investigators believe that the hazards to life during acute hypersplenic thrombocytopenic crises outweigh the danger of developing subsequent SLE. Best and Darling (224) undertook a critical analysis of the existing data on this issue, and they concluded that splenectomy does not lead to the dissemination of latent SLE but actually is beneficial to some patients with drug-resistant cytopenias.

Clinical Presentation

The clinical manifestations of thrombocytopenia in SLE generally are similar to those seen in patients with ITP or other causes of thrombocytopenia, and they depend on the platelet count. When the platelet count is below 50,000/mL, spontaneous bleeding or purpura may occur. In addition to the platelet count, however, other factors, including qualitative platelet defects and platelet age, are important in the development of spontaneous bleeding (266). Bleeding usually presents as petechiae and/or ecchymoses, especially in the lower limbs, which experience increased capillary pressure. Nasal and buccal mucosal hemorrhage, heavy menstrual blood flow, epistaxis, and gum bleeding also may be present. Spontaneous bleeding into the brain is the most feared complication and can be fatal.

Chapter 27 discusses the immunologic properties of antiplatelet antibodies in ITP and SLE.

Treatment

The mainstay of drug therapy is systemic corticosteroids, 1.0 to 1.5 mg/kg/day of prednisone equivalent. Corticosteroid therapy is considered to be the equivalent of medical splenectomy, because it prevents the sequestration of antibody-coated platelets in the spleen (266). In most patients, a clinical response is seen within 1 to 8 weeks. High-dose intravenous pulse methylprednisolone also has been used for profound thrombocytopenia in SLE, but its superiority over conventional steroid therapy has not been established (267). Moreover, repeated courses may result in a diminished platelet response (268).

In idiopathic ITP, splenectomy generally is recommended for patients who fail to respond to systemic corticosteroids or for those who require moderate doses of steroids to maintain the platelet count (266). Splenectomy removes the major site of destruction of damaged platelets and the source of antiplatelet antibodies. In SLE patients, however, it is preferable to try other agents before recommending splenectomy for steroid-resistant thrombocytopenia, because of the increased risk of severe infections following splenectomy, the apparent efficacy of other agents and the disputed value of splenectomy (226 ,269).

Danazol, which is an androgenic steroid with few virilizing effects, has been shown to be effective in some patients with SLE and thrombocytopenia refractory to steroids, cytotoxic drugs, and/or splenectomy, and it is given at an average dose of 200 mg, three or four times a day. Often, however, danazol cannot be discontinued without recurrence of the thrombocytopenia (270 ,271). Danazol has been used in the therapy of thrombocytopenia associated with antiphospholipid antibody syndrome (272). A retrospective study of 59 SLE patients (15 subjects had “incomplete” SLE) with autoimmune thrombocytopenia reported a high long-term remission rate with prednisone combined with either hydroxychloroquine or danazol (273).

Intermittent intravenous cyclophosphamide was shown to be effective in the treatment of thrombocytopenia in seven patients with SLE refractory to splenectomy or steroids or requiring excessive doses of steroids (274 ,275). Other agents that have been reported to be useful in the treatment of thrombocytopenia, although in a limited number of patients with SLE, include azathioprine (276), cyclosporine (277), dapsone (278), vincristine (279), and mycophenolate (280). In chronic ITP not associated with SLE, azathioprine has been found to be effective in 64 of

patients who are refractory to steroids and/or splenectomy. Clinical response was delayed in many patients, requiring a course of at least 4 months on the drug (281).

Extracorporeal immunoadsorption of plasma to remove IgG and circulating immune complexes using staphylococcal- A-silica columns has been reported to be effective in refractory ITP (282). The mechanism is unclear but the procedure may decrease platelet activation (283).

IV γ globulin also is effective, but its effect may not be long-lasting (284). As in idiopathic ITP, gamma globulin is most useful in the treatment of life-threatening bleeding or in preparing the patient for urgent surgery or elective cesarean section (285 ,286 ,287). A prospective, randomized, clinical trial showed that intravenous IgG offers no advantages over systemic corticosteroids as the primary form of therapy in untreated patients with ITP, including patients with SLE (288). My group also has used γ globulin successfully as an adjunctive measure patients with SLE and thrombocytopenia who failed to respond optimally to corticosteroids and had a concomitant serious bacterial infection.

Maier et al. (289) studied the mechanism of action for intravenous IgG in SLE-associated thrombocytopenia. Five of seven patients with lupus who received IV IgG showed a greater than 50% increase in platelet count. The beneficial response was not dependent on the reduction of circulating platelet-binding IgG or circulating immune complexes, suggesting that blockade of the Fc receptor may be the major mechanism of action.

Anti-D immunoglobulin IV has been reported to be an effective treatment of nonsplenectomized children and adults with chronic or acute ITP in 70% of patients (290). The mechanism of action is not clear but in part it works via blockade of the reticuloendothelial system with autologous red blood cell antibody complexes. Treatment with anti-D immunoglobulin may be a means of preventing splenectomy and for long-term maintenance for ITP. Although its use in lupus thrombocytopenia has been described (291), there are no controlled drug trials in SLE.

The effectiveness of splenectomy in the treatment of steroid-resistant thrombocytopenia in SLE is controversial. Holman and Dineen (292) followed ten patients who underwent splenectomy for thrombocytopenia. Two died postoperatively, and eight had excellent results, with up to a 30-year follow-up. Breckenridge et al. (293) reported that 9 of 16 patients with SLE had normal platelet counts without medications a year after splenectomy. A similar experience was reported by others (262 ,294). Splenectomy in refractory thrombocytopenia associated with primary and SLE-related antiphospholipid syndrome showed a high rate of good and long-term response (295 ,296). In a retrospective analysis of 18 SLE patients with ITP who underwent splenectomy, only 65% had a sustained long term remission (273). In contrast, Hall et al. (297) found that only two of 14 splenectomized thrombocytopenic patients with SLE refractory to drug therapy went into remission at a mean follow-up of 6 years. A controlled but retrospective study of splenectomy in SLE patients with ITP and/or AIHA by Rivero et al. (226) found that splenectomy does not prevent recurrent episodes of thrombocytopenia. Moreover, the splenectomized patients had higher frequency of cutaneous vasculitis and infection after the surgery.

When feasible, laparoscopic is preferred over open splenectomy because of fewer complications and shorter postoperative stay (298). Splenic irradiation has been reported to be effective in the treatment of older patients with steroid-resistant ITP, including SLE (299). This modality may be useful in selected older patients because of the high morbidity that is associated with splenectomy in this age group.

Rituximab, a chimeric anti-CD20 monoclonal antibody, has been reported to be useful in a retrospective study of 35 patients with severe idiopathic immune thrombocytopenia refractory to conventional therapy, with an overall response rate of 44% (300). Rituximab has also been reported to be efficacious in autoimmune thrombocytopenia associated with SLE and with antiphospholipid syndrome (301 ,302). Controlled studies are needed to better clarify the efficacy and safety of rituximab in SLE and other nonmalignant hematologic disorders.

In summary, profound and persistent immune thrombocytopenia (less than 50,000/mL) in patients with SLE should be treated with systemic corticosteroids. Patients who become refractory to steroids or who experience undesirable side effects should be given a trial of azathioprine or danazol. Rituximab is a promising agent. Monthly intravenous cyclophosphamide therapy probably is preferable for patients with multisystem involvement, especially nephritis. Splenectomy may be necessary in some patients, although its long-term sequelae are not completely understood and disputed.

Amegakaryocytic Thrombocytopenia

Acquired thrombocytopenia that is associated with decreased numbers of megakaryocytes in the bone marrow, amegakaryocytic thrombocytopenia (AMT) is a rare disorder with different causes and pathogenetic mechanisms. A few cases of amegakaryocytic thrombocytopenia associated with SLE have been reported (303 ,304). In a well-studied patient with SLE, peripheral T lymphocytes, but not serum, inhibited autologous colony-forming megakaryocytes in vitro, suggesting an underlying cell-medicated pathogenetic mechanism (305).

Kuwana et al. (306) described IgG antibodies to c-Mpl, the thrombopoietin receptor, in a subset of SLE patients with thrombocytopenia and megakaryocytic hypoplasia. The autoantibody maybe pathogenic by blocking the the intereaction between thrombopoetin and its receptor resulting in impaired thrombopoesis.

Acquired Abnormalities of Platelet Function

Acquired abnormalities of platelet function include disorders of platelet adhesiveness to the vessel wall and subendothelial

matrix, platelet aggregation, and platelet secretion. Platelet function, as measured by in vitro qualitative tests and by bleeding time, is affected by several factors, including aspirin and NSAIDs, common foods, spices, the presence of systemic conditions (including SLE and chronic renal failure, lymphoproliferative diseases, and other hematologic disorders), and circulating antiplatelet antibodies (307). These factors should be considered when interpreting the results of qualitative tests of platelet function in an individual patient.

Activation of normal platelets can be induced by adhesion to collagen and by soluble agonists such as epinephrine and adenosine diphosphate (ADP). The activation process involves a complex system of metabolic reactions acting in concert to stimulate platelet aggregation and granule secretion. Regan et al. (308) found that platelets from 12 of 21 patients with SLE failed to aggregate in response to collagen and showed impaired aggregation with ADP and epinephrine. These abnormalities are similar to those induced by aspirin, but none of their patients was on aspirin or any drug that is known to affect platelet function. Others have confirmed these abnormalities in SLE (309 ,310).

In 1980, Parbtani et al. (311) found that the concentration of serotonin and adenine nucleotides, as stored in the dense granules of platelets, was reduced in patients with acquired platelet function defects, including SLE. Weiss et al. (312) extended this observation and reported that levels of substances stored in platelet-dense granules, including thromboglobulin, were decreased in SLE. Because these findings were similar to those observed in patients with congenital storage pool deficiency, it has been suggested that the platelet defect in SLE represents an acquired storage pool disease. The reduction of the intraplatelet concentration of serotonin in SLE has been confirmed by other investigators (309 ,310 ,311 ,313 ,314 ,315). Although the concentration of plasma serotonin is normal in patients with SLE, the urinary excretion of serotonin is increased (311).

The low concentration of intraplatelet serotonin has been shown to correlate with disease activity and been taken to indicate in vivo platelet activation in SLE (310 ,315). This is supported further by the findings of elevated levels of plasma ß-thromboglobulin and decreased amounts of platelet factors III and IV (248 ,316 ,317). ß-Thromboglobulin, which is a specific constituent of granules, is released on stimulation of platelets.

The mechanism of in vivo platelet activation in SLE is not known. Parbtani et al. (311) suggested that the functional platelet defects result from the circulation of exhausted platelets following their in vivo exposure to factors that induce a release reaction, such as damaged endothelium, thrombin, and immune complexes. The globulin fraction of SLE sera has been shown to contain factors that cause the release of serotonin from normal platelets (313). These plasma factors probably include circulating immune complexes and specific antiplatelet antibodies. Additionally, the level of platelet-activating factor (PAF), which is a mediator of inflammation with a wide range of biologic activities (including platelet activation), is increased in the plasma of patients with SLE and active disease (318). PAF, which is synthesized by a number of cell types after immunologic stimulation, including monocytes, macrophages, granulocytes, platelets, and endothelial cells, may be involved in SLE (319).

Studies in experimental models of immune complex glomerulonephritis have established that platelets participate in the pathogenesis of renal injury. Platelets can facilitate the deposition of immune complexes and augment the subsequent inflammatory response. The involvement of platelets in SLE nephritis also has been examined. By infusing radiolabeled autologous platelets to patients with diffuse lupus nephritis, Clark et al. (320) found the sequestration of platelets not only in the spleen and liver but also in the kidneys, suggesting intrarenal platelet consumption. Complexes of DNA and specific anti-DNA that are important in lupus glomerulonephritis have been identified on the surface of platelets of patients with SLE (321). Moreover, Duffus et al. (322) have localized platelet surface antigens and platelet factor IV at sites of glomerular injury in SLE. Persistent platelet activation may also contribute to increased thrombosis risk in SLE (323).

Thrombotic Thrombocytopenic Purpura

Thrombotic thrombocytopenic purpura (TTP) is a diffuse disorder of the microcirculation of unknown cause that is characterized by a pentad of fever, thrombocytopenic purpura, microangiopathic hemolytic anemia (MAHA), fluctuating neurologic findings, and renal dysfunction. It is a rare disorder, usually seen in females 10 to 40 years of age, and may follow a prodrome of upper respiratory tract symptoms or arthralgias and myalgias (324). Patients present with nonspecific constitutional symptoms, including malaise, fatigue, weakness, and fever. Therefore, the other manifestations appear in rapid sequence, often baffling the clinician by the subtle appearance and diversity of features. Delays in establishing a correct diagnosis are not uncommon. Half of patients experience neurologic symptoms at presentation, most commonly headaches, confusion, and paresis. MAHA is diagnosed by the presence of fragmented red blood cells (i.e., schizocytes), nucleated red blood cells, an elevated lactate dehydrogenase (LDH) level, reticulocytosis, negative Coombs test, indirect hyperbilirubinemia, and hemoglobulinuria. Severe thrombocytopenia is a characteristic finding. Coagulation parameters generally are normal or show only mild abnormalities, such as elevation of fibrin split products. Pathologically, intravascular microthrombi consisting of fibrin and platelet aggregates in capillaries and precapillary arterioles in several organs are found. No histologic evidence of vasculitis is seen. Systemic corticosteroids, antiplatelet agents, splenectomy, plasma infusions, and plasmapheresis are among the therapies that are used (325). Before 1965, cumulative

experience showed that over 90% of patients succumbed to the disease (326). In the last 20 years, the introduction of effective therapy has led to a dramatic improvement in the survival rate to between 70% and 80% (324 ,325).

The association between TTP and SLE has been debated for years (326). In 1964, Levine and Shearn (327) reviewed the English-language literature, emphasizing the possible relationship to SLE. They presented two cases of their own showing overlapping features and analyzed 147 cases that were reported with adequate autopsy protocols. In 34 cases (23%), evidence of concomitant SLE was found. Libman-Sacks endocarditis was noted in 25 patients, onion-ring changes in the spleen in 8, wire-loop glomeruli in 7, and a positive LE-cell test in 5. Patients with features of both SLE and TTP appeared to constitute a clinical variant and had a higher female predominance, a higher frequency of biologic false-positive tests for syphilis, elevated serum globulin levels, splenomegaly, arthritis and arthralgia, and pleuritis. Amorosi and Ultmann (328) found 13 cases of SLE among 271 cases of TTP reviewed. In contrast, other investigators concluded that the combination of SLE and TTP is rare (329).

There has been an increase in the number of published reports of the association (330 ,331 ,332 ,333). When prior reports were reviewed using the 1982 ACR criteria for SLE, Musio et al. (334) identified 41 reported patients with SLE associated TTP. The patients were divided into three groups: 30 (73%) patients who developed TTP after the diagnosis of SLE; 6 (15%) patients had TTP preceding SLE, and 5 (12%) patients presented simultaneously with TTP and SLE. TTP developed from 3 months to 25 years after the diagnosis of SLE in the first group. SLE antedated TTP for several years in three patients with hemolytic uremic syndrome variant (335 ,336). In half of the patients, TTP occurred in the setting of active SLE, while in the other half it occurred during an inactive phase (330). TTP can antedate the onset of SLE and also can occur as a terminal event in SLE (326 ,337 ,338 ,339 ,340 ,341 ,342). Dixit et al. (343) reported a patient with probable SLE, C2 deficiency, and chronic relapsing TTP.

Several mechanisms have been proposed to explain the microvascular thrombosis in acquired TTP, including the presence of serum platelet-aggregating factor, circulating immune complexes, endothelial injury, defects in the fibrinoloytic system, and prostacyclin abnormalities (344). Autoantibodies to microvascular endothelial cells (345) and to CD36, a single chain membrane polypeptide (also known as GPIV) that is expressed in platelets and endothelial cells, have been reported in TTP (346). In SLE, Itoh et al. (347) suggested the role of antiplatelet antibodies in the development of TTP in some patients.

The formation of platelet aggregates in the microcirculation in TTP is mediated by large multimer forms of von Willebrand factor. Synthesized by endothelial cells and released into the circulation the von Willebrand factor binds to platelets causing aggregates, thrombocytopenia, and hemolysis. A specific enzyme, the von Willebrand Factor protease (ADAMTS 13) cleaves the large forms of von Willebrand factor (348). Tsai and Lian (349) found IgG antibodies in TTP patients that inhibited the protease enzyme and the resulting defect in the proteolysis of multimers of Van Willebrand factor can lead to platelet thrombosis. Rick et al. (350) used a functional assay for ADAMTS 13 activity and inhibitory antibody level in an SLE patient to confirm the diagnosis of TTP. Serial measurement of ADAMTS 13 and the inibitor showed correlation with the clinical course of TTP.

The treatment of TTP associated with SLE is similar to that for idiopathic acquired TTP. Systemic corticosteroids (347) and plasma infusion (351), with or without plasmapheresis, have been used successfully in a few cases. A Canadian study of 102 patients has shown that plasma exchange alone with fresh-frozen plasma is more effective than plasma infusion in the treatment of TTP (352). Fourteen of 41 (34%) SLE patients with TTP died, and the mortality rates were significant in all forms of therapy: plasma exchange (32%), plasma infusion (20%), steroids alone (20%) (334). A review of 53 reported cases of co-existent SLE and TTP suggests that cytotoxic agents may be helpful in those with severe disease refractory to plasmapheresis (353) Rituximab was found to be efficacious in the treatment of relapses in a small number of TTP patients (354 ,355) presumably by inhibiting the synthesis of the autoantibodies to von Willebrand Factor cleaving protease (ADAMTS13).

White Blood Cell Disorders

White Cell Count and Leukopenia

The characteristic change in the leukocyte count in SLE, which first was observed in 1923 by Goeckerman (356), is a depression of the white blood cells to between 2,000 and 4,500/mL. In large series of SLE patients reported in the literature, leukopenia has been observed in over 50% (2 ,7 ,8 ,9 ,10 ,46).

Leukopenia, which is defined as a white blood cell count of below 4,500/mL, was noted in 43% of 520 patients of Dubois and Tuffanelli (2) and in 66% of 150 patients of Estes and Christian (7). Severe leukopenia, with counts of below 2,000/mL, was uncommon. Of 122 patients with SLE who were studied serially by Harvey et al. (10), 75 patients had counts of below 5,000/mL. Larson (46) found that 18% of 200 patients had leukocyte counts of below 4,500/mL on two or more occasions, and that the usual range of leukopenia was between 2,500 and 3,500/mL. Among 111 hospitalized patients with SLE, Michael et al. (101) found a white blood cell count of below 500/mL in 66 (60%) patients at some time. In a series of 142 patients studied by Ropes (11), a white cell count of less than 4,000/mL was found in 67%, but in 12% the white cell count was always normal.

Leukopenia in SLE has been shown to be significantly associated with a high frequency of skin rash, lymphopenia,

and elevated anti-DNA titer. Anemia, fatigue, arthritis, anemia, and elevated sedimentation rate also were more common in patients with leukopenia (357). A leukocyte count of 4,000/mL, and occasionally as low as 2,500 mL, may occur in patients with active and untreated discoid LE. This may arise after treatment of the skin lesions with antimalarials. Leukopenia and fever can be a rare manifestation of allergic reaction to prednisolone (358).

Differential White Cell Count

An increase of nonsegmented neutrophils in patients with SLE was first reported by Rose and Pillsbury (359). This increase was observed in patients with normal as well as elevated total white cell counts (101). Estes and Christian (7) noted a normal differential count in 50% of their patients with leukopenia. These data are similar to the findings of other investigators.

Of 111 patients who were studied by Michael et al. (101), 64% had an increase in nonsegmented neutrophils together with an increase in mature granulocytes. Also, 14 patients had one to seven myelocytes when first seen, and a few others showed a similar increase in later counts. This occurred more frequently in those with normal or low white cell counts than in those with elevated total leukocyte counts. Before the institution of therapy, 24% of 105 patients studied by Harvey et al. (10) had neutrophilic granulocyte counts of 80% to 89%, and 5% of patients had counts of over 90%.

Eosinophilia

In my experience, eosinophilia is uncommon in patients with SLE without concomitant parasite infection, allergic reaction, or some other known cause. Eosinophilia is not mentioned in several series of patients in the literature (7 ,9 ,357). In contrast, earlier workers observed that eosinophilia is not rare in SLE. In a series of 200 patients studied by Larson (46), 10 had counts of 3% or more, and 5 patients had persistent eosinophilia in excess of 10%. Harvey et al. (10) found eosinophilia of 3% or more in 15 of 46 patients who were studied before therapy. Of these, 2 patients had counts of 17% and 24%, respectively, associated with extensive skin lesions and not attributable to causes other than SLE. Direct eosinophil counts were performed in 60 patients. Counts of less than 50/mL were found in 31 patients, of between 50 and 100/mL in 11, of between 100 and 200/mL in 10, of between 200 and 400/mL in six, and of more than 400/mL in only two. Ropes (11) observed a high prevalence of eosinophilia; an eosinophil count of more than 5% was seen in 21 of 142 patients at some time along the course of the illness. Michael et al. (101) noted an eosinophil count of over 3% in six of 111 untreated patients (17).

Two rare causes of eosinophilia, hypereosinophilic syndrome with diarrhea, and Loffler endocarditis have been reported in SLE (360 ,361).

Basophils

Hunsiker and Brun (362) enumerated basophils in patients with various skin disorders and found a moderate reduction of basophils in those with discoid LE and a marked diminution in patients with SLE. Egido et al. (363) found the absolute basophil counts in SLE to be inversely related to anti-DNA antibodies and the level of circulating immune complexes and to be directly related to serum complement level. Basophils and tissue mast cells possess Fc receptors that are specific to IgE antibodies. If such antibodies are cross-linked by binding to antigen, basophils and mast cells degranulate, releasing granules that contain vasoactive substances and producing a hypersensitivity allergic reaction. High levels of IgE have been found on the surface of basophils in SLE patients. More important, SLE basophils underwent degranulation when incubated with soluble DNA antigen, suggesting the presence of cell-bound, specific IgE anti-DNA antibodies. Although glomerular deposits of IgE have been reported in SLE, the pathogenic role of these antibodies remains to be established.

Granulocytopenia

Granulocytopenia occurs infrequently in patients with SLE and can have a number of causes, including drug reaction, severe infection, decreased bone marrow production, and destruction mediated by antigranulocyte antibodies. At times, the total white blood cell count may decrease to levels as low as 1,000/mL without any apparent cause other than SLE. Nevertheless, in this situation, the physician should always be aware of the possibility that the granulocytopenia may be the result of medications used in SLE, such as antimalarials (10 ,364). A prospective study, however, showed a high prevalence (47%) of neutropenia at some time during the course of lupus, although it was severe (<1,000 polymorphonuclear neutrophils [PMNs] per mL) in only six of 126 patients (4%) who were studied (120).

My group has observed agranulocytosis in SLE caused by atabrine, levamisole, and triethylenemelamine (365). Agranulocytosis in SLE has occurred because of reaction to sulfadiazine (366). McDuffie (367) reported three patients with SLE who developed agranulocytosis secondary to medications that are not commonly associated with blood dyscrasias: hydroxychloroquine, dextropropoxyphene, and nitrofurantoin. Bone marrow examination showed the absence of myeloid precursors in two patients and aplastic changes in the third.

Both humoral and cell-mediated immune mechanisms are important in the pathogenesis of neutropenia in SLE. Starkebaum et al. (368) examined the in vivo neutrophil kinetics of a patient with lupus and severe neutropenia, and they found changes that were indicative of increased peripheral destruction of granulocytes combined with ineffective granulocytopoiesis by the bone marrow. Elevated levels of surface-bound IgG were detected on the patient's neutrophils. Monomeric IgG antibodies, but not immune complexes,

isolated from the serum of the patient opsonized normal neutrophils for in vitro ingestion by other phagocytic cells. These observations provide a basis for the role of humoral factors in some cases of neutropenia. Additionally, Hadley et al. (369) found an inverse correlation between the neutrophil count and the ability of SLE sera to opsonize granulocytes for recognition and clearance by human monocytes. Impairment of reticuloendothelial system function in SLE (370) may allow antibody-sensitized granulocytes to remain in the circulation. Neutropenia in SLE has been reported to be associated with anti-Ro/SSA antibodies. Moreover, these antibodies bind to a 64-kd membrane protein that cross-react with the 60-kd Ro antigen, implying that anti-Ro/SSA antibodies may mediate neutropenia in SLE (371). Anti-La/SSB antibodies from SLE patients have also been shown to bind and penetrate normal PMN and impair phagocytosis and enhance apoptosis and IL-8 production. Whether anti-La/SSB antibodies contributes to the neutropenia remains to be established (372).

The number of progenitor cells of granulocyte and monocytes in the bone marrow (colony-forming unit culture [CFU-C]) has been found to be reduced in patients with SLE, and this correlated with the peripheral granulocyte and monocyte counts (127 ,373). Moreover, T lymphocytes suppressed the generation of autologous bone marrow CFU-C in vitro, suggesting a role of cell-mediated mechanisms in the impairment of granulopoiesis in SLE.

Courtney et al. (374) found an increased number of apoptotic neutrophils in SLE patients, especially those with anti-dsDNA and active disease as well as those with neutropenia. Whether increased neutrophil apoptosis contributes to neutropenia in SLE or not remains to be investigated.

Severe neutropenia in SLE is responsive to systemic corticosteroid therapy (375). Recombinant human granulocyte colony-stimulating factor (rhG-CSF) has been used successfully in combination with pulse methylprednisolone or with low-dose prednisone in the treatment of lupus neutropenia (375 ,376).

In the presence of infection in the patient who is not receiving systemic corticosteroids, leukocyte counts often may rise to between 15,000 and 20,000/mL. Unfortunately, other patients with SLE may have intercurrent infections without a demonstrable rise in the white cell count, so this valuable guide cannot be depended on in these cases. Marked leukocytosis with counts higher than 30,000/mL has been observed by my group and others in the presence of concomitant infections. During treatment with corticosteroids, the usual range of leukocytosis is 15,000 to 25,000/mL regardless of the initial white blood cell count.

Bone Marrow Granulocyte Reserve

The leukocytosis that occurs following the administration of endotoxin, etiocholanolone, or glucocorticoids primarily is caused by the release of PMNs from bone marrow reserves (377). The amount of PMN reserve is considered to be important in the host defense against infections, and it also serves as a guide in predicting the ability of the patient to tolerate a myelotoxic drug. Paulus et al. (378) found that the granulocyte reserve, as measured by etiocholanolone injection, in patients with SLE and nephritis was higher in those who were on azathioprine than in those on no medications or in patients on combined prednisone and azathioprine therapy. Kimball et al. (379) found that 62% of 59 patients with SLE had an abnormally low granulocyte reserve when challenged with etiocholanolone. No correlation among deficient granulocyte reserve and other clinical or laboratory parameters was found. Most of their patients were on corticosteroids, however, which now are known to challenge the bone marrow reserves (380), so that possibly the administration of etiocholanolone did not augment the maximally stimulated bone marrow, resulting in a subnormal response (381). This subnormal response to etiocholanolone of untreated patients with SLE suggests that the disease itself can suppress bone marrow reserves. Evidence suggesting the role of one or more circulating humoral factors for this abnormality has been described (379).

The number of bisegmented neutrophils (i.e., Pelger anomaly) was significantly higher and the lymphocyte count lower in patients with leukopenia associated with SLE and other autoimmune disorders (382).

Lymphocyte Counts

Lymphopenia is one of the most common hematologic findings in SLE (8 ,383). Early investigators noted lymphopenia but failed to emphasize it, probably because relative percentages rather than absolute numbers of lymphocytes in the peripheral blood were used (10 ,101). The mechanism of lymphopenia in SLE is not clear. Delbarre et al. (384) followed lymphocyte counts in 19 patients with SLE, and they noted that lymphopenia developed in 84% during the acute stage and was associated with an increased sedimentation rate. At remission, the lymphocyte count increased and the sedimentation rate fell. Grigor et al. (8) found absolute lymphopenia to be more common than leukopenia.

Rivero et al. (383) examined the significance of lymphopenia in 158 patients with SLE. At diagnosis, 75% of these patients had a significantly reduced absolute lymphocyte count, but on follow-up, additional patients became lymphopenic. Therefore, the total cumulative frequency of absolute lymphopenia was 93%. Lymphopenia was found to be independent of, although contributory to, leukopenia, so these two findings were not primarily interrelated. Absolute lymphopenia was correlated with disease activity, and patients with an absolute lymphocyte count of less than 1,500/mL at diagnosis had a higher frequency of fever, polyarthritis, and CNS involvement but a lower prevalence of thrombocytopenia and/or hemolytic anemia. Life-table analysis, however, showed no adverse influence of lymphopenia on survival of patients with lupus (120).

Table 41-2: Red Cells, White Blood Cells and Platelets in SLE

Anemia occurs in over 50% of patients at some time during the course of the disease; anemia can result from one or a combination of factors including chronic disease, autoimmune hemolysis, iron deficiency, and chronic renal failure.
Autoimmune hemolytic anemia characterized by the presence of warm-reacting immunoglobulin G (IgG) antibodies to erythrocytes develops in up to 16% of patients and may be the presenting manifestation of the disease; a combined warm- and cold-antibody type of autoimmune hemolytic anemia occurs occasionally.
Leukopenia is common and may result from active disease or a drug reaction; lymphopenia is a characteristic finding in untreated, active SLE; in vitro studies of granulocyte function generally are abnormal in SLE.
Leukocytosis in SLE is associated with a concurrent infection or corticosteroid therapy.
A platelet count of lower than 150,000 cells/mL is seen in 7% to 52% of patients (mean, 14%); in vitro platelet functions including aggregation in response to collagen are frequently abnormal in SLE.
Autoimmune thrombocytopenic purpura is not uncommon in SLE; 3% to 15% of patients diagnosed as having idopathic autoimmune thrombocytopenic purpura go on to develop classic SLE.
Thrombotic thrombocytopenic purpura is a rare, life-threatening complication of SLE.

Lymphopenia was found to be associated with the presence of anti-Ro, anti-La, anti-Sm, and anti-RNP in SLE and cutaneous LE (385).

Table 41-2 summarizes the changes in red cells, platelets, and white cells in patients with SLE.

Granulocyte Function in SLE

To understand the importance of granulocyte function as a factor in the susceptibility of patients with SLE to infections, studies have been undertaken to examine the phagocytic, opsonizing, chemotactic, and oxidative functions of neutrophils and monocytes. Most of these studies have concluded that in general, granulocyte function in SLE is abnormal, but the specific qualitative and quantitative abnormalities reported in them were either inconsistent or contradictory.

Phagocytosis and Opsonization

Brandt and Hedberg (386) reported that the ability of neutrophils of SLE patients to ingest yeast particles is significantly lower than those of RA patients and of healthy subjects. Phagocytic activity was not reduced when normal granulocytes were suspended in normal plasma, and the lowest phagocytic activity tended to be associated with neutropenia. Orozco et al. (387) confirmed this observation and found that the phagocytosis of *Escherichia coli* by SLE leukocytes decreased by 62% in those with active disease. In contrast to Brandt et al. (386), they also observed that the phagocytic activity normalized if the SLE granulocytes were incubated with fresh normal serum rather than with SLE serum. The presence of a serum factor that inhibits phagocytosis in SLE also has been reported by others (381 ,388), although the nature of this factor is not known. Sera from SLE patients with active disease failed to support normal granulocytes in phagocytosis, indicating a defect in opsonic capability. The low serum complement level, rather than a deficiency in natural antibodies, was the limiting factor in the deficient serum opsonic activity of SLE sera (387). The initial rate of phagocytosis of lipopolysaccharide-coated paraffin droplets by neutrophils from untreated patients with active SLE was significantly lower than that in normal subjects (389). In contrast to these results, Al-Hadithy et al. (390) found the phagocytosis of *Candida* sp. by neutrophils of SLE patients to be normal when the patients were considered as a group, but 20 of their patients had impaired phagocytic values. Additionally, Hallgren et al. (388) described an increased ability of SLE granulocytes to phagocytose IgG-coated latex particles in a serum-free system, to exclude the effects of circulating inhibitors. Phagocytosis of *E. coli* particles by monocyte and granulocytes in SLE was not reduced when compared to age-matched healthy controls in a whole blood system (391).

Chemotaxis and Migration

The in vitro chemotactic response of the neutrophils in SLE, when compared as a group to that of healthy subjects, has been observed to be normal (392 ,393). Clark et al. (393) noted that the generation of chemotactic factors in SLE serum clearly was depressed, and that the defect correlated with an elevated titer of anti-DNA antibodies, low serum immunoglobulin level, and high frequency of infections. Alvarez et al. (392) showed that the generation of serum chemotactic activity by the classic pathway of complement, but not by the alternative pathway, was impaired in patients with SLE. Serum inhibitors of chemotaxis, including a specific inhibitor of C5-derived chemotactic activity, also have been found in SLE patients (390).

Oxidative Functions

Wenger and Bole (394) investigated the reduction of nitroblue tetrazolium (NBT) dye by peripheral blood leukocytes of patients with SLE, and they found the activity to be low in resting leukocytes. When the leukocytes were stimulated by allowing them to phagocytose latex particles, an incremental increase in NBT dye reduction, comparable to

that seen in healthy individuals, was noted. On the other hand, neutrophils from patients with SLE who were infected failed to demonstrate the anticipated increase in NBT dye reduction. Others have confirmed the abnormal NBT dye reduction test results in patients with SLE (389 ,395). This test is considered to be a measure of the oxidative events associated with phagocytosis and intracellular killings, so it further supports the role of abnormal granulocyte function in the increased incidence of infection found in those with SLE.

The apparent inconsistencies in the studies of in vitro SLE granulocyte function by various investigators probably reflect differences in methodology and in the selection of patients. This also emphasizes the importance of other factors that affect these tests, such as the use of steroids and other drugs, activity of SLE, and the presence of inhibitory factors in the serum. The clinical significance of in vitro functional abnormalities is not entirely clear, but in vivo studies in SLE using the Rebuck skin window technique have shown abnormalities in granulocyte functions (396). Whether the observed abnormalities in granulocyte function are primary cellular defects or secondary to the disease process is not completely clear. Hurd et al. (397) have postulated that the ingestion of immune complexes by SLE neutrophils can alter their functions. In vitro exposure of normal granulocytes to SLE sera, especially those from patients with active disease, resulted in increased adhesiveness, aggregation, and oxygenation activity (398 ,399 ,400).

Neutrophils from patients with lupus, and especially those with active disease, express increased amounts of adhesive molecule ß-integrin CD11b/CD18 but not L-selectin (401). Exposure in the circulation to complement products may lead to the intravascular activation of neutrophils (402). Activated neutrophils then can contribute to the development of vasculitis, transient pulmonary dysfunction, CNS involvement, and leukopenia, as well as to an increased susceptibility to infections.

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Chapter 42
Gastrointestinal and Hepatic Manifestations

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Gastrointestinal Involvement

Although gastrointestinal (GI) manifestations are common in patients with systemic lupus erythematosus (SLE) (Table 42-1), their incidence varies with the interest and methods of the observer who is studying the illness. For example, two major studies failed to mention GI complications (1 ,2), and another made only brief reference to it (3). Abdominal symptoms and signs may be the result of SLE, medications that are used to treat SLE, or intercurrent processes.

Historical Notes

Osler (4) was impressed with the frequency of GI manifestations or, as he called them, GI crises. He believed that they might mimic any type of abdominal condition. In 1939, Reifenstein et al. (5) found evidence of peritonitis in 13% to 18% autopsied SLE cases, with perihepatitis and enlargement of the liver in one third. The most feared GI complication of SLE is lupus enteritis, caused by vascular involvement of the bowel wall, with infarction or hemorrhage. The first modern description of this was by Klemperer (6) in 1941.

Prevalence

GI complaints were the initial presentation in 10% of Dubois' patients (7); 25% to 40% had protracted symptoms. Haserick et al. (8) divided the GI symptoms of SLE among 87 patients into three groupings: none (63%), minor (29%), and major (8%). We have found this breakdown to be clinically useful. Subclinical involvement of the GI tract also is common. For example, Landing et al. at Children's Hospital of Los Angeles found chronic mucosal infiltration in 96% of 26 autopsied children with SLE (9). Younger patients with lupus are more susceptible. Among 272 patients with SLE, the prevalence of GI manifestations ranged from 10% in children to none over the age of 50 (10). Sultan et al. published a recent review on GI manifestations in SLE and found anorexia to be the most common reported manifestation with a prevalence of 36% to 71% in published studies (11).

Pharyngitis, Dysphagia, and Esophagitis

Persistent sore throat is not an infrequent finding, especially in children (12). (Chapter 29 discusses mucous membrane lesions and other features of oral pathology.) Dysphagia occurs in 1% to 6% (7 ,13 ,14 ,15 ,16 ,17 ,18) and heartburn in 11% to 50% of patients (13 ,19). In a literature review, Zizic (17) related that although only 5% of patients with SLE complained of dysphagia, 25% had impaired esophageal peristalsis, compared with 67% of patients with scleroderma. Several studies using esophageal manometry noted aperistalsis or hypoperistalsis of the esophagus in approximately 10% of patients with SLE (18 ,19 ,20 ,21 ,22 ,23). Aperistalsis sometimes correlated with the presence of Raynaud phenomenon. Gutierrez et al. (24) compared esophageal motility in 14 patients with SLE and 17 with mixed connective-tissue disease (MCTD). A definite correlation was found between Raynaud phenomenon and hypoperistalsis, with the latter being more common in MCTD. The SLE group had only a slightly decreased lower esophageal sphincter pressure. Esophageal motor dysfunction in SLE also can produce diffuse spasm and result in symptoms of chest pain (25). The aperistaltic group can show atony and dilatation of the esophagus on upper GI radiography (26). Dysphagia is usually a result of complications of acid reflux disease, but a rare association of a bullous skin disease called *epidermolysis bullosa acquisita* involving the esophagus has also been reported (27).

Ramirez-Mata et al. (28) performed esophageal manometric studies in a group of unselected patients with SLE and noted abnormalities in 16. Absent or abnormally low contractions were found at the upper one third in 7 patients, at the lower two thirds in 3, in the entire esophagus in 2, at the lower esophageal sphincter in 2, and at the lower two thirds plus the lower sphincter in the remaining 2. They found no relationship among the presence of esophageal dysfunction and activity, duration, or therapy of SLE. Interestingly, 5 of the 34 patients who had normal studies complained of dysphagia and heartburn. Upper esophageal skeletal muscle fiber atrophy also was found in 2 of 26 autopsies on children with SLE (9). One report confirmed these

findings and suggested that hypoperistalsis or aperistalsis may be caused by an inflammatory reaction in the esophageal muscles or by ischemic or vasculitic damage to Auerbach plexus (19). Esophageal imaging with gastrograffin, computed tomography (CT) scanning, or endoscopy is required to make the diagnosis of esophageal ulceration or perforation from systemic vasculitis (18).

Table 42-1: Systemic Lupus Erythematosus and the Gastrointestinal Tract

1. Gastrointestinal (GI) symptoms are common in systemic lupus erythematosus (SLE). Secondary causes such as concurrent disease, stress, and medication must be ruled out.
2. Sore throat and oral ulcers are common.
3. Dysphagia is present in 2% to 6% of patients, especially in association with Raynaud's phenomenon.
4. Anorexia, nausea, vomiting, or diarrhea may be prominent in one third of patients when the disease is active. Chronic intestinal pseudoobstruction (CIPO) causes the above symptoms and is a disturbance of the enteric nervous system. Inflammatory bowel disease, infection, and concomitant drug administration must be ruled out as other causes.
5. The incidence of peptic ulcer disease was 6% in patients presenting with acute abdominal pain; it usually is caused by antiinflammatory medication.
6. Ascites is found in 8% to 11% of patients. If a result of nephrosis, cirrhosis, or congestive heart failure, it is a painless transudate. Exudative causes might be painful and include serosal inflammation. Patients with lupus peritonitis often are steroid-responsive.
7. Pancreatitis is a serious complication of SLE. It is associated with pancreatic vasculitis, activity of SLE in other systems, and rarely, with subcutaneous fat necrosis. Mild elevation of pancreatic enzyme levels may occur in SLE without pancreatitis; high levels suggest pancreatitis. Steroids are the treatment of choice, but they (along with thiazide diuretics and azathioprine) can induce pancreatitis.
8. Abdominal pain, distension, and tenderness warrant a search for ischemia or bowel ulceration especially in patients with a SLEDAI of 4 or more. In the outpatient settin g, these signs may suggest the presence of small-bowel bacterial overgrowth (SIBO).
9. Malabsorption syndromes are rare but do occur.
10. Mesenteric or intestinal vasculitis is a life-threatening complication of SLE, usually associated with multisystem activity. High doses of steroids are required, and surgical intervention is indicated if extensive bowel infarction (with hemorrhage) and/or large intestinal perforations occur. Patients succumb from complications of obstruction, perforation, or infarction.

The treatment of esophageal symptoms is the same as that of esophageal problems associated with Raynaud disease. Small and frequent meals, avoidance of postprandial recumbency, and the administration of antacids, proton pump inhibitors, H_2 antagonists, or parasympathomimetic agents play a therapeutic role.

Anorexia, Nausea, Vomiting, and Diarrhea

The most common cause of anorexia, nausea, vomiting, and diarrhea in SLE is related to the use of salicylates, nonsteroidal anti-inflammatory drugs (NSAIDs), antimalarial drugs, corticosteroids, and cytotoxic agents. These symptoms even can occur for weeks after therapy is stopped. When caused by the disease, manifestations are persistent and are not explained by other factors.

Anorexia occurs in 49% to 82% of patients (7 ,13 ,29), especially if untreated. Nausea has been reported in 11% to 38% (7 ,13 ,15 ,29 ,30). When medications are excluded as a cause, however, the incidence is approximately 8% (16). Vomiting can be prominent (7 ,8 ,13 ,14 ,29 ,30); my own group has observed it as a symptom in 7% of patients (16). Diarrhea occurs in 4% to 21% of patients (7 ,13 ,14 ,29 ,30), and children have an increased incidence of all these symptoms (31).

Motility Disorders

Recently, motility problems have been associated with SLE. Hirschsprung disease has been reported in two patients with neonatal lupus (33). Chronic intestinal pseudo-obstruction (CIPO) reflects a dysfunction of the visceral smooth muscle or the enteric nervous system (31 ,32 ,33 ,34). Hill reported the second case of CIPO in lupus where a pathologic

specimen was available and was found to have smooth muscle atrophy in the bowel wall as the etiology for the altered motility (32). Symptoms and signs of this complication in SLE include a subacute onset of abdominal pain and distension associated with vomiting and constipation and a distended tender abdomen with hypoactive or absent bowel sounds and lack of bowel sounds. Radiologic examination reveals dilated, fluid-filled bowel loops and occasionally bilateral ureteral dilatation with a reduced bladder capacity. Antroduodenal manometry demonstrates intestinal and esophageal hypomotility. Nojima et al. described two patients with CIPO who had antibodies to proliferating cell nuclear antigen (PCNA) but no other specific antibodies or clinical manifestations of SLE (35). Treatment of CIPO usually involves high doses of steroids, broad-spectrum antibiotics, and promotility drugs. Perlemuter et al. reported the use of octreotide in a dose of 50 µg twice a day subcutaneously in CIPO in SLE and scleroderma (36). The symptoms of CIPO resolved in the three patients receiving treatment within 48 hours. Recurrence of symptoms responded to increasing the dose of octreotide.

Abdominal Pain and Acute Abdomens

Abdominal pain is found in 8% to 37% of patients with SLE (7 ,12 ,13 ,14 ,16 ,30), with the lowest incidence being reported in series that exclude medication-related symptoms. In 412 consecutive admissions to Cleveland hospitals for collagen vascular diseases, 63 patients had abdominal complaints; of these, 48 had SLE. Pain was present in 85%, and fever was noted on examination in 76% of patients and peritoneal signs in 10%. Corticosteroids were being given to 64%. An acute cause was determined in 33 patients, including duodenal or gastric ulcer, gastritis, and pancreatitis. Mesenteric vasculitis was present in 3 patients; in 16, the pain was of undetermined cause. Surgery was performed on 21 patients; in 11, it was exploratory. Al-Hakeem et al. identified 13 patients with a principal diagnosis of abdominal pain out of 88 patients with SLE who were admitted to the hospital during a 15-year period (37). Diagnoses accounting for abdominal pain–included adhesions (3), diverticulitis (3), cholecystitis (2), perforated ulcer and colon, gastroenteritis, duodenitis, and inflammatory bowel disease (1 each). Nine of the 13 patients required surgery. In another survey of 63 procedures, 16% morbidity and 6% surgical mortality rates were recorded (38). Rojas-Serrano evaluated the causes of emergency room consultation for patients with SLE and found that abdominal pain was the third most frequent reason accounting for 18 out 180 lupus patients visiting the emergency room (39). Min et al. studied the etiology of acute abdominal pain in patients visiting an emergency room (40). Twenty-six patients with SLE and abdominal pain made 44 visits to the emergency room. Twenty-seven (59.1%) of these visits were for ischemic bowel disease. Other diagnoses included pancreatitis, serositis, splenic infarction, angioedema, renal vein thrombosis, pelvic inflammatory disease, upper GI bleeding, and ectopic pregnancy. CT scanning and ultrasound help establish the diagnosis of ischemic bowel disease.

Small Intestinal Bacterial Overgrowth

In the outpatient setting, abdominal pain in SLE may be from serositis, small-bowel bacterial overgrowth, or non-SLE causes. Serositis can present as an acute surgical abdomen as reported by Wakiyama et al. (41). Low et al. reported the characteristics of lupus serositis on barium radiograph and CT imaging (42). The small-bowel barium series showed segments of spiculation with tethering, angulation, and obstruction. CT scanning demonstrated ascites and asymmetric thickening of the small-bowel wall. Albano et al. investigated the prevalence of small intestinal bacterial overgrowth in 14 SLE patients using a lactulose hydrogen-breath test (43). Symptoms of small-bowel bacterial overgrowth such as bloating (50%), diarrhea (64%), constipation (42%), and abdominal pain (42%) were present in these SLE patients without any clear identifiable cause on history and physical examination. Breath hydrogen above 20 million parts per million (ppm) with two distinct peaks of hydrogen production was diagnostic for small intestinal bacterial overgrowth (SIBO). Twelve patients (86%) were found to have SIBO by predefined criteria. Apperloo-Renkema et al. (44 ,45) documented that patients with SLE have lower colonization resistance to indigenous bacteria of the intestinal tract. IgG-class antibacterial antibodies to fecal microflora are decreased in active SLE, which suggests sequestration in immune complexes that could play a role in mesenteric vasculitis. Small-intestine bacterial overgrowth (SIBO) in SLE may be a result of decreased neutralizing antibodies to intestinal flora.

Helicobacter Pylori Infection in SLE

Sawalha et al. studied the prevalence of seropositivity against *H. pylori* and four other control antigens in 466 SLE patients and compared them with 466 control patients matched for age (+/-3 years), sex, and ethnicity. Most of the control patients were from the same pedigree multiplex for SLE. The frequency of sereopositivity to *H. pylori* only was lower in the SLE cohort compared to the control and this difference could be explained by the lower prevalence in African-American patients (38.1 versus 60.2; OR = 0.4, p = 0.0009, 95% CI 0.24–0.69) (46). The mean age of onset of illness in the *H. pylori* group was significantly older (34.4 versus 28, p = 0.039) compared to the *H. pylori* seronegative SLE patients. Though no conclusions can be drawn from this study, a relationship between SLE and a decreased seroprevalence of *H. pylori* infection in African-American lupus is evident. Direct biopsies of the gastric antrum were not performed in this study.

Acute Abdomen Associated with SLE

Abdominal pain and tenderness in patients with SLE can be the first manifestations of an intraabdominal disaster. Patients presenting with abdominal pain, even without tenderness, need an aggressive and comprehensive evaluation, including a complete blood count, amylase-level determination, blood-chemistry profiles, and abdominal radiography. If free air, moderate amount of free fluid, acidosis, or hyperamylasemia without pancreatitis is present, diagnostic laparoscopy should be performed. If pseudo-obstruction and/or thumbprinting of the bowel are seen without free peritoneal fluid, appropriate work-up and treatment should be instituted. Specialized tests, such as an upper GI, barium enema, CT, magnetic resonance imaging (MRI), gallium and indium white-cell scanning, and visceral angiography, may be helpful in specific cases.

Patients suspected of having an intraabdominal crisis should be placed on nothing by mouth and supported with intravenous fluids while undergoing these initial diagnostic evaluations. If peritonitis is suspected, broad-spectrum antibiotics should be administered. Symptoms of hypotension or third spacing warrant monitoring the urine output with a urinary catheter and inserting a central venous catheter or pulmonary artery catheter if required. Aggressive fluid replacement, antibiotics, and steroid stress dose coverage precedes laparoscopic or open surgical exploration. Steroid therapy can mask bowel ischemia and perforation. The best application of diagnostic laparoscopy is in the evaluation of a patient with equivocal findings. It can avoid an unnecessary laparotomy and offer an aggressive diagnostic approach.

Peptic Ulcer Disease

The incidence of peptic ulcers in SLE has been reported as being from 4% to 21% (9 ,15 ,47 ,48), but these studies antedated the present era of endoscopy and gastroprotective therapy. Perforated ulcers have been reported (8 ,9 ,47 ,48) and were found in 3 (5.8%) out of 55 SLE patients presenting with an acute abdomen in a more recent report (49). Therapy with acetylated salicylates and NSAIDs probably is a more frequent cause of peptic ulcer disease than is active SLE. Siurala et al. (50) performed gastric biopsies on 17 patients with SLE; 4 had superficial gastritis and 8 had atrophic gastritis in this 1965 report. Forty years and ten gastroprotective agents later, this area is overdue for re-examination. Junca et al. investigated the prevalence of intrinsic factor and pernicious anemia in 30 patients with SLE and 45 control patients (51). Pernicious anemia characterized by the presence of low-serum cobalamin concentration, macrocytic anemia, and the presence of intrinsic factor antibody was found in only 1 patient (3.3%), although 23% had low cobalamin levels and 10% had intrinsic factor antibody out of the 30 patients with SLE.

Inflammatory Bowel Disease

Ulcerative Colitis

Persistent diarrhea may result from ulcerative colitis that is rarely associated with SLE. Dubois noted concurrent disease in 2 of his 520 patients (7), and my own group has noted concurrent disease in 2 of our 464 patients with idiopathic SLE (16). Kurlander and Kirsner (52) elegantly documented the clinicopathologic correlations and remarked that lupus colitis and ulcerative colitis can be indistinguishable. Lupus colitis can be focalized to a single, small area (53). In 1965, Alarcon-Segovia et al. (54) reviewed the literature extensively and collected 19 cases of concomitant SLE and ulcerative colitis. They also presented 8 additional patients in detail from their Mayo Clinic experience, which accounted for four of their SLE cases. Additionally, 100 patients with ulcerative colitis were evaluated for SLE, which was found in 3 patients. Both sulphasalazine and olsalazine have been associated with the development of a drug-induced SLE (55 ,56). Folwaczny et al. found an increased prevalence of positive antinuclear antibody (ANA) in patients with Crohn disease and ulcerative colitis compared to first-degree relatives and normal controls (57). Eighteen percent of Crohn and 43% of ulcerative colitis patients had a positive ANA test whereas 13% of relatives of Crohn patients and 24% of relatives of ulcerative colitis patients had a positive test. Two percent of healthy controls had a positive test.

Regional Ileitis

Concurrence of SLE and regional ileitis (i.e., Crohn disease) is surprisingly rare and has been reported in about 10 patients (58). Evidence that inflammatory-bowel disease may respond to methotrexate, azathioprine, anti–tumor necrosis factor therapy, and antimalarial drugs is intriguing, and this emphasizes the importance of initiating studies to delineate the relationship between inflammatory-bowel disease and SLE.

Collagenous Colitis

Collagenous colitis is a distinct disorder that is characterized by colonic lymphocytic infiltration of the surface epithelium. Patients have watery diarrhea, but a normal endoscopic appearance and radiographic findings (59). This recently described condition responds to sulfasalazine and corticosteroids. Several reports have associated this condition with lupus (60 ,61 ,62 ,63).

One case of Canada-Cronkhite syndrome associated with SLE has appeared (64).

Celiac Disease in Association with SLE

Gluten sensitive enteropathy is one of the most common autoimmune conditions with a prevalence of 1:100.

An overlap of this condition with SLE has not been cited frequently. Zittouni et al. reported 5 patients with celiac disease who had lupus concomitantly (65). Lupus occurred before and after the diagnosis of celiac disease in one patient respectively. Eighty percent of the patients had positive serologic tests for celiac disease and three out of five patients had abdominal symptoms. Mader et al. screened 61 patients fulfilling ACR criteria for SLE for the presence of anti-endomysial antibodies (AEAs) and antigliadin (AGLA) antibodies and compared the prevalence of seropositivity with 35 healthy controls (66). None of the lupus patients or the controls had AEA but 27 (44.3%) lupus patients and 6 (17.1%) control patients had AGLA. A positive correlation was found between the presence of AGLA and arthritis in lupus. Other manifestations including cutaneous rashes and individual components on the SLEDAI were negatively correlated. The authors conclude that the presence of AGLA is an epiphenomenon, although small intestinal biopsies were not done to rule out celiac disease. They recommend AEA as the preferred screening test for celiac disease in association with SLE. Similar results were reported by Rensch et al. in their SLE cohort of 103 patients where a false-positive rate of 23% was reported for the antigliadin antibody (67).

Protein-Losing Enteropathy and Malabsorption

The presence of severe diarrhea and marked hypoalbuminemia (reported to be as low as 0.8 g/dL) without proteinuria should raise the suspicion of a protein-losing enteropathy. Approximately 32 reports of clinically evident protein-losing enteropathy have been published, along with several literature reviews (49 ,55 ,68). Siurala et al. (50) studied the small intestine in patients with SLE and diarrhea. Among small intestinal biopsies in 19 cases, 12 were normal, and villous atrophy was noted in 2. Radiographic signs as well as signs of intestinal malabsorption were found in 7 patients. Subclinical cases can occur. Hizawa et al. described the radiologic findings in six SLE patients with PLE (69). He reported that there were two types of PLE, four patients presenting with acute onset of enteritis with abdominal pain, nausea, vomiting, and watery diarrhea, and the remaining two patients having mild diarrhea and developed progressive hypoalbuminemia. The first clinical presentation was associated with irregular spiculation and thickening, and thumb printing suggestive of ischemia, whereas the latter had thickened folds with nodules in them, which at biopsy were shown to be lymphangiectasia. Both groups responded well to steroids.

The villi can be lustrous and swollen and may be of various sizes (70). Increased fecal excretion of intravenous radiolabeled albumin is the best quantitative study for following disease activity, although one report (71) has suggested that α_1-antitrypsin clearance also can monitor response to therapy.

The cause of protein-losing enteropathy is unknown, but several theories have cited vascular damage, bacterial overgrowth, fat malabsorption, abnormalities in bile salt metabolism, thrombosis, and mesenteric venulitis as possibilities (70 ,72 ,73). Intestinal permeability may be altered in most patients with SLE as measured by Cr-51-labeled ethylenediaminetetraacetic acid (EDTA) scans (68 ,74). Most patients have abdominal pain, and this can be the initial manifestation of their SLE (72). Cases have been reported of protein-losing enteropathy in association with Sjögren (75) syndrome, interstitial cystitis (76), red blood cell aplasia (77), and as the initial manifestation of the disease (78). It may occur more often in children than in adults (79 ,80). Laboratory investigation may reveal normal lymphocyte counts, elevated serum cholesterol levels, low serum complement levels, antiribonucleoprotein (anti-RNP) antibody (81), and sterile paracentesis fluid with low white-cell counts (82). Lymphangiectasia is uncommon.

Response to corticosteroids is nearly universal but resistant cases may require cyclosporine or intravenous pulse cyclophosphamide. Octreotide, a somatostatin antagonist is useful in treating protein losing enteropathy by decreasing intestinal blood flow and by modulating activated inflammatory cells by binding to the surface somatostatin receptor. Medium chain triglycerides are also useful, because they are carried through the portal system, thereby decreasing lymphatic flow, and also by virtue of being absorbed via the large bowel thus correcting lipid deficits in this condition. Some patients also may require a gluten-free diet (68 ,83 ,84 ,85).

Mader et al. investigated a cohort of 21 SLE patients for malabsorption with a screening D-xylose absorption test, examination of the stool for fat droplets and with histologic examination of a specimen of the duodenum obtained during endoscopy (86). Two patients (9.5%) had evidence of malabsorption manifested by an abnormal D-xylose absorption and excessive fecal fat excretion. Two other patients showed excessive fecal fat excretion. One of the patients with malabsorption had abnormal small bowel histology with flattened villi and an inflammatory infiltrate. There was no excessive deposition of immunoglobulins in the mucosa on immunoperoxidase staining. The etiology of the malabsorption was uncertain.

Ascites and Peritonitis

Ascites can be the initial presentation of SLE. It occurs in 8% to 11% of patients, often as a manifestation of the nephrotic syndrome (15 ,31 ,87 ,88 ,89). Sterile peritonitis was observed in 3 of 704 European patients with lupus (90). In an excellent review of ascites in SLE, Schousboe et al. (78) classified ascites as either acute or chronic. Acute causes include lupus peritonitis, infarction, perforated viscus, pancreatitis, mesenteric vasculitis (91), and hemorrhagic and bacterial peritonitis (92). Chronic causes of ascites include

lupus peritonitis, congestive heart failure, pericarditis, nephrotic syndrome, Budd-Chiari syndrome, protein-losing enteropathy, underlying malignancy (93), cirrhosis, and tuberculosis. Ascitic fluid can be inflammatory or noninflammatory. Noninflammatory lesions are always painless and associated with transudative fluid; most patients have nephrotic syndrome. Peritonitis usually is inflammatory and exudative. It generally is painful, but not always (89 ,94 ,95). Peritoneal tissue can contain immune complex deposits and inflammatory infiltrates (96 ,97). ANA, anti-DNA, and low complement levels can be present in peritoneal fluid (96 ,98 ,99). Reports also have appeared of concurrent familial Mediterranean fever (100 ,101) and of oligoclonal protein bands (102 ,103) inducing ascites in patients with SLE. Fetal ascites in babies with Ro/SSA-positive mothers may result from congestive heart failure or from various immune mechanisms (104). Low et al. reported the characteristics of lupus serositis on barium radiograph and CT imaging (105). The small-bowel barium series showed segments of spiculation with tethering, angulation, and obstruction. CT scanning demonstrated ascites and asymmetric thickening of the small bowel wall.

Ascites caused by lupus peritonitis usually is steroid-responsive. Other causes may require additional interventions (78), including azathioprine (106) and cyclophosphamide (107). Gentle diuresis is an important adjunctive measure that often provides symptomatic relief, provided that renal function is not impaired by this approach.

Pancreatitis

Prevalence

Pancreatitis can be the initial manifestation of SLE (108). First reported by Dubois in 1953 (29), it presents with severe epigastric pain radiating to the back, nausea, vomiting, an elevated serum amylase level, and dehydration. Seven of 704 European patients with SLE had a history of pancreatitis (90). Neither Fries and Holman (13) nor Ropes (14) noted pancreatitis in any of their combined experience with 350 patients. Ropes attributed this to the sparing use of steroids. Rothfield (109), however, observed pancreatitis in 8 of 365 patients. Of 168 consecutive SLE admissions to the University of Alabama Medical Center, 7 (4%) had pancreatitis; 5 of these 7 succumbed (110).

Etiopathogenesis and Clinical Presentation

Corticosteroids are not considered to play a role in the in causing pancreatitis in SLE patients. Hernandez-Cruz et al. reviewed their database of SLE patients and found 18 patients with 26 episodes of pancreatitis with an average SLEDAI score of 6.5 at the time of the acute pancreatitis episode (111). Eleven out of the 26 episodes were severe episodes and 4 patients died—3 of pulmonary hemorrhage and 1 from septicemia. The most common cause was felt to be medication use (8 episodes) and hypertriglyceridemia, alcohol, and cholelithiasis were thought to be the etiology in 4, 2, and 2 patients, respectively. Pascual examined their database of SLE patients from July 1984 to July 2001 and found 49 separate acute pancreatitis episodes in 35 lupus patients giving a prevalence of 3.5% (112). Of the 49 episodes, 14 (28.5%) were considered to be biliary disease and 10 (20.4%) were considered to be toxic-metabolic (alcohol, increased triglycerides, uremia, etc) in etiology. The remaining 17 (34.7%) were considered to be idiopathic or because of SLE. Mex-SLEDAI was significantly higher in the idiopathic group (median of 9 (3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19) in the idiopathic versus 5 (0–23) in the toxic metabolic and 3 (0,1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18) in the biliary group). A case control analysis with a ratio of controls to cases ratio of 4 to 1 showed an increased prevalence of acute pancreatitis in SLE (46% versus 14%). The odds ratio for the severity of pancreatitis and mortality was significantly higher in pancreatitis associated with SLE than in non-SLE controls (OR 8.6 versus 7.5). In conclusion, pancreatitis is a more often a severe and often fatal manifestation of lupus than an illness from other factors such as biliary disease or medications including corticosteroids, Similar conclusions were drawn by Derk et al. in their review of all hospital admissions for lupus patients to Thomas Jefferson Unversity Hospital between 1982 and 2002 (113). Of the 2,947 hospitalized SLE patients, 25 (0.85%) had acute pancreatitis. The majority (76%) has active SLE pm admission with a average involvement of 4.4 organs. Eighteen of the 25 patients had an increase in their corticosteroid doses with improvement of their clinical and laboratory parameters.

Pancreatitis can be the initial presentation of SLE and may appear in childhood (114 ,115 ,116). Several cases of panniculitis and subcutaneous fat necrosis have been reported as being associated with SLE and pancreatitis (117 ,118 ,119), as has type I hyperlipidemia (120) with increased levels of chylomicrons and thrombi in pancreatic arteries, because of antiphospholipid antibodies (121 ,122 ,123 ,124).

Corticosteroids, azathioprine, and thiazide diuretics are used in the treatment of SLE and may induce attacks of pancreatitis that are independent of the disease (125 ,126 ,127). Cases of pancreatic vasculitis were documented in the presteroid era (5). In 25 lupus necropsies, 8 cases of pancreatitis were found (128 ,129 ,130). Four of these had pancreatic vasculitis, and 4 were thought to have steroid-induced disease. Pancreatitis also can be caused by hypovolemia, ischemia, cholecystitis, alcoholism, carcinoma, and viral infections, all of which can occur in SLE.

Mild elevations of serum amylase levels may be noted in patients with SLE in the absence of pancreatitis. Hasselbacher et al. (131) studied 25 patients with SLE but without pancreatitis and 15 non-SLE controls. Amylase levels were elevated in 5 patients, and 6 had macroamylasemia, compared with none of the controls. The mean amylase level in the SLE group was 161.7 mg/dL, compared with 116.4 mg/dL in the control group; this difference was statistically significant. Macroamylasemia results from

decreased renal clearance of an immunoglobulinamylase complex. The presence of a pathogenic autoantibody to amylase was proposed. Tsianos et al. (132) found elevated pancreatic and salivary amylase components in 11 of 36 unselected patients with SLE. Thirty-five percent of 20 children with SLE, most of whom were asymptomatic, had elevated serum cationic trypsinogen levels; this is indicative of subclinical pancreatic dysfunction (133). These reports have associated active SLE with elevated amylase levels without abdominal pain.

In their definitive study on lupus pancreatitis, Reynolds et al. (134) combined a literature survey with a review of 20 patients (75% were female). The mean age of their group was 34 years, and the mean disease duration was 3.75 years. The mean prednisone dose was 11 mg, and 5 patients also were taking azathioprine. Of the 20 patients, 8 had recurrent attacks of pancreatitis, and amylase levels did not correlate with renal function or steroid doses. The mean duration of each episode was 15.5 days. The chief clue to the cause (i.e., lupus vs. drug induced) was that most of the patients with SLE-induced pancreatitis had multisystem SLE involvement (an average of 6.2 organ systems were involved) and responded well to increased steroid administration.

Management

Treatment includes immediate discontinuation of nonessential drugs that can induce pancreatitis (e.g., azathioprine, diuretics), intravenous hydration, nothing by mouth, antibiotics if needed, and sparing use of analgesics. The decision to use high dose corticosteroids is difficult if the patient has evidence of active SLE and is on glucocorticoid therapy. Careful observation is essential.

The prognosis of pancreatitis associated with SLE is very poor especially with those with active SLE with involvement of multiple systems. Two literature reviews have been published (135 ,136). One reported on 66 patients, 26 of whom were not taking steroids (133). Nearly 75% of the 66 patients had a fatal outcome. Pancreatitis may induce diabetes (see Chapter 39).

Mesenteric and Intestinal Vasculitis, Melena, and Bowel Hemorrhage

Prevalence and Presentation

Mesenteric vasculitis, with or without infarction, is one of the most serious complications of SLE. Although Ropes (15) found peritoneal involvement (from serositis) at autopsy in 63% of patients, with adhesion being common, only a small percentage had mesenteric vasculitis or acute abdomen. Vitali et al. (90) found that 1.1% of 704 European patients with lupus had intestinal vasculitis. Conversely, Landing et al. noted ischemic bowel in 60% of 26 necropsies on children (9). Melena has been observed in from 1% to 6% of patients with SLE (7 ,13 ,14). Most mesenteric vasculitis presents with cramping or with constant abdominal pain, vomiting, and fever. Diffuse direct and rebound tenderness usually are present.

Four detailed and authoritative studies of lupus enteritis and other abdominal complications have been published (17 ,137 ,138 ,139). Zizic et al. (138) detailed five patients with large bowel perforation. All had active SLE and mesenteric or intestinal vasculitis. The presentation was insidious, with lower abdominal pain. Abdominal rigidity was present in only one patient. Most had nausea, vomiting, diarrhea, and bloody stools. All had tenderness, and most had rebound tenderness and distention. Bowel sounds were diminished or absent. Prior or concurrent administration of steroids masked symptoms in some, and it may have promoted the bowel wall thinning that led to perforation.

Chase et al. (137) recounted 15 cases of acute surgical abdomen in 140 patients with SLE. An increased incidence of peripheral vasculitis, central nervous system (CNS) disease activity, ischemic necrosis of bone, thrombocytopenia, and rheumatoid factor was present. Of the 15 patients, 11 underwent exploratory laparatomies, 9 were found to have vasculitis, and 2 were found to have polyserositis. Eight patients died, primarily from complications of infarction or perforation.

Shapeero et al. (140) reviewed the hospital records of 141 patients with SLE who were admitted to the Hospital of the University of Pennsylvania over a 20-year period. Of these, 68 had abdominal symptoms, and 20 were thought to have ischemic abdominal disease. In 9 patients, this was confirmed radiographically by pseudo-obstruction of the gastric outlet, duodenal stasis, effacement of mucosal folds, spasticity, and thumb printing. Of these 20 patients, most had anorexia, nausea, vomiting, postprandial fullness, and abdominal pain. Only 10 had melena, 35 had fevers, and 50 had guarding. Additionally, 20 had leukocytosis, and 65 were anemic. All responded to steroid therapy.

Lupus enteritis can produce gastritis, mucosal ulceration, bowel edema with ileus, hemorrhagic ileitis, intussusception, perforation, and/or infarction (8 ,14 ,141 ,142 ,143 ,144 ,145). Spontaneous hemoperitoneum can be secondary to thrombocytopenia (146) or to ruptured aneurysms (147 ,148). Colonic diverticula (146) and mesenteric vasculitis (149 ,150) may induce perforation. Antiphospholipid antibodies may play a prominent role in inducing intestinal infarction (150 ,151 ,152 ,153 ,154). Medina et al. studied the relationship between SLEDAI scores and the sources of an acute abdomen in 51 SLE patients (155). Patients with intraabdominal vasculitis (19 patients) or thrombosis (3 patients) had higher SLEDAI scores than 14 active SLE patients with non-SLE related acute abdomens (mean 17.5 [range, 13 to 24] versus 8.2 [range, 5 to 11]). Fifteen patients with inactive SLE (SLEDAI 1.7, range, 0 to 4) had intra-abdominal pathology that was diverse and not related to lupus. Buck et al. found that a SLEDAI greater than 8 appeared to indicate bowel vasculitis in patients with SLE admitted to the

hospital for abdominal pain without peritoneal signs (156). The authors emphasize imaging and early laporotomy in active SLE with high SLEDAI scores whether an acute abdomen is present or not. When all patients with SLE who are hospitalized are evaluated, lupus patients with GI vasculitis differed from SLE patients hospitalized for abdominal pain without vasculitis only with regard to having leukopenia at the time of perforation (157). Lupus patients presenting with an GI syndrome with abdominal pain, vomiting and diarrhea as a result of serositis and bowel involvement often resolve with immunosuppressive therapy without surgical intervention (158). SLEDAI scores in this cohort requiring hospitalization were lower at 4 or above.

Laboratory, Pathogenetic, and Radiographic Findings

Laboratory evaluations are not particularly helpful. Acute-phase reactants and general indicators of active SLE usually are present. Paracentesis may be useful in ruling out pancreatitis or infection.

Radiographic changes include pseudo-obstruction of the gastric outlet, duodenal stasis, effacement of the mucosal folds, and thumbprinting. Thumbprinting represents bowel submucosal edema or hemorrhage on a barium or gastrografin enema; this finding is relatively specific for ischemic bowel disease. Similar findings can be found using CT with contrast. CT of the abdomen has identified intra-abdominal abscesses, lymphadenopathy, serositis, bowel-wall thickening, edematous and distended loops of bowel, pancreatic pseudocysts, and enlarged liver and spleens in patients with SLE (159 ,160 ,161). Ko et al. published their findings on radiologic assessment of lupus mesenteric vasculitis (162). Of the 15 patients with mesenteric vasculitis, CT scans done within 3 to 4 days of the onset of abdominal pain revealed the characteristic palisade and comblike pattern of mesenteric blood vessels with vasculitis in 11 out of 15 patients. Peritoneal enhancement of ascitic fluid (11 patients), small-bowel wall thickening (10 patients) and a double halo or target sign (8 patients) were other common signs of mesenteric vasculitis (Fig. 42-1). Abdominal ultrasounds can show bowel-wall thickening (163). Gallium scans and indium-111 white-cell scans can light up areas of inflammation and sepsis (164).

The histologic characteristics and distribution of mesenteric vasculitis are similar to those that are seen in polyarteritis nodosa (138 ,165). The colon and small bowel often are involved with vasculitis in the submucosa. This results in ulcerated mucosa, submucosal edema, necrosis, and infarction (166). Two cases of appendiceal arteriolitis (167) have been documented. Pneumatosis cystoides intestinalis may coexist with necrotizing vasculitis (168 ,169 ,170 ,171 ,172 ,173), and although usually benign, it occasionally can cause perforation.

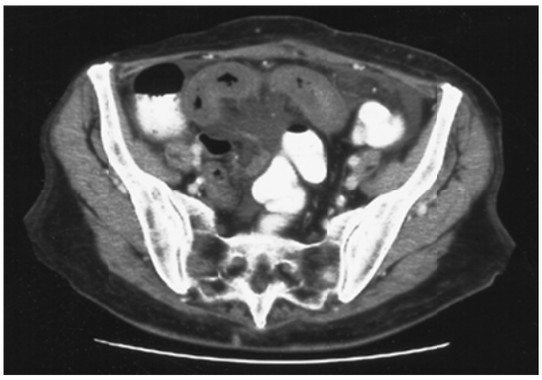

Figure 42-1. Lupus vasculitis involving mesenteric arteries causing bowel edema and necrosis of the small and large intestine. Histological examination showed vasculitis with small-vessel thrombosis. (Printed with permission from Cedars-Sinai Medical Center, Los Angeles.)

Treatment and Outcome

The treatment of choice for lupus enteritis is 1 to 2mg/kg/day of parenteral methylprednisolone (8 ,144 ,174 ,175 ,176 ,177 ,178), or its equivalent, in addition to complete bowel rest. If a rapid response is not noted, surgical intervention may become necessary in cases of perforation or large areas of ischemia (140 ,144 ,175). Mesenteric vasculitis has a high mortality rate. One survey of patients with SLE undergoing surgery documented the widely held belief that in and of itself, steroid therapy increases the risk of postoperative complications (178). Grimbacher et al. reported successful treatment of relapsing intestinal vasculitis with intravenous pulse cyclophosphamide therapy (179). Table 42-1 presents a summary of SLE and the GI tract.

Liver Abnormalities

Hepatomegaly

Enlargement of the liver was present in 10% to 32% of patients in the series listed in Table 42.1 , with the frequency decreasing over the last three decades. An enlarged liver was present in 28 of 108 children with SLE who were studied by King et al. (12), and Ropes reported a palpable liver in one half of her patients (15). The liver usually extends 2 to 3 cm below the costal margin, but it occasionally can reach the iliac crest. Tenderness is uncommon unless viral hepatitis or peritonitis is present. Hepatomegaly and tenderness may be present with normal liver function tests. An enlarged liver can be histologically normal (180 ,181).

Jaundice

Jaundice was present in 1% to 4% of the patients listed in Table 42-1 and in 9 (3%) of Rothfield's 375 patients (109).

The most common causes of jaundice in SLE are hemolytic anemia and viral hepatitis; cirrhosis and obstructive jaundice from a biliary or pancreatic mass are responsible for the remainder.

Vascular Lesions: Hepatic Vasculitis, Portal Hypertension, the Budd-Chiari Syndrome, and Antiphospholipid Antibodies

Dubois described the first case of hepatic arteritis in 1953 (29). It was found in one of 58 necropsies reported by Ropes (15) and in 11 of 52 necropsies in Japan where it was specifically looked for (182) (Fig. 42-2). This rare complication of SLE (183) can be associated with ruptured hepatic aneurysms (184 ,185).

Five specific complications are attributable to antiphospholipid antibodies: (1) Budd-Chiari syndrome, (2) hepatic veno-occlusive disease, (3) nodular regenerative hyperplasia (182 ,186), (4) liver infarction, and (5) transient elevation of hepatic enzymes resulting from multiple fibrin thrombi (187).

The Budd-Chiari syndrome is occlusion of the hepatic veins with secondary cirrhosis and ascites. It almost always is caused by thromboses in patients with antiphospholipid antibodies (188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196). This usually leads to portal hypertension, which rarely is seen by itself. In one study, the disease was associated with antibody to proliferating-cell nuclear antigen (168). Hepatic infarction may be associated with pregnancy (197 ,198). The diagnosis of nodular regenerative hyperplasia may be missed on clinical grounds as well as by ultrasound or CT imaging but show up as high signal on T1-weighted images and isotense on T2-weighted images (199).

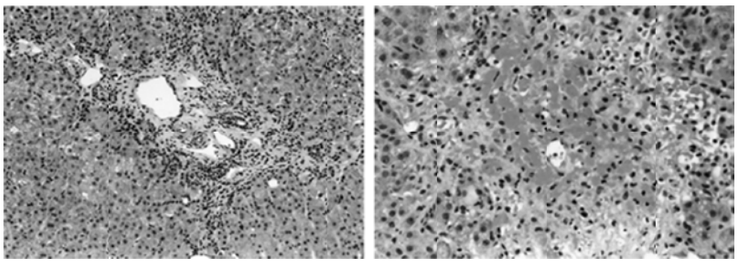

Figure 42-2. Biopsy of the liver in a 14-year-old Asian girl with classic systemic lupus erythematosus. Patient had a subacute onset of severe hepatitis (transaminases >tenfold) with negative hepatitis virus serologies and other hepatic autoantibodies and strongly positive antiribosomal P antibody. Patient had a complete recovery with high-dose steroids and azathioprine (H&E; 1:200). (Printed with permission from Cedars-Sinai Medical Center, Los Angeles.)

Neonatal Lupus Liver Disease

Lee et al. (200) found liver disease in 4 of 35 cases of neonatal lupus. Transient, although often severe, cholestasis was the principal finding, and no liver-specific antibodies were found. In a series of four cases (201), hepatic fibrosis was found along with giant-cell transformation, ductal obstruction, extramedullary hematopoiesis, and cholestasis.

Liver Function Test Abnormalities: Clinicopathologic Correlates

Liver function tests usually are obtained incidentally as part of blood chemistry panel. In SLE, nonspecific liver enzyme elevations are seen in a minority of patients and usually are of little significance. In our own experience, most liver function test abnormalities in SLE result from the administration of NSAIDs or methotrexate, or they are elevated because of increased muscle enzyme levels. Pathologic changes also are nonspecific and mild.

Rothfield (109) found elevated liver enzyme levels at diagnosis in 30% of patients with SLE, and Gibson and Myers (202) reviewed liver disease in 81 patients with SLE. Of these, 45 (55%) had abnormal liver function tests at some point, and 27% had enlarged livers. These abnormalities were accounted for by nonhepatic sources in nine patients, were drug-induced in 14, and from congestive heart failure in three. Of 19 biopsies that were reviewed, 7 were normal, 5 had portal-inflammatory infiltrates, 1 had a fatty liver, and 1 had chronic active hepatitis. Only 3 of the 81 patients ever had transaminase levels exceeding 100 mg/dL. In another survey (203), elevations in liver function tests were associated with disease activity and liver membrane autoantibodies.

Peliosis hepatis is a condition where blood-filled spaces occur in the liver from diverse causes causing injury from drugs and infections on the flow of blood from the sinusoids to the centrilobular veins. Three cases have been reported in association with lupus with reported improvement with immunosuppressive treatment (204).

Altomonte et al. (205) compared 18 females with SLE but without known liver disease to 20 healthy controls. Significant differences included the following: delayed Bromsulphalein excretion (27%), elevated fasting serum bile acid levels (50%), and increased γ-glutamyl transpeptidase levels (38%). Miller et al. (206) followed 260 patients with SLE and 100 controls for 12 months. Of the 60 patients with SLE and abnormal liver function testing, 41 could be traced to an identifiable cause (aspirin in 27, alcohol in 6, and others in 7). In 12 of 15 patients with elevated transaminase levels, subclinical liver disease was a probable cause.

Runyon et al. (207) noted that 124 of 206 patients with SLE who they tested had abnormal liver-enzyme values; liver disease was identified in 43 patients. Biopsies were performed on 33 patients; 3 of the 206 patients died in hepatic failure. The ultimate diagnosis was steatosis in 12 patients, cirrhosis in 4, chronic active hepatitis in 3, chronic granulomatous hepatitis in 3, centrilobular necrosis in 3, chronic persistent hepatitis in 2, and microabscess in 2. None were positive for hepatitis B antigen. The pathology was thought to be drug-induced in 21% of patients. Corticosteroids were beneficial in 8 of the 12 patients who received them. One third of 216 patients with SLE at Johns Hopkins had abnormal liver function tests over 1,717 visits, and elevations of their liver enzymes correlated with disease activity (208). On the other hand, severe liver disease can be present in patients with SLE and only minimal laboratory abnormalities (209).

Tsuji et al. reviewed the records of hospitalized lupus patients over a decade and found 73 patients with elevated transaminases (210). Of these, 43 (58.9%) did not have an identifiable cause of elevated transaminases and was attributed to active SLE. Of the identifiable cause of elevated liver enzymes, 7 (9.6%) were identified to have hemophagocytic syndrome on the basis of a marked elevation of serum ferritin levels (mean, 14,671 mg/dL; range, 370–84,651). This group also had the highest elevation in liver enzymes. Viral infection were not ruled out as the cause of hemophagocytic syndrome in this retrospective review. Maeshima reported a case of established systemic lupus who was admitted with a very low white blood cell (WBC) count and high transaminases (211). The ferritin level was 2306 ng/mL and a bone marrow biopsy was consistent with hemophagocytosis. Epstein-Barr and cytomegalovirus infections were ruled out and the patient responded to betamethasone and cyclosporin A treatment.

Ropes (15) reported on 58 necropsies in SLE. Of these, 50% had an enlarged liver, moderate to marked fatty infiltration was observed in 44%, and portal congestion was noted in 47%. Hematoxylin bodies were seen in 3, arteritis in 1, and hemosiderosis in 1. Fatty livers usually are associated with corticosteroid therapy, and several reports have commented on the presence of nodular regeneration and hyperplasia in SLE (190 ,212). The patients in these reports had normal liver function test results; this underdiagnosed finding could be secondary to steroid or danazol administration. Concentric membranous bodies in hepatocytes are found in hepatomas but occasionally are seen in lupus, and they reflect increased protein synthesis during regeneration (213).

Van Hoek reviewed the causes of elevated liver enzymes in SLE and found that medications such as nonsteroidal anti-inflammatory medication, aspirin, or azathioprine were the most common etiology (214). Liver-function test abnormalities may result from non-liver–related causes such as unconjugated hyperbilirubinemia, hemolysis or hepatitis resulting from immunologic, infectious, or drug-related causes. Hepatitis resulting from SLE was most likely to be lobular and associated with autoantibodies such as anti-double-stranded DNA and antiribosomal P antibodies. In contrast, autoimmune hepatitis was more likely to be periportal (chronic active hepatitis) with rosetting of liver cells and dense lymphoid infiltrates and often has specific autoantibodies to anti-liver-specific protein or have anti-liver–kidney–microsomal antibody. Both conditions are associated with features of autoimmunity such as polyarthralgia, hypergammaglobulinemia, and a positive ANA.

In summary, most patients with SLE and elevated liver function tests have liver biopsy specimens that reveal nodules, mild fatty changes, or mild fibrosis. Rarely, features of chronic active hepatitis are found.

Lupus Hepatitis

Definition and Clinical Features

Lupus hepatitis may be defined as an insiduous, rarely acute onset of transaminitis in patients who fulfill American College of Rheumatology (ACR) criteria for SLE, frequently have a positive test for ribosomal P antibody, and biopsy findings of lymphocytic infiltration of periportal areas with isolated areas of necrosis (214) (Fig. 42-2).

Ohira et al. found that 15 (44.1%) of the 34 SLE patients with liver dysfunction were positive for ribosomal P protein antibody (215). Of these 34 patients, 16 had SLE associated hepatitis and 11 out of 16 (68.8%) were positive for ribosomal P protein antibody whereas a control group of 20 patients with autoimmune hepatitis were negative for the antibody.

Our group found evidence for autoimmune hepatitis among 22 of 464 patients with SLE (4.7%) who fulfilled ACR criteria for SLE (16). Hepatitis was present in 2.4% of 704 European patients with lupus (90), and SLE was present in six (4.2%) of a group of Japanese patients with chronic active hepatitis (216). Chronic active hepatitis was found in 2.4 of 1,468 autopsied Japanese patients with lupus (182).

Patients usually present with nonspecific symptoms of fatigue, malaise, and anorexia. Jaundice is present in fulminant hepatitis. Mild liver enlargement, jaundice, or ascites in severe cases and other joint- and organ-threatening manifestations of SLE are found on physical examination.

Laboratory and Serologic Abnormalities

Elevations in serum glutamic-oxalocetic transminase (SGOT) and serum glutamatic pyruvate transaminase (SGPT) usually are less than two- to threefold but in severe cases, marked elevation in transaminases (more than tenfold) with mild increase in bilirubin and alkaline phosphatase may be seen. Antibodies such as a positive ANA, dsDNA, Smith, and hypergammaglobulinemia are seen. Antibody to ribosomal P protein is a strong marker for lupus hepatitis (217 ,218). Patients with autoimmune hepatitis and primary biliary cirrhosis characterized by antibody to smooth muscle, liver-kidney microsomal and mitochondria rarely fulfill criteria for SLE. Of 89 patients with lupoid hepatitis who were followed at the Mayo Clinic, 43 had arthritis, 10 had thrombocyotopenia, 9 had pleurisy, and 8 had leukopenia. Malar rash, pericarditis, neuritis, hemolytic anemia, and proteinuria were observed in 2 patients or less. Only 9 fulfilled the ACR criteria for SLE (219 ,220). The overwhelming majority of patients are women, and an increased association with human leukocyte antigen (HLA) haplotypes B8 and DR3 has been noted (221). In a comparison to 50 patients with SLE and 50 with chronic active hepatitis, 95% of the SLE group and 20% of the chronic active hepatitis group fulfilled ACR criteria for SLE (222).

Type I Autoimmune (Lupoid) Hepatitis

In 1955, Joske and King (223) first called attention to the coexistence of LE cells in patients who had apparent viral hepatitis. Mackay et al. (224) continued their studies and, in 1956, coined the term lupoid hepatitis, believing it to be a manifestation of SLE. Despite steroid treatment, however, these patients all succumbed to liver failure an average of 3 years after presentation (225 ,226). Many early studies were conducted before the availability of ANA, smooth muscle antibody, antimitochondrial antibody, or hepatitis virus serologic tests. These reports are of historic interest and were reviewed on pages 91 to 93 of the second revised edition of this text (227). Mackay's 1990 review also is useful (228).

Definition and Clinical Features

Type I autoimmune (lupoid) hepatitis is defined serologically and histologically, and it is a subset of chronic active hepatitis (229). Histologic hepatic changes include periportal piecemeal necrosis, dense lymphoid infiltrates, and prominence of plasma cells. Serologically, patients have a positive ANA and high levels of γ globulins, and antibodies to smooth muscle may be found. Chronic active hepatitis is associated with HLA-B8, DR3, and DR4, and it has many causes, including viral hepatitis A, B, or C; drug-induced hepatitis; Wilson disease; alcoholism; primary biliary cirrhosis; and α1-antitrypsin deficiency, all of which must be ruled out (220 ,230).

Autoimmune hepatitis has an insidious onset. Generally found in a young or middle-aged female who complains of fatigue, malaise, anorexia, and low-grade fevers, there are usually no physical findings at first. Hepatosplenomegaly, jaundice, and signs of cirrhosis or liver failure occur late.

Sherlock (231) observed that 42% of patients with chronic active hepatitis have keratoconjunctivitis sicca and xerostomia. She thought that the incidence of ulcerative colitis, which is reported to be 10%, could be as high as 30%; it was found in five of Mackay's first 40 patients with lupoid hepatitis (225 ,226 ,232).

Laboratory and Serologic Abnormalities

Liver enzyme, γ globulin, alkaline phosphatase, and bilirubin levels are elevated, the albumin level is decreased, and the prothrombin time may be prolonged (221 ,233 ,234). LE preparations usually are positive, but they may become negative with claimed improvement (235). The ANA of autoimmune hepatitis has specificities for histones and granulocytes (221). ANAs are positive in approximately 10% of patients with nonautoimmune, chronic active hepatitis (235). Anti–single-stranded DNA (anti-ssDNA) is found in approximately 0% to 16% of patients with chronic active hepatitis (214 ,235 ,236 ,237 ,238). Other autoantibodies, such as anti-dsDNA, anti-Sm, anti-RNP, anti-Ro/SSA, anti-La/SSB, and anticardiolipin antibody, are found in 0% to 5% of patients (236 ,238 ,239).

Smooth-muscle antibodies and antimitochondrial antibodies were present in 81 (30%) of Mackay's series (240) and in 64 (16%) of those reported by Leggett et al. (241). Antimitochondrial antibodies to M5 may cross-react with antibodies to phospholipids and yield false-positive readings in SLE (242). Smooth-muscle antibodies have specificity for actin (228). Antibodies to bile canaliculi have been claimed but not confirmed (240 ,243).

Other autoantibody systems that are found in autoimmune hepatitis include antibodies to liver-kidney-microsomal autoantigen, soluble cytoplasmic autoantigen, and M2 mitochondrial autoantigen. Five percent of patients with autoimmune hepatitis have false-positive antibodies for hepatitis C (231).

Association with Nephritis

Several studies have noted nephritis in patients with autoimmune hepatitis (244 ,245 ,246). Hepatitis B surface antigenemia is associated independently with glomerulonephritis (247).

In one previous report (248), five patients with SLE who were hepatitis-B surface antigen positive underwent renal biopsy and found lupus nephritis present in two and hepatitis B antigen-associated nephritis in three. Lai et al. compared clinicopathologic features of 22 patients with hepatitis B virus membranous nephropathy with 26 patients with Class V lupus membranous nephropathy (249). Other than being male and lacking the autoantibodies of lupus patients, there were no reliable distinctions between the two groups and antisera to hepatitis B virus antigens is required to identify the antigens in the glomerular pathology. Renal tubular acidosis has been found in chronic active hepatitis (232 ,250 ,251).

Treatment and Prognosis

Steroids remain the mainstay of treatment, prolonging life and making the patient more comfortable (221 ,232 ,252 ,253). In a controlled study comparing prednisolone alone (40 mg daily at initiation of treatment and 15 mg daily maintenance), azathioprine alone (150–200 mg daily), and prednisolone azathioprine combined (10 mg and 100 mg daily, respectively), Mackay (254) reported that the relapse rate was lowest and overall survival longest in the combined-treatment group. All three regimens suppressed the disease, and indices of liver function showed improvement. Between-group comparisons for certain indices showed prednisolone alone to be superior to azathioprine alone. It was concluded that prolonged suppressive treatment has ameliorative effects while treatment is maintained, but that a long-term cure is infrequent.

Mistilis and Blackburn (255) recommended a daily dose of 60 mg of prednisone for the first 2 or 3 weeks, with gradual reduction over several months to a maintenance level of 5 to 20 mg daily. Steroids alone did not improve survival but did reduce early mortality. This group recommended a daily dose of 1.5 mg/kg of azathioprine to control disease activity, but other controlled studies have questioned the efficacy and safety of azathioprine (250).

Newer treatments for chronic, active hepatitis include interferon, colchicine, and liver transplantation. Our group has shown that patients with SLE can safely undergo liver transplantation (256).

The prognosis of autoimmune hepatitis varies depending on the patients included in the survey. The 5-year survival wasreported by Mackay to be 65% in 1968 and 80% in 1988 (231 ,240). Poulsen et al. examined a sicker hospital patient registry between 1977 and 1993 and identified 96 patients with a discharge diagnosis of lupoid hepatitis (Poulsen). After a mean follow-up of 7.5 years, only 50% of patients were alive. The standardized mortality rate was 3.7 (95% CI, 22.1–100.9) compared to the general population. The risk of dying from liver cirrhois was increased by 51.2%. Patients with autoimmune hepatitis have a increased risk of developing hepatocellular cancer with the risk found to be 1 out of a cohort of 212 patients followed for 1732 patient-years. One of out 88 patients with cirrhosis developed liver cancer after 1002 patient-years of observation (mean-123+/-9 months) (257).

Other Causes of Hepatitis in Systemic Lupus Erythematosus

Hepatitis A and B Infection

SLE cohorts have the same incidence as control groups of hepatitis virus A or B antigens and antibodies (248 ,258). Autoimmune hepatitis rarely is observed in patients who are hepatitis B surface-antigen positive (234 ,258 ,259 ,260). Seventy-six patients with SLE in Singapore were tested for hepatitis B virus (HBV) infection; 15 (19.7%) had one or more of the three serologic markers for infection (260). This was comparable to the 19% positivity in 100 sex- and age-matched, healthy individuals. Lu et al. investigated the prevalence of hepatitis B in patients with SLE in Taiwan, which is a hyperendemic area for hepatitis B infection (261). The study also examined the level of interferons (IFNs) in both these disorders, which has been found to be low in both. The prevalence of HBV infection was lower than in the general population (3.5% versus 14.7%). The six patients out of the 173 SLE patients who had co-existing HBV infection and SLE had less active SLE with lesser degree of proteinuria and lower autoantibody levels than patients with SLE, but no evidence of HBV infection. These patients and patients with HBV infection had near normal levels of IFN-γ levels when compared to SLE patients. However, their levels of IFN-α were lower than in normal controls as well as patients with SLE. This suggests that subjects with low IFN-α levels are at increased risk for HBV infection and that IFN-γ, which is probably induced by the HBV infection, ameliorates the activity of SLE patients who have coexistent HBV infection. Abu-Shakra et al. found no evidence of HBV infection in 96 SLE patients in Israel where the prevalence of HBV infection in the general population is 2% (262).

Hepatitis C

Some autoimmune hepatitis may be associated with the hepatitis C virus (HCV) (263). Antibodies to hepatitis C were found in four of 71 patients with SLE in an Italian clinic (264). Bronson et al. published a case report of a 35-year-old woman who developed an acute onset of SLE with manifestations of arthralgia, malar rash, low-titer dsDNA antibody, negative tests for cryoglobulin, and diffuse proliferative glomerulonephritis coincident with a HCV infection (265). McMurray et al. reviewed the serologic and clinical manifestations associated with HCV infection (266). Autoantibodies that are specific for autoimmune hepatitis such as anti–smooth-muscle antibody are seen in up to 66% of patients with HCV infection (267). Nonspecific antibodies such as low-titer ANA (30%), anticardiolipin antibody (22%), and rheumatoid factor (76%) also are found in

chronic HCV infection prompting the investigation of the prevalence of HCV infection in patients with SLE. Kowdley et al., Karakoc et al., Abu-Shakra et al., and Chavez et al. investigated the prevalence of HCV infection in patients with SLE (262 ,267 ,268). The true prevalence of HCV was estimated to be close to that in the general population (0.5% to 1% in most studies). Chavez et al. reported a fourfold increase in the prevalence of hepatitis C in their cohort of 112 SLE patients even after eliminating 4 out 10 RIBA-III positive cases with HCV viral load testing (269). A case-control study was performed by Perlemutter et al. comparing 19 patients with SLE with hepatitis C virus (HCV) coinfection with 42 randomized SLE patients matched for age and sex but negative for HCV infection (270). The two groups were similar with respect to the prevalence of organ involvement and the levels of antinuclear and doublestranded DNA antibodies and C3 complement. The total hemolytic complement and C4 complement levels were higher in the SLE-HCV cohort along with a higher prevalence of mixed cryoglobulinemia. Serum ALT levels were elevated in 12 patients and cirrhosis was found on biopsy in 2 out of 11 SLE-HCV patients who were biopsied. Interferon or ribavirin therapy did not flare up SLE and conversely SLE or cumulative steroid treatment of up to 20 g per patient did not cause an increase in liver failures in the SLE patients who were infected with HCV. Conversely, although 25% of a cohort of 122 consecutive patients with SLE tested positive for cryoglobulins, a cryocrit greater than 1% was associated with hepatitis C infection (271).

Table 42-2: Systemic Lupus Erythematosus and the Liver

1. Hepatomegaly occurs in 10% to 31% of patients with systemic lupus erythematosus (SLE) and is seen in 50% at necropsy.
2. Jaundice is present in 1% to 4% of patients and is secondary to hemolysis, hepatitis, or pancreatitis.
3. Hepatic vasculitis is uncommon.
4. Budd-Chiari syndrome is associated with the presence of antiphospholipid antibodies.
5. Elevated liver enzyme levels are found in 30% to 60% of patients with SLE at some time. Most are caused by infections, salicylates, or NSAIDs. Enzyme levels greater than three times normal are rare.
6. Lupus hepatitis usually is insiduous in onset, varying in severity and frequently associated with ribosomal P antibody. These patients usually fulfill criteria for SLE and have biopsy findings of periportal infiltration of lymphocytes and isolated degeneration of hepatocytes.
7. Autoimmune hepatitis (lupoid hepatitis) is a form of chronic active hepatitis with malaise, arthralgia, fever, anorexia, jaundice, and negative hepatitis viral studies. Antimitochondrial and antismooth muscle antibodies often are present. Abnormalities associated with SLE, such as lupus erythematosus cells and antinuclear antibody are found. Most of these patients should be classified as being in a subset of chronic active hepatitis. Only 10% fulfill ACR criteria for SLE. Biopsy findings include piecemeal necrosis and are identical to chronic active viral hepatitis B and C.
8. The prevalence of Hepatitis B and C infection in SLE patients is not different to the prevalence in the general population.

Drug-induced autoimmune hepatitis has been reported after ingestion of the laxative oxyphenisatin (272) or after chlorpromazine (273). The picture can be confusing (without liver biopsy), especially because patients with lupus can develop viral hepatitis (as can any otherwise healthy person). Aspirin and NSAIDs are used to treat SLE but are hepatotoxic, and their effects can mimic those of chronic active hepatitis (274 ,275). Perihepatitis has been reported as well (276 ,277). Minocycline use has been reported to be associated with drug-induced lupus and autoimmune hepatitis in about 12 cases. An additional case of minocycline-induced SLE was described in Angulo et al. after taking the drug for 1 year for acne. A 22-year previously healthy woman developed arthritis, elevated liver function tests, and a positive ANA, anti–smooth-muscle antibody, and an antihistamine antibody (278). The hepatitis resolved after stopping the minocycline. The arthritis resolved after taking tapering doses of prednisone over 18 months.

Table 42-2 presents a summary of SLE and the liver.

Biliary Abnormalities: Cholecystitis, Cholangitis, and Biliary Cirrhosis

Gallbladder disease is no more common in patients with SLE than it is in the general population. Cholecystitis and serositis can be difficult to tell apart (279). Cystic duct artery vasculitis commonly is seen in polyarteritis, but only a few reports have noted this in SLE (280 ,281 ,282). Kamimura et al. reported a case of acalculous cholecystitis in a SLE

patient with abdominal pain and fever (283). Examination revealed positive Murphy's sign and pericholecystic edema with no gallstones on imaging. Treatment with corticosteroids resulted in rapid improvement of her symptoms and resolution of the pericholecystic edema in 5 days. The authors report that there were six previous cases of acalculous cholecystitis reported in SLE patients. The authors recommend surgical treatment if there is gall bladder distension on radiographs.

Four cases of sclerosing cholangitis and SLE have appeared (284 ,285 ,286).

Primary biliary cirrhosis (PBC) is associated with females who have features that are consistent with the calcinosis cutis, Raynaud phenomenon, esophageal motility disorder, sclerodactyly, and telangiectasia (CREST) syndrome and antimitochondrial antibodies. Only ten patients with PBC who developed SLE later have been reported in the past (287 ,288 ,289 ,290 ,291 ,292 ,293 ,294). In one of the patients recently reported, the diagnosis of SLE antedated the diagnosis of PBC, which has not occurred in the previous cases.

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Chapter 43

Systemic Lupus Erythematosus in Childhood and Adolescence

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Children and adolescents with systemic lupus erythematosus (SLE) represent both a special challenge and a special opportunity. Early onset allows us to observe the natural history of SLE and investigate potential etiologies, free from the confounding factors that frequently are present in older patients (1). The impact of SLE on children and adolescents, however, often is profound. Recognition of the special considerations that relate to ongoing physical and emotional growth directly influences the choice of medications and the likelihood of success. Satisfactory outcome for a child or adolescent with SLE is not 5- or 10-year survival, into the earliest years of adulthood, but 50- or 60-year survival, which more closely approximates the normal human lifespan. Every aspect of the medical care for a child or adolescent with SLE must take this into account.

Awareness of the complex interaction between the child's illness and the needs of his or her family is critical to successful care. Children and adolescents are emotionally immature individuals just beginning to formulate their concept of self. They are extremely vulnerable to the psychological impact of both chronic illness and medications that dramatically alter their appearance (Fig. 43-1). Family and peer-group pressures, which are difficult even for normal children, may be overwhelming for the child or adolescent with SLE. The interaction of these special needs with the complexities of SLE makes caring for children and adolescents with SLE a unique process. Optimal results require excellent medical care coupled with multidisciplinary patient and family education and support. Compliance is one of the most profound determinants of outcome for children with SLE. It should not be taken as a given that the child and family will comply. Without extensive educational efforts it is more likely that they will not comply and this may have a significant negative impact on the long-term prognosis.

Childhood-onset SLE often is described as more severe than adult-onset disease (2), and early age of onset has been correlated with a worse prognosis (3). Other studies, however, have suggested an improved prognosis for children and adolescents with SLE (4 ,5). In published series, a large proportion of both children and adolescents with SLE have significant renal or central nervous system (CNS) involvement (6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14), but many cases of mild SLE in children and adolescents probably go unreported, either because they are not recognized or they never warrant referral to specialized centers. Delayed diagnosis, because physicians failed to consider SLE in the differential diagnosis of a young or male child, is one of the greatest risks to children and adolescents with SLE. Severe damage to the evolving self-image and sense of worth is another complication of SLE that is unique to children and adolescents. Both of these problems may profoundly affect the prognosis.

English-language reports of children with SLE appeared as early as 1892 (15). Sequeira and Balean (16), writing from the London Hospital in 1902, noted that the disease commences early in life in a much larger proportion of cases than is commonly believed. Eight children with SLE were among 71 cases they reported. Further reports of SLE in childhood continued to appear throughout the 1920s, 1930s, and 1940s (17 ,18 ,19 ,20 ,21 ,22).

The modern era began in the 1950s and 1960s, when series of children with SLE began to be published. Zetterstrom and Berglund (23) described 10 patients in 1956, and Gribetz and Henley (24) described an additional 15 in 1959. Between 1960 and 1968, more than 150 additional children with SLE were described (2 ,8 ,12 ,25). The total number of children with SLE in published series now far exceeds 500 (5 ,6 ,7 ,9 ,11 ,13 ,14 ,26 ,27 ,28).

In the presteroid era, childhood-onset SLE was a rapidly evolving, and usually fatal, multisystem disease. Since the 1960s, however, corticosteroids and improved pediatric care have resulted in greatly enhanced survival (4 ,29). SLE now is a common diagnosis in every large pediatric rheumatology program. Systematic management and vigorous treatment protocols are rapidly improving the outlook for children with even the most severe disease (30 ,31 ,32). With proper care, most children and adolescents with SLE now have an excellent prognosis.

Epidemiology

Despite the many published cases of childhood SLE, its true incidence and prevalence are unknown (33). Fessel's 1973 survey of 126,000 members of the Kaiser Permanente

plan did not report any patients with onset of SLE before 15 years of age (34). Siegel and Lee (35) estimated the annual incidence of SLE in childhood to be 0.6 per 100,000. Hochberg (36) found a similar incidence in Baltimore. Studies coordinating multiple regional pediatric rheumatology centers in New England and in Canada found a similar annual incidence of approximately 0.4 per 100,000 (36 ,37 ,38). The Canadian study (38) was complicated by wide differences in the incidence of SLE in the data from various reporting institutions. Every effort to provide an accurate determination of the incidence of SLE has been complicated by the recognized variations in racial incidence and geographic referral patterns. Although better numbers have been provided by countries where every child with a specific diagnosis is reported to a central registry, these countries have lacked the ethnic diversity that is found in larger countries, making their data inapplicable. Efforts to develop a central registry for children with SLE to provide demographically more diverse information are underway.

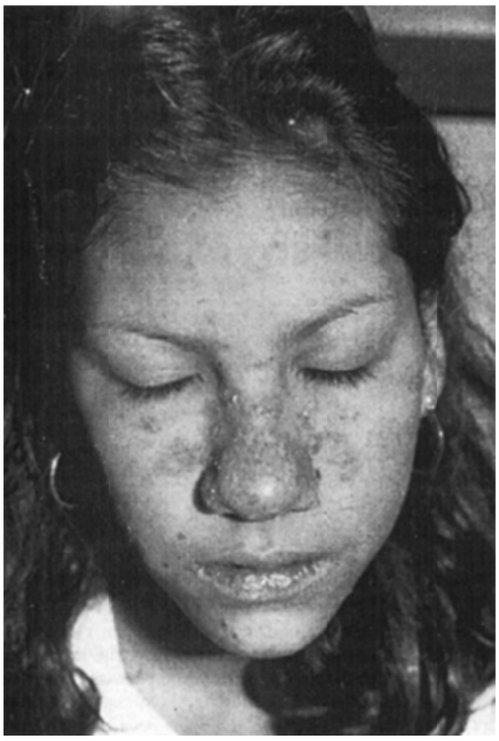

Figure 43-1. Markedly altered facial appearance resulting from skin manifestations in a teenage female with systemic lupus erythematosus.

Because of the difficulties in assessing the true incidence of SLE, the prevalence of childhood SLE can only be estimated. Figures from the regional referral center in Los Angeles suggest there are between 5,000 and 10,000 children with SLE in the United States (5). A similar estimate arises from the assumption that children with SLE are 0.10 as common as children with juvenile rheumatoid arthritis (JRA) (33). (The corresponding prevalence of SLE is 5 to 10 per 100,000 children (39 ,40 ,41 ,42), again suggesting 5,000 to 10,000 children with SLE in the United States.) Unfortunately, these figures represent only a first approximation of the true prevalence of SLE in children and adolescents. The influences of sex and racial origin on the occurrence and manifestations of SLE are widely recognized (5 ,35 ,43 ,44). In childhood, the influence of race is striking. The age and sex-adjusted prevalence of SLE in African American, Asian, and Hispanic children were more than threefold that of white children at one large center (5). Female sex, age, and race were even more striking influences when the incidence of SLE among pre- and postpubertal children was considered. For all male children, the frequency of SLE rose from 1 per 100,000 male children aged 1 through 9 years to 1.61 per 100,000 male children aged 10 through 19 years (i.e., a 60% increase with puberty). For white females, the increase with puberty was from 1.27 to 4.40 per 100,000 (246%); for African-American females, 3.72 to 19.86 per 100,000 (434%); and for Asian females, 6.16 to 31.14 per 100,000 (406%). In contrast, the increase for Hispanic females was only 4.62 to 13.00 per 100,000 (181%) (5). Although based on a limited sample, these data suggest a marked variation in the influence of sex hormones and puberty on the predisposition to SLE among different races.

Causative Factors

The cause of SLE remains unknown. The availability of parents and siblings who live in the same household as children with SLE, however, provides a unique opportunity to evaluate environmental and genetic hypotheses. Many studies have demonstrated an increased frequency of immunologic abnormalities in first-degree relatives of both adults and children with SLE (45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,56 ,57). These studies have been interpreted as evidence both of an infectious cause and of a genetic predisposition. Current evidence suggests that both environmental and genetic factors are important with preliminary data suggesting that Epstein-Barr virus (EBV) infection may have a pivotal role in the susceptible host (1). (These theories are discussed in detail in other chapters.)

Much of the evidence for a genetic predisposition to SLE has come from studies of twin and sibling pairs. Many twin pairs have onset of SLE in childhood or adolescence. Identical twins with the onset of SLE as early as 3 years of age have been reported (58). The percentage of identical twins who are concordant for lupus may be as high as 70 (59), but this figure has been challenged. When both twins develop SLE, they typically have the onset of disease at a similar and earlier age when compared to nonidentical siblings who develop SLE (16.5 + 7.9 vs. 26.2 + 20.5 years) (60).

Additional evidence for a genetic component of SLE has come from the study of immunologic abnormalities in family members of children with SLE. Expression of this genetic component appears to be promoted by female sex hormones. In a study of 34 families having children with SLE, Ro/SSA antibody-positive mothers were more likely than Ro/SSA negative mothers to have Ro/SSA antibody-positive daughters (7/11 vs. 4/18, $P < 0.05$ probands excluded). There was no association between Ro/SSA antibody-positive fathers and sons: however, Ro/SSA antibody-positive fathers had fewer male children than expected (5 sons/12 daughters for Ro/SSA antibody-positive fathers vs. 19 sons/19 daughters for Ro/SSA antibody-negative fathers, $P < 0.05$) (61). The explanation for the decreased number of male offspring remains under investigation.

The frequent occurrence of serologic abnormalities in the families of children with SLE strongly supports the hypothesis that family members of children share genes that predispose to the development of autoimmune disease. Formulated in response to the occurrence of multiple autoimmune diseases within some families (62 ,63), the “supergene” hypothesis proposes the existence of a single gene or group of genes that predisposes to autoimmune disease. The subsequent occurrence of autoimmune disease in carriers is determined both by the interaction of these predisposing factors with the remainder of the genome and by environmental events. In such children the development of autoantibodies may precede the development of clinically evident disease by decades (64). This finding makes the routine reassurance of the family of a child with a significant antinuclear antibody titer, but no clinical findings, difficult. Additional, new evidence suggests that interferon-regulated genes may play a key role in the development of active SLE, but their full significance has not yet been elucidated (65 ,66). The increased frequency and amount of antibodies to Ro/SSA in mothers of children with SLE may be evidence of altered immunoregulatory gene expression in otherwise well individuals (51). Ro/SSA antibody expression is linked to histocompatibility antigens in patients with SLE and Sjögren syndrome (67).

Antiphospholipid antibodies are an additional immunologic abnormality, which occurs with increased frequency in children with SLE and in their family members (68 ,69 ,70). Because the antiphospholipid syndrome often appears to be independent of SLE, the explanation for this association is unclear. The report of Mujic et al. (71) describing an adolescent with evidence of antiphospholipid antibodies at age 16 who did not manifest SLE until 17 years later is a reminder that SLE may evolve over an extended period of time.

An increased frequency of SLE in childhood occurs in children with defects of the immune system. This is especially true of genetic defects in the complement system (most often C2 or C4 deficiency) (60 ,72 ,73 ,74 ,80). Such an association may occur because the genes controlling C4 synthesis are closely linked to the human leukocyte antigen (HLA) histocompatibility complex, or because the complement defect directly increases the likelihood of autoimmune disease (75 ,76 ,77 ,78 ,79 ,80 ,81 ,82). HLA linkages in this region have been demonstrated for adults with SLE, but they have not been independently evaluated in children (83 ,84). A Turkish family has been reported in which homozygous C1q deficiency was associated with the development of SLE in one sibling and IgA nephropathy in another (85). Interestingly a common pattern of hypergammaglobulinemia, cytopenias, and rash, which may be associated with other findings suggestive of SLE, has been described in a group of individuals with CD95 (Fas/APO- 1) mutations (86).

IgA deficiency is another defect of immune function that occurs more frequently than expected among children with SLE (6 ,87 ,88). In one study (87), IgA deficiency was found in only 0.03% of the normal population but in 4.6% of children with SLE. The association of SLE in childhood with defects in the immune system strongly suggests that defective antigen processing predisposes to the development of SLE. The development of discoid lupus (and in one case, SLE in children with chronic granulomatous disease and their mothers) is another observation suggesting an association between defective antigen processing and the development of SLE (79 ,89 ,90).

Despite the associations between defective immunoglobulin and complement function and SLE, treatment with intravenous immunoglobulin therapy has not been of benefit. Most patients with SLE are hypergammaglobulinemic, even if IgA-deficient. Further, because IgA is a secretory immunoglobulin, intravenous immunoglobulin therapy does not restore effective mucosal immunity, and administering intravenous immunoglobulin to IgA-deficient individuals may be associated with dangerous allergic reactions.

Children and adolescents with SLE are a valuable resource for large-scale efforts to understand the genetics and molecular biology of this disease. The opportunity to study SLE in populations such as young white males (who lack the known predisposing factors) may facilitate the identification of new risk factors. Each risk factor is a piece in the puzzle that ultimately will lead to understanding the pathogenesis of SLE (51).

Clinical Manifestations

Unexplained fever, malaise, and weight loss are the most common manifestations of SLE in children and adolescents. However, these manifestations are nonspecific and are often underreported by physicians in specialized rheumatic disease clinics. Because these nonspecific symptoms may be associated with many chronic illnesses, the physician should actively seek evidence of arthritis or a photosensitive rash, hematuria or proteinuria, hypergammaglobulinemia, and hypocomplementemia. Any of these findings should prompt consideration of SLE, but one cannot rely on their presence. On initial evaluation, the patient and family often do not describe findings such as arthritis of the small joints of the hands, alopecia, or photosensitivity. Unless they are specifically questioned, they do not relate these findings to the primary complaint. The reported

frequency of many complaints varies widely among series of children with SLE, and this variation reflects not only selection and referral criteria but also the care with which the complaint was sought and validated by the investigators (Table 43-1) (91).

Table 43-1: Clinical Manifestations of Systemic Lupus Erythematosus in Children and Adults*

	Cases (n)		
Parameter	Cassidy et al. (6)	King et al. (12)	Pistiner** et al.
Renal involvement	86	61	28
Hypertension	28	—	25
Musculoskeletal findings	76	79	91
Cutaneous	76	70	55
Photosensitivity	16	—	37
Hair loss	20	—	31
Oral, nasal ulceration	16	—	19
Cardiac involvement	47	17	12
Pulmonary involvement	36	19	12
CNS involvement	31	13	11
Anemia	47	—	30
Leukopenia	71	—	51
Thrombocytopenia	24	—	16

*The findings from Cassidy et al. and King et al. represent two large pediatric series; those from Pistiner et al. represent a large adult series.

**From Pistiner M, Wallace DJ, Nessim S, et al;. Lupus erythematosus in the 1980s: a survey of 570 patients. *Semin Arthritis Rheum* 1991;21:55–64.

Most children with SLE present with chronic illness, but some children and adolescents with SLE are acutely ill at presentation. These children may present with seizures, psychosis, uremia, profound anemia, pulmonary hemorrhage, or sepsis as the initial manifestation (92). Often, the diagnosis of SLE is not considered until the clinicians note that the child is not recovering as expected despite adequate therapy for the presenting event.

Confirming the diagnosis of SLE in children and adolescents is based on criteria developed by the American Rheumatism Association (ARA) for use in adults (93). Classification as definite SLE is based on the fulfillment of four criteria, but the diagnosis should not be discarded automatically in children who meet only three. Although the ARA criteria are useful guidelines, fulfillment of four criteria does not exclude other diagnoses; likewise, failure to fulfill four does not exclude SLE. Antinuclear antibody (ANA) testing is useful, but a positive test is not sufficient for the diagnosis of SLE in childhood. Correlation of this information with the titer of antibody and the age of the child improves the predictive value of a positive test (94). ANA-positive children who fulfill at least one other criterion should be periodically re-evaluated. Definite SLE may manifest decades after the initial presentation (64 ,95).

Renal Disease

Renal disease is evident in approximately two thirds of children and adolescents with SLE (2 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,96). Renal manifestations range from mild glomerulitis with a normal urine sediment to sudden renal failure (92 ,97). The most common signs of renal involvement are hematuria, proteinuria, and hypertension. Although children and their families may complain of malaise, headache, swollen feet, and/or swollen eyelids (if nephrotic syndrome is present), the signs of renal involvement commonly are silent in childhood.

Renal biopsy of children and adolescents with SLE without regard to clinical manifestations demonstrates varying degrees of renal involvement in nearly every case (97 ,99). Although most children with a normal urine sediment have only mild glomerulitis, diffuse proliferative glomerulonephritis (DPGN) may be present. The significance of silent DPGN is uncertain. Series reporting follow-up of silent nephritis in SLE have described a benign prognosis (97 ,98 ,99), and this makes proper interpretation of such biopsies and the importance of detecting silent DPGN uncertain. Most investigators agree that renal biopsy may be deferred if the creatinine clearance and urinalysis are normal. However, renal biopsy should be performed whenever necessary to confirm the diagnosis, to investigate unexplained changes in renal function, and when considering or monitoring the effects of aggressive therapy (31 ,100).

Renal involvement in childhood SLE is categorized according to the World Health Organization (WHO) criteria (101).

Mild glomerulitis is the most benign form, followed by focal segmental glomerulonephritis and membranous glomerulonephritis (102). DPGN carries the greatest risk of chronic renal failure. DPGN was the most frequent abnormality in children who underwent biopsy because of abnormal urine sediment (58), but in a series in which all children with SLE underwent biopsy, only 20% of children had DPGN (4 ,7 ,11 ,13 ,29 ,103). Combining the data from several large series, 42% of children (108 of 256) had DPGN at the time of initial biopsy, 26% had either mild glomerulitis or no abnormality, 25% had focal glomerulitis, and 6% had membranous glomerulonephritis.

Focal glomerulonephritis and membranous glomerulonephritis generally are benign, but either may progress to DPGN, with ultimate renal failure (4 ,10 ,13 ,29 ,101 ,104 ,105 ,106). Repeat renal biopsy should be performed in these patients if renal function continues to deteriorate or they manifest persistent hypocomplementemia. Long-term studies indicate that renal scarring (i.e., chronicity index) is a better predictor of ultimate outcome than the WHO classification (101 ,106 ,107). In the absence of scarring, active disease (including glomerular crescents) is not automatically associated with a poor prognosis (105 ,106); however, good outcome for these children is contingent on aggressive management of their renal disease to prevent the development of scarring (discussed later). Most children with SLE do not develop renal disease after the first 2 years following diagnosis (2 ,7), but one third of those who ultimately do develop significant renal disease lack evidence of renal involvement at presentation.

The sudden onset of renal failure in a child with SLE may result from active nephritis (92), but alternative explanations must be appropriately excluded. Renal vein thrombosis and renal artery thrombosis are other causes of sudden renal deterioration in children with SLE, and both are more frequent in association with anticardiolipin antibodies (108 ,109). Drugs and self- or family-administered health food supplements that interfere with glomerular filtration or are directly nephrotoxic also must be considered when seeking the cause of sudden onset renal failure. A mild rise in the blood urea nitrogen level (BUN) usually follows the initiation of acetylsalicylic acid or other nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with renal involvement, but some children with SLE are unusually sensitive to their effects. An unexpectedly sharp rise in the BUN following initiation of NSAIDs should prompt further investigation for previously unsuspected renal involvement.

Mild clinical manifestations of renal involvement usually are well controlled with corticosteroids and diuretics. Persistent renal disease may require immunosuppressive therapy (116). Chronic glomerular scarring is prevented by cyclophosphamide over the intermediate term (31 ,111 ,112). The major concern of the physician caring for a child with lupus nephritis is preserving sufficient renal function to support normal growth and development. For adolescent females, this includes the preservation of adequate renal function to support pregnancy. These concerns dictate intervention before significant renal compromise has occurred. Physicians who normally care for adults must be reminded that the normal serum creatinine level of children is much lower. Levels of serum creatinine elevation that might represent minimal impairment in an adult may indicate severe renal compromise in a child.

Current treatment regimens for children and adolescents with SLE have led to a steady improvement in 5- and 10-year renal function survival (30). It is not yet clear, however, whether these improvements result in significantly enhanced survival 20 and 30 years following diagnosis. Maintaining adequate renal function is important for children and adolescents with SLE. In contrast to adults, they do poorly on long-term dialysis (101). Children with SLE coming to dialysis with active disease often die of sepsis or other complications within the first year. However, those who have gradually developed global glomerular sclerosis often do well with dialysis and subsequent renal transplantation.

Children whose proteinuria and hematuria improve with corticosteroid therapy but whose creatinine clearance slowly deteriorates are of particular concern. Often, these children do well over a 5-year period but progress to renal failure between 5 and 10 years following diagnosis. Routine monitoring of creatinine clearance and, if deterioration is evident, early intervention, are important. In the event of chronic deterioration, the clinician should intervene aggressively while adequate function can still be preserved. Adult series suggest that maintaining a creatinine clearance of 70 mL/min per 1.75 m^2 is adequate (111 ,113), but intervention at this point may not preserve sufficient renal function for the satisfactory growth and development of children and adolescents.

Optimal therapy for children and adolescents with lupus nephritis remains uncertain. In large part, this results from the failure of many investigators to properly stratify the patients in their studies (102). The systematic use of intermittent intravenous cyclophosphamide has been successful in children with DPGN and useful for children with membranous glomerulonephritis (31 ,116 ,117). Others have reported excellent results with the combined use of large doses of prednisone and, when necessary, azathioprine (9 ,29 ,114). When 10-year renal survival is considered, the systematic use of intravenous cyclophosphamide appears to offer the best outcome (117) Individual physicians who proclaim excellent results in their institution with other regimens rarely have prospective data to support their claims. Reservation of routine use of cyclophosphamide only for those with well established severe disease is generally associated with a poor outcome (118). The consistent failure of reporting centers to stratify patients according to age, race, sex, and severity of disease despite the fact that these factors are all recognized to impact survival and the varied populations served by various centers make comparative analysis difficult.

At present, a large-scale study of staging criteria for children with SLE is underway (102). Use of these criteria should improve our ability to assess the various therapeutic regimens that have been advocated for children with lupus nephritis. Routine use of intravenous cyclophosphamide has

many advantages, including accurate assessment of patient compliance and clinical status at each dosage interval. Poor compliance is a major determinant of poor outcome (115). Additionally, periodic inpatient cyclophosphamide therapy allows the physician to monitor renal function status and clinical status before each immunosuppressive drug dosage, thus minimizing complications. New regimens for the treatment of SLE continue to appear on the horizon. Although consistent use of high dose mycophenolate mofetil has been recommended for control of lupus nephritis in adults (119), its sustained benefit is unproven. Rituximab is a new biologic agent directed against activated B cells bearing the lymphocyte marker CD20. There are several case reports published regarding its use in SLE and several small series have been presented (120 ,121 ,122). The ultimate utility of rituximab seems likely, but further evaluation is required. Other agents that block or eliminate activated B cells (such as BLyS antagonists) are also being evaluated. New regimens utilizing a combination of “conventional” agents and the newer biologic agents may hold the greatest promise.

All of the recommended regimens for treatment of lupus nephritis fail in some patients. Continued efforts to improve care are necessary for these children and adolescents, who may relentlessly progress to renal failure and, often, death. Systematic therapy modeled on the experience of pediatric oncologists may hold the key to enhanced survival for children and adolescents with severe lupus nephritis (116 ,123). Evolution of current therapeutic regimens to include multiple agents that are given at fixed intervals to induce a remission of disease, followed by prolonged maintenance therapy, seems to provide the best long-term outcome.

Low-dose oral methotrexate has been an effective adjunctive therapy in a few children (124), but it has not proven to be successful when used in isolation. Low-dose oral methotrexate may be a beneficial addition to immunosuppressive regimens to provide improved maintenance after the induction phase (e.g., children who have reached 3-month dosage intervals on cyclophosphamide). Higher dosages of intravenous methotrexate have been used successfully in protocols for the treatment of children who have failed conventional immunosuppressive therapy (125).

Autologous stem-cell transplantation has been proposed and utilized for a variety of autoimmune diseases including some children with SLE (126 ,127 ,128 ,129). This technique may hold great promise, but this technique is associated with a significant mortality and the majority of the reported responses have not persisted over time. Whether the beneficial effect results from the stem-cell transplantation or is from the immunosuppressive chemotherapy given at the time of stem-cell transplant is under active investigation.

Central Nervous System Manifestations

Psychosis, sudden personality change, seizures, chorea, transverse myelitis, peripheral neuropathy, and pseudotumor cerebri all may be presenting manifestations of SLE in childhood (130 ,131 ,132 ,133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142). Most series have reported CNS involvement in 20 to 30 of children (2 ,6 ,11 ,13 ,29). If carefully sought, mild evidence of CNS involvement is present in up to 45 of children and adolescents (23 ,141). In every instance appropriate investigation should be undertaken to exclude stroke as the etiology of sudden CNS changes, even in the patient who is not known to be anticardiolipin antibody positive.

Subtle CNS changes, including impaired judgment and poor short-term memory, are the most common CNS manifestations of SLE (132 ,137 ,143 ,144). These alterations often are ascribed to steroid therapy or situational stress, but they occur with greater frequency in SLE than in other chronic childhood rheumatic diseases that require similar corticosteroid therapy. Adolescents with SLE often have difficulty complying with their medications or appointments, and they often alienate friends and family in ways that are inconsistent with their prior behavior. Physicians must be acutely aware of these changes, because they may have disastrous consequences. A trial of increased corticosteroids may be beneficial in children with SLE whose behavior has become erratic or uncharacteristic, even in the absence of objective findings. Others have argued for reducing the corticosteroids in such circumstances, but this rarely is effective.

Delirium, hallucinations, seizures, and coma are the most common objective neurologic signs in childhood. Psychosis that is unrelated to corticosteroids typically occurs in 4 to 10 of children (2 ,7 ,13 ,28 ,29 ,141). Caeiro et al. (6) reported significant neuropsychiatric findings in 30 of children in an English series. The reported frequency of neuropsychiatric manifestations in children and adolescents with SLE is lower than that in adults (145). This may be a true finding, but it more likely represents a decreased appreciation of neuropsychiatric involvement in childhood.

Chorea is more frequent in children than in adults with SLE (145). Although it is infrequent, it has been documented as being the initial manifestation of childhood SLE in multiple reports (135 ,140 ,146 ,147 ,148), perhaps because it is such a striking finding. Of children with SLE, 4% to 10% are affected by chorea at some point (2 ,7 ,23 ,28 ,29 ,141). This increased incidence may reflect an increased sensitivity of the basal ganglia to damage by autoreactive antibodies or vascular events accompanying SLE in childhood (149).

Most often, acute CNS involvement occurs early in the natural history of childhood SLE (150). Frequently, it first becomes evident during, or worsens immediately after, initiation of corticosteroid therapy. The explanation for this is uncertain, but these symptoms frequently resolve with pulse methylprednisolone therapy. Late-onset CNS involvement more often results from stroke, uremia, or an infectious process (151).

Both sudden onset of optic neuritis and acute sensorineural hearing loss may occur in children with SLE (152 ,153). However, the most striking CNS damage in children and adolescents with SLE typically results from seizures or strokes, including cerebral vein thrombosis. These complications may occur in the presence or absence of anticardiolipin antibodies (154 ,155 ,156).

Cognitive defects and aberrant behavior present a more difficult management problem. Aberrant behavior may have dramatic effects on social acceptance, grades, and compliance, thereby directly affecting both self-image and long-term prognosis. Efforts to ascribe behavioral change to a single cause rarely are successful (132 ,137 ,143). Unfortunately this often results in failure to aggressive treat these problems with resultant progression.

Nonspecific problems in children with diffuse CNS involvement most likely represent the combined effects of SLE, situational factors, and corticosteroid therapy. When such symptoms are present, increasing the corticosteroid dosage more often is successful than a dramatic reduction.

No single objective test for the presence of CNS-SLE is accurate in childhood. Computed tomography (CT) of children and adolescents with SLE who have received long-term corticosteroids commonly demonstrates diffuse cortical atrophy (157 ,158 ,159). Alterations in cerebrospinal fluid protein or sugar levels or cell count cannot be relied on (132 ,141 ,150), but these studies often are necessary to exclude infection and other explanations for altered CNS function (151). Single-photon emission CT may be a more sensitive test for cerebral perfusion abnormalities in these children (160), but other studies suggest that magnetic resonance imaging (MRI) is more sensitive (161). Antibodies to ribosomal P have been found to correlate with CNS manifestations of SLE in adults, but their presence correlates less reliably with CNS disease in children and adolescents (162 ,163).

Treatment of CNS manifestations in children and adolescents with SLE is a challenge. Because the manifestations may result from corticosteroid therapy, physicians frequently hesitate to increase the dosage; nonetheless, this often is the most effective therapy. For severe CNS manifestations, pulse methylprednisolone therapy often is effective. When other measures fail, intravenous cyclophosphamide frequently is beneficial. Children with short-term psychosis or coma often respond to therapy, but when significant impairment has been present for long periods, the prognosis is guarded. It is important to respond aggressively when faced with continuing evidence of CNS deterioration. Chronic “mild” problems for which intervention is not felt to be warranted often progress to dementia over time.

Psychosocial Concerns

Psychologic reactions that relate to the many issues affecting children and adolescents with SLE often are confusing. Children with SLE commonly demonstrate an impaired quality of life that is impacted by the activity of their disease (164 ,165). Adolescents who are afflicted with chronic disease are caught between their need to establish an independent personality and the dependency of the sick role. Just as they are struggling to assert their independence, they must be taken for doctor's visits, forced to undergo examinations and blood tests, and required to take unpleasant medications. This situation is intensified by the almost universal need for dosages of corticosteroids that increase acne and produce obvious cushingoid facies. It is the unusual adolescent who does not rebel under these circumstances. This rebellion may take the form of noncompliance with scheduled physician visits, overt or covert medication noncompliance, or familial disruption. The physician who expects the adolescent with SLE to act like an adult should expect an unsatisfactory patient-physician relationship that often results in a poor outcome.

Anger frequently is the adolescent's predominant response to his or her situation. It is important to remember that there is no well-defined target for this anger. The adolescent obviously is angry about having SLE, but the disease has no direct embodiment. The physician, the medications, and the required examinations, however, all are direct manifestations of the disease and thus easy targets for the adolescent's rage. This may be expressed overtly by refusing to cooperate, but it is more difficult to deal with when covert and unrecognized.

There is no single, successful method for dealing with adolescent rebellion in the setting of chronic illness. Because adolescents frequently believe that important information is being kept from them, it is important to emphasize honesty, trust, and integrity. The physician cannot demand these from the adolescent patient without promising to provide them in return. Often, the situation is handled best by excusing the family from the room, because there may be many issues that the adolescent is afraid to voice in front of parents or siblings. It often is useful to ask the patient directly what the physician or family has done to provoke the behavior. Frequently, it takes only a few minutes of conversation to elicit a recognition that the anger is primarily over being ill. Dealing with this honestly and directly is a key step in developing a healthy patient-physician-family relationship.

For some children, no amount of discussion and reassurance is sufficient. Often, this is in response to unspoken fears or needs in the family of which the physician may not be aware. In these circumstances, it is best to recommend family counseling. Individual counseling of the adolescent furthers their feeling of having been singled out and often is counterproductive (unless it develops out of initial family-centered care). Situations where both honest discussion and family counseling fail, are unusual. When they do occur, it is important to determine whether the adolescent behavior may be a manifestation of unrecognized cerebritis, for which increased medication may be required. If a satisfactory patient-family-physician relationship cannot be established despite every possible effort, then referral to another physician or center may be indicated. Because this forces the adolescent and family to reevaluate their conduct and initiate new relationships, it may be beneficial even when no additional steps are taken.

Pulmonary Manifestations

Pleurisy and pleural effusions are the most common pulmonary manifestations (166 ,167). Severe manifestations, including pneumothorax, pneumonia, chronic restrictive

lung disease, pulmonary hypertension, and acute pulmonary hemorrhage, may occur (166 ,167 ,168 ,169 ,170 ,171 ,172 ,173 ,174). Pleuritic chest pain, pleural effusions, and chronic interstitial infiltrates affect from 10% to 30% of children with SLE (2 ,6 ,11 ,13 ,14 ,28 ,29). When a series of Canadian children with SLE was reviewed for manifestations of respiratory involvement, 17 of 24 patients (77%) had evidence of pulmonary involvement (166).

Chronic pulmonary involvement may result in slowly progressive diaphragmatic dysfunction and restrictive lung disease. These changes appear as malaise and dyspnea on exertion (166 ,168 ,172 ,175 ,176). Diaphragmatic dysfunction may contribute to frequent infection (166 ,168). Diaphragmatic involvement also may be more common than previously recognized. Changes ranging from wide variation in fiber size to calcinosis were common in autopsy specimens of children dying from SLE (169). It is useful to note both pulse rate and respirations as part of the routine examination. Gradual increases in either or both parameters recorded over time may be the earliest clue to developing cardiac or pulmonary dysfunction, which are not clinically evident to either the patient or physician.

Significant restrictive lung disease may be present in children with normal chest radiographs. A study of 15 children with SLE by Trapani et al. found pulmonary involvement in 6 who were without pulmonary symptoms (177). Children with dyspnea or tachypnea at rest should be monitored with periodic pulmonary function testing. As the ability to ameliorate renal and CNS manifestations of SLE in children and adolescents improves, chronic pulmonary involvement is becoming an increasing concern.

The most common fatal complication of pulmonary involvement in children and adolescents with SLE is pneumonia (170). Pneumonia was the primary cause of death for 9 of 26 children with SLE coming to autopsy in one reported series; pulmonary hemorrhage contributed to the death of 5 others. In contrast, renal failure and CNS involvement were the primary causes of death in only 4 and 3 children, respectively.

Pulmonary hypertension, denoted by accentuation of the second heart sound, is an ominous finding in children and adolescents with SLE. Once established, it progresses steadily to right-sided heart failure and death (168). Pulmonary hemorrhage may occur in the setting of preexisting pulmonary hypertension or in isolation (168 ,171 ,172 ,175). Sudden, unexplained pallor and tachypnea often indicate the onset of pulmonary hemorrhage (162), which if left untreated is rapidly fatal.

Minor manifestations of pulmonary involvement normally respond to corticosteroids (166 ,167 ,178). Deaths from pneumonia, in which *Escherichia coli, Klebsiella sp.,* or *Staphylococcus aureus* were the predominant organisms, illustrate the need for broad-spectrum antibiotic coverage (170). *Pneumocystis carinii* and other nonbacterial organisms may be present (179). When pneumonia is superimposed on active pulmonary SLE, the contributions of infection and active SLE cannot be differentiated with certainty. Both antibiotics and increased doses of corticosteroids may be appropriate.

Children with pulmonary hypertension may benefit symptomatically from the addition of calcium channel blocking agents to reduce pulmonary vascular resistance. No therapy is known to reverse the course of this complication. Cytotoxic drugs have been ineffective, except in rare anecdotal reports. Pneumonia is a frequent complication in children with established pulmonary hypertension and may progress rapidly to sepsis. Massive pulmonary hemorrhage may respond to large doses of corticosteroids with ventilator support and, perhaps, plasmapheresis or extracorporeal membrane oxygenation (171 ,179).

Musculoskeletal Manifestations

The arthritis of SLE generally is nondeforming and responds well to anti-inflammatory medications. Significant arthritis at presentation is found in 40% to 60% of children and adolescents with SLE, and it occurs in over 80% of children with SLE at some point (2 ,6 ,7 ,11 ,13 ,14 ,29 ,41). Usually, the arthritis affects the small joints of the hands and feet, with swelling and pain on motion. Asymptomatic knee effusions frequently are present in children with active disease who may not have arthritis elsewhere.

Rarely, children with well-documented JRA and erosive changes develop definite SLE (180). These children appear to have two independent diseases. Although this is an infrequent occurrence, the greater than expected frequency of this overlap suggests that SLE and JRA share some common genetic predisposition.

Avascular necrosis is the most significant musculoskeletal complication of SLE in children and adolescents, and it may result from SLE alone, corticosteroid therapy, or their interaction. A cross-sectional radiographic study of 35 children with SLE found evidence of avascular necrosis in 40% (181). However, these children were drawn from a program that routinely uses high-dose corticosteroids (i.e., 2 mg/kg/day). The frequency of avascular necrosis in a general population of children and adolescents with SLE is unknown.

Avascular necrosis usually affects the hips and knees of children with SLE. Children report gradual onset of progressive discomfort in the affected joints, and the initial evaluation may prove negative. MRI and, later, routine radiography ultimately reveal evidence of osteonecrosis. Although no clear association of avascular necrosis with the total dosage of corticosteroids or their mode of administration has been found, the incidence of avascular necrosis is far higher in children who have received corticosteroids for prolonged periods (181 ,182).

Meaningful muscle involvement is rare in children with SLE. Diffuse weakness may be the result of steroid myopathy (183). Mild elevations of serum creatinine phosphokinase levels often are seen but rarely are associated with clinical weakness. Antibodies to the acetylcholinesterase receptor may produce a myasthenia gravis–like picture, and transplacental passage of antibodies to this receptor is

reported to have caused weakness in the child of a mother with SLE (184). Dermatomyositis, which may be associated with a positive ANA, arthritis, a heliotropic rash, and significant proximal muscle weakness, must be excluded if significant weakness is present. The presence of antibodies to double-stranded DNA (dsDNA) does not automatically exclude the diagnosis of dermatomyositis, but hypocomplementemia is not expected in children with this condition.

Dermatologic Manifestations

Rashes occur frequently in children with SLE (2 ,6 ,7 ,11 ,13 ,14 ,28 ,29), but only 30% to 50% ever manifest the typical butterfly rash (11 ,28) (Fig. 43-2). Vasculitic involvement of the hard palate frequently accompanies the facial rash of SLE, and these lesions are a useful confirmatory sign if the cause of the facial rash is in question. Cutaneous lesions may take the form of recurrent urticaria, bullae, vasculitic nodules, or chronic ulceration. Vasculitic lesions frequently are a manifestation of active disease. Other dermatologic manifestations may wax and wane without exacerbation of systemic disease.

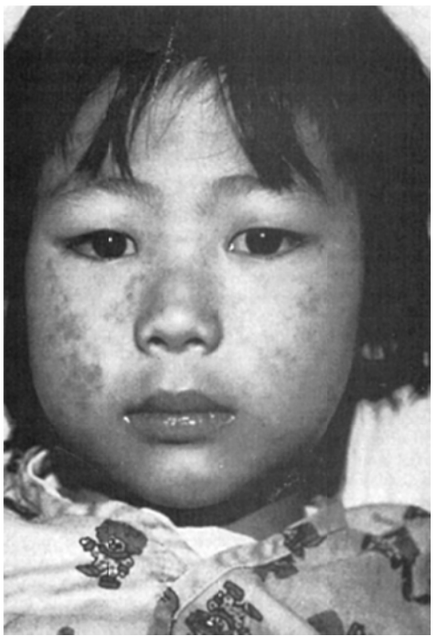

Figure 43-2. Typical bilateral malar rash in a young Asian female with systemic lupus erythematosus.

Bullous lesions resembling bullous pemphigoid are the predominant manifestations of SLE in some children (185). Boys with this manifestation predominate. Often, they have mild systemic disease, and renal involvement is rare. Little information is available about these children in the literature. Dapsone often is helpful but has not been uniformly useful (186).

Dermatologic manifestations usually are not of long-term significance. Most respond to treatment without significant scarring. All the dermatologic lesions of SLE may be aggravated by sun exposure, and children with SLE should be counseled to use sun-blocking agents and to avoid unnecessary sun exposure, which may provoke increased systemic disease activity. Definite photosensitivity occurs in 16% of children (187).

It is important to discuss photosensitivity and its attendant precautions with great sensitivity. Adolescents often resent being told they cannot go to the beach or other all day outdoor activities (e.g., theme parks) with their friends. One must make every effort to accentuate the positive. For example we encourage our patients to participate in these activities in the evening when the risk of significant ultraviolet exposure is less. However, we recommend long sleeves, hats, and sun block at all times. One also must be sure to emphasize the exact nature of the risk. A recent patient suffered severe skin irritation after going to a tanning salon. They professed not to understand that this too was ultraviolet exposure and included in the photosensitivity precautions explained to them.

Discoid lupus erythematosus (DLE) is unusual in childhood. Most children referred for DLE have systemic manifestations when questioned and examined carefully. Some children with DLE progress to SLE, but this is rare (171). Isolated DLE is of concern because of associated disfigurement and psychologic effects. In the past dermatologic lesions of SLE in childhood have been treated primarily with topical corticosteroids. However, with sustained use these have adverse affects on the skin. More recently topical ointments containing tacrolimus or related compounds have been found to be effective. However there may be an increased risk of skin cancer if their use is sustained (188).

Cardiac Manifestations

Cardiac manifestations rarely are prominent in children and adolescents with SLE, but occasionally, they are catastrophic (189). Pericarditis, myocarditis, and mild valvular involvement are common and may not be symptomatic (6 ,7 ,13 ,14 ,190 ,191 ,192 ,193). Clinically evident pericarditis or myocarditis occurs in 10% of children (11 ,13 ,14 ,28), but occasional series report a higher frequency (2 ,7 ,170). Children with SLE may develop cardiac tamponade, but this complication is uncommon (194). Severe and recurrent pericarditis may warrant surgical intervention.

Many children with SLE are anemic and develop flow murmurs. Libman-Sacks endocarditis may occur in childhood, however, and this predisposes to bacterial endocarditis.

In large series of patients with SLE, bacterial endocarditis occurs with a greater-than-expected frequency (170 ,178 ,195). All children with significant valvular lesions must receive antibiotic coverage for dentistry and other invasive procedures; some recommend routine bacterial endocarditis prophylaxis for all patients with SLE.

Circulating lipid abnormalities occur in adolescents and young adults with SLE and may contribute to premature myocardial infarctions and coronary arteritis, and septic thrombosis. These lipid abnormalities are in part related to prolonged corticosteroid therapy (105 ,178 ,196 ,197 ,198 ,199 ,200). Preliminary studies to determine whether statins are safe and effective for children with SLE are underway (201). The association of prolonged corticosteroid therapy with premature myocardial infarction is well documented and raises significant questions about the long-term safety of high-dose corticosteroid regimens.

Gastrointestinal Manifestations

Mild gastrointestinal (GI) involvement is common in children and adolescents with SLE; 30% to 40% manifest hepatomegaly or splenomegaly at diagnosis (7 ,13 ,14). Chronic abdominal pain, anorexia, weight loss, and malaise also are frequent presenting complaints (2 ,7 ,11 ,13 ,14 ,29) that often resolve with corticosteroid therapy. In some cases the onset of the abdominal pain may be acute (202 ,203). Abdominal pain that is unresponsive to corticosteroids may result from small-vessel vasculitis that may not be detected by routine testing (204). These children may respond to a further increase in their corticosteroid dosage (14). Retroperitoneal fibrosis is a rare cause of abdominal pain in children with SLE (205). More often, abdominal pain is the result of pancreatitis that is induced either by SLE, corticosteroids, or both (2 ,13 ,206 ,207 ,208 ,210). Fulminant pancreatitis resulting in death has occurred.

Pneumatosis cystoides intestinalis may be discovered radiographically in patients who have complained of abdominal pain without evident explanation for weeks or months (13 ,209 ,210 ,211). This may be the result of chronic ischemia. Frank bowel ischemia often is found at autopsy (209). Although severe ischemia probably is a terminal event, its frequency suggests that the bowel often is compromised by lesser degrees of vascular insufficiency during life in children with severe SLE. This may be the explanation for some children with unexplained chronic abdominal pain.

Less frequent GI manifestations of SLE include hepatitis and ileitis (2 ,209). Protein-losing enteropathy and marked hyperlipoproteinemia (212 ,213) also have been reported. The relationship of these manifestations to SLE is uncertain. GI irritation secondary to drugs used in treating SLE is frequent, and aspirin-induced hepatotoxicity is particularly common. Severe gastritis and ulcers may occur as well. Additional rare manifestations of SLE in childhood include the simultaneous occurrence of GI inflammation and cystitis (214).

Although infarction of the spleen may produce acute abdominal pain, splenic involvement in SLE usually is asymptomatic. Functional asplenia is a very worrisome complication, because it is associated with increased susceptibility to infection (215). The presence of Howell-Jolly bodies on the peripheral smear should alert clinicians to the possibility of functional asplenia and prompt hospitalization if the child is febrile without adequate explanation.

Infection

Infection is a major cause of both morbidity and mortality for children and adolescents with SLE (2 ,6 ,11 ,13 ,29). Platt et al. (29) documented 55 separate infections occurring in 70 patients over a mean follow-up of 9 years. Sepsis was a contributing cause of death in 25% to 85% of deaths in various series (2 ,6 ,11 ,13 ,29), and it was a cited factor in 35 of 83 deaths (42%) occurring in 374 children collected from six large studies (2 ,6 ,11 ,13 ,29).

The increased frequency of sepsis most likely results from the combined effects of SLE and the drugs that are used to mediate it (216 ,217). The frequency of infection increases with increasing steroid dosage (216). Not only do bacterial infections increase, but opportunistic infections and infections caused by viruses, fungi, and related organisms are more common in children with SLE (29 ,170). The indiscriminate use of immunosuppressive drugs also may contribute to the increased incidence of infection; however, careful use of periodic intravenous cyclophosphamide accompanied by a reduced dosage of corticosteroids often leads to a reduced frequency of infections. In contrast to children taking a prescribed daily dosage of azathioprine or other immunosuppressive agents, children receiving periodic intravenous cyclophosphamide therapy can be intensively screened prior to receiving each dosage of the immunosuppressive agent. For all children with SLE potentially fatal infections, including both bacterial endocarditis and meningitis, occur with a greater-than-expected frequency (151 ,195). Functional asplenia, decreased phagocytosis, poor complement metabolism, and corticosteroid effects all may contribute to this problem.

Hematologic Manifestations

The most common hematologic manifestation of SLE in children and adolescents is anemia. Usually, this is not a Coombs-positive hemolytic anemia with a reticulocytosis; rather, it is a microcytic anemia of chronic disease. Leukopenia and thrombocytopenia are common but not invariably present. Sickle cell anemia is not directly associated with SLE, but it is common in African Americans, who have an increased incidence of SLE. When SLE and sickle cell disease occur together, the similarity of symptoms between the two illnesses may produce confusion. If the physician cannot distinguish the etiology of problems with certainty, he or she may have to treat as appropriate to both conditions.

Children often are seen who have ANAs and thrombocytopenia, which has been labeled idiopathic thrombocytopenic purpura (ITP). A false-positive biologic test result for syphilis or prolonged partial thromboplastin time (PTT) in this setting may suggest SLE. Children with ITP may have antibodies to Sm, Ro, La, or RNP. All children with serologic markers such as these should be carefully followed and periodically re-evaluated for evidence of systemic disease including periodic testing for hypocomplementemia, renal impairment, and proteinuria or hematuria. Some of these children ultimately develop SLE (218 ,219) (Table 43-2). In the absence of other manifestations of SLE, therapy for these children is similar to that for ITP alone.

Menorrhagia may be the presenting feature of SLE in teenage females. Prolonged bleeding or a prolonged PTT resulting from the lupus anticoagulant may be the initial manifestation of SLE in a patient who is being screened for other reasons. However, these findings alone do not establish the diagnosis of SLE. Management of these complications is the same for children and adolescents as for adults.

Anticardiolipin antibodies (aCL) occur in children with SLE with a similar frequency to that of adults (68 ,69 ,220). They are associated with an increased risk of thrombosis and CNS disease (221 ,222). Children with high titer aCL, lupus anticoagulant, and thrombocytopenia may be the group at highest risk of thrombosis (223). Others have suggested that SLE patients with the SA1 anti-DNA idiotype may be at increased risk of vascular complications in the setting of aCL (224). The risk for children with low titer aCL in the absence of lupus anticoagulant appears to be low (225). Low-dose aspirin therapy may be beneficial in reducing the risk. Children have been reported who presented with acute thrombosis and were found to be anticardiolipin antibody-positive but who did not have additional findings to support the diagnosis of SLE (226). Proper categorization of such children is uncertain, because over time, some have gone on to develop SLE (71).

Table 43-2: Incidence of Serologic Antibodies in 92 Children with Systemic Lupus Erythematosus*

Antibody	Incidence (%)	
	Ouchterlony	ELISA
Ro/SSA	16	46
La/SSB	11	17
Sm (RNP)	27	58

*The presence of these antibodies did not correlate with disease activity, except that Ro/SSA antibodies by Ouchterlony were significantly more common in children younger than 10 years of age (11 of 28 vs. 4 of 64, $P < .001$). Children younger than 10 years of age also had a significantly higher mean ELISA titer of Ro/SSA antibodies.
ELISA, enzyme-linked immunosorbent assay.

Laboratory Evaluation

No laboratory feature of SLE in children and adolescents is unique to this age group. For clinicians, the diagnosis of SLE is strongly suggested by the constellation of hypergammaglobulinemia, leukopenia, anemia, and thrombocytopenia. A positive ANA is confirmatory, but none of these findings is essential.

ANAs are present in over 90% of children and adolescents with SLE (6 ,11 ,14 ,192 ,227). Antibodies to various other nuclear and cytoplasmic antigens also are found (50 ,228 ,229 ,230 ,231 ,232 ,233 ,234 ,235). One study that compared the incidence of antibodies to DNA, Sm, and RNP found a lower frequency in children with SLE than in a simultaneously studied population of adult patients with SLE (50). Antibodies to Ro/SSA and La/SSB were found in similar numbers of adult- and childhood-onset patients. These antibodies also are found with increased frequency in the relatives of children with SLE (50). Their presence in asymptomatic relatives has been variously interpreted as being evidence of environmental exposure or genetic predisposition. Recent findings suggest that one may be genetically predisposed to manifest anti-Ro antibodies in response to viral infections such as Epstein-Barr virus suggesting that both environmental exposure and genetic predisposition play an important role in the development of SLE.

Antibodies against dsDNA are both sensitive and specific for active SLE in childhood (228 ,230) but may occur in other conditions (228). Decreased serum levels of the third component of complement (236) correlate well with active SLE in childhood, but neither decreased C3 levels nor antibodies to dsDNA can be relied on as a specific indicator of active renal disease (228 ,237).

Decreased C4 levels often are correlated with decreases in C3, but they may occur in isolation. Decreased C4 levels also frequently are found in the relatives of children with SLE. This may be evidence of the C4 null allele, a manifestation of subclinical disease, or both (30 ,75 ,238 ,239).

Hypergammaglobulinemia frequently is present in children with SLE but also may be found in various chronic inflammatory states (2 ,6 ,11). IgA deficiency occasionally is seen, as is panhypogammaglobulinemia (6 ,87 ,88). Panhy-pogammaglobulinemia is a common complication of cyclophosphamide therapy, but it also occurs in patients with SLE who have not received immunosuppressive agents (88).

False-positive test results for syphilis formerly were found in many children with SLE (2 ,14), but more recent studies report fewer false-positive results (6 ,11). In the United States, it is important to warn the family that positive results may be reported to the public health department. Unwarranted investigation can be halted if questions are referred to the physician. The diagnosis of SLE, however, does not exclude the possibility of treponemal disease. False-positive fluorescent treponemal antibody (FTA) test results may occur because of nonspecific agglutination resulting from hypergammaglobulinemia (240), but FTA-positive individuals in whom the possibility of treponemal

disease cannot be reliably excluded should receive appropriate therapy.

Therapy

NSAIDs provide useful control of the arthritis and musculoskeletal manifestations of SLE in children and adolescents (Table 43-3). Renal function and blood pressure must be monitored because of their known effects on glomerular filtration, but significant undesired effects are infrequent. Acetylsalicylic acid (i.e., aspirin) often is used in low dosage (5 mg/kg/day) in children with anticardiolipin antibodies. Antiinflammatory doses of aspirin (80 mg/kg/day) have been advocated, but children with SLE are very susceptible to salicylate-induced hepatotoxicity. Alternate NSAIDs are preferable.

Hydroxychloroquine sulfate (Plaquenil) and chloroquine routinely are used in children and adolescents with SLE (2 ,7 ,8 ,11 ,12 ,14). They are believed to have a useful steroid-sparing effect at a dose of 7 mg/kg/day. Although rare ocular toxicity is a concern, it was not reported in children or adolescents in any of these series (Table 43-4).

Intravenous pulse methylprednisolone (30 mg/kg daily, up to 1 g) given as an intravenous infusion has been used to control flares of nephritis (241) or CNS disease. Therapy was associated with dramatic short-term improvement in renal disease, but was not superior over the longer-term, to daily prednisone (241). Long-term benefit from pulse methylprednisolone is more likely in children with acute CNS involvement and other manifestations of SLE that appear to result from an acute event. Although rare, side effects may occur with pulse methylprednisolone, including significant hypotension, hypertension, and pancreatitis. Deaths have rarely occurred.

Table 43-3: Immunosuppressive Treatment of Childhood SLE

Drug(s)*	Suggested Dosage	Useful For	Remarks
NSAIDs,	—	Mild disease	Monitor for idiosyncratic effects of NSAIDs on renal and central nervous system (CNS) function.
Prednisone	1–2 mg/kg/d	More severe or unresponsive disease.	Rarely exceed 80 mg/day; may be divided up to q.i.d., if necessary.
Methylprednisolone	30 mg/kg/d, IV	Acute manifestations of CNS or renal disease	Maximum: 1,000 mg for 3 days
Cyclophosphamide	500–1000 mg/m2/mo for 7 months, then every 3 months for 30 additional months	DPGN	May be helpful for some children with severe nephrotic syndrome or CNS disease

*Other agents have been used, with differing reports of their efficacy.
DPGN, diffuse proliferative glomerulonephritis.

Table 43-4: Dosages of Medications Commonly Used for Children with SLE*

NSAIDs
- Naproxen 10–15 mg/kg divided BID (Usual maximum, 500 mg BID)
- Tolmetin 20–40 mg/kg divided BID (Usual maximum, 600 mg BID)
- Diclofenac 1–3 mg/kg divided BID (Usual maximum, 75 mg BID)
- Ibuprofen 20–40 mg/kg divided TID or QID (Usual maximum, 800 mg TID) may be associated with idiosyncratic reactions in SLE.

Other Drugs
- Hydroxychloroquine 7 mg/kg up to 200 mg/d (Some centers use 400 mg/d maximum dose)
- Dapsone 1 mg/kg up to 100 mg/d

Immunosuppressives
- Azathioprine 1–3 mg/kg/d (Usual maximum 100 mg/d)
- Cyclophosphamide (See text for intravenous administration.

Not recommended PO because of hemorrhagic cystitis risk)
- Methotrexate 10 mg/m2/wk (Safety and efficacy in SLE not yet established)

*These typically are used dosages only. Full prescribing information provided by the manufacturer should be consulted for possible side effects, interactions, and other consequences of the use of these medications.

In the 1970s, most children were treated with high-dose corticosteroids (2 mg/kg/day) followed by a gradual tapering once their disease came under control (2 ,78 ,11 ,14 ,28). Children with continuing active disease and evidence of renal involvement received immunosuppressive agents (7 ,9 ,13 ,29). Cushingoid facies, cataracts,

avascular necrosis, and other complications were common (9 ,11 ,28).

In the late 1980s, systematic use of intravenous cyclophosphamide became common. It has been argued that corticosteroids are preferable to cytotoxic agents, because corticosteroids do not have life-threatening side effects. However, overt suicide resulting from the psychosocial stresses of Cushingoid facies and chronic disease has occurred, and covert suicide in the form of noncompliance (e.g., stopped corticosteroids against medical advice) is not uncommon (29).

Although the therapeutic role of cytotoxic drugs remains controversial, data supporting their safety and efficacy have become increasingly convincing (31 ,242 ,243 ,244). Concerns regarding sterility, risk of infections, and risk of neoplasia have limited their use to children with significant disease activity that is unresponsive to acceptable doses of corticosteroids (6 ,7 ,9 ,10 ,13 ,14 ,29 ,110 ,245). Immunosuppressives have been used in those with CNS disease, with varying results (6). Although a few centers report good results using high-dose prednisone and azathioprine over both 5- and 10-year periods (9 ,29), others have had less success with this regimen. Controlled trials in adult patients with SLE have found cyclophosphamide to be as effective as, and less toxic than, the combination of cyclophosphamide and azathioprine (110 ,111). Proper stratification of patients at study entry may be the key to resolving these issues (Table 43-5). We have found the systematic use of cyclophosphamide to be associated with a far greater and faster improvement in clinical parameters, and sense of well being, although allowing a more rapid reduction in corticosteroid dosage. A large number of children initially treated with systematic intravenous cyclophosphamide for a period of 3 years, are now off all immunosuppressive agents and disease free (112). As noted in the section on nephritis, a number of new biologic agents have begun to be used in the treatment of patients with SLE. Their ultimate safety and utility remain uncertain. It is hoped that the combined use of a variety of agents (much as is used in the treatment of childhood neoplasms) will ultimately lead to development of a regimen with maximum efficacy and minimum toxicity.

For children and adolescents with SLE, the desire to avoid iatrogenic injury must be balanced against the goal of sustained survival. Cyclophosphamide administered with vigorous intravenous hydration and careful inpatient monitoring has proven to be both safe and effective (31 ,110). Although sterility and late-onset neoplasia are theoretic risks, neither has been documented in children receiving cyclophosphamide with rigorous intravenous hydration. In contrast, avascular necrosis, cataracts, and Cushingoid facies commonly are experienced by children receiving high doses of corticosteroids over a prolonged period.

At the Hospital for Special Surgery in New York City, children who fail to respond adequately to corticosteroid therapy receive intravenous cyclophosphamide according to a well-defined protocol. Children initially receive 500 mg/m^2 of cyclophosphamide, followed by monthly increases to 750 mg/m^2, and then 1,000 mg/m^2 if the WBC count does not fall below 2,000/mL at its nadir. (It should be noted that 875 mg/m^2 often is the maximum dose that does not produce too great a fall in white count for children smaller than 1 m^2. In addition, the dosage should not exceed 40 mg/kg, because larger doses have been associated with cardiac toxicity in oncologic studies.) The induction phase of cyclophosphamide therapy continues with monthly cyclophosphamide infusions for a total of seven doses. Many children do not show evidence of major improvement until after the third monthly dose. If a child has deteriorated despite 6 months of therapy with cyclophosphamide, however, further therapy may not be warranted.

Children who respond well to monthly cyclophosphamide continue on therapy at 3-month intervals at the same dosage for an additional 10 maintenance doses. After 36 months of therapy, a repeat renal biopsy is performed and the cyclophosphamide discontinued if no evidence of active disease is found. Some children demonstrate renewed disease activity (e.g., decreasing hemoglobin, decreasing complement levels, falling creatinine clearance, increasing hematuria or proteinuria) following the transition from monthly therapy to therapy every 3 months. These children are treated with three additional monthly doses of cyclophosphamide, following which therapy every 3 months is resumed. Acute flares are managed with intravenous bolus doses of methylprednisolone for 3 days (30 mg/kg/day, up to a maximum of 1,000 mg) as necessary.

Most children who complete 36 months of therapy are withdrawn from cyclophosphamide and do well on low-dose corticosteroids. Some of these initial children are now alive, more than 15 years later, off all medications despite having biopsy-proven DPGN when therapy was initiated. Children who respond to monthly cyclophosphamide but who deteriorate whenever the frequency of therapy is decreased to every 3 months or flare after the initial course of cyclophosphamide has been completed, represent a different situation. Children with persistent disease activity without established chronic renal damage currently are treated with an aggressive program of combination therapy, using both intravenous cyclophosphamide and intravenous methotrexate. For those with only moderate disease activity but persistent hypocomplementemia, therapy with mycophenolate mofetil (up to 1 g BID) may be beneficial. For those children with severe disease recurrence, we have combined a 9-month course of intravenous cyclophosphamide with high-dose intravenous methotrexate (300 mg/m^2). This regimen has allowed us to bring four previously resistant patients under good disease control. In these children, methotrexate should be initiated carefully at 50 mg/m^2 or less and gradually increased if significant renal compromise is present. They should receive daily folic acid, and may require folinic acid “rescue” if there is significant renal compromise. Creatinine clearance of less than 60 mL/minute may be associated with increased risk of methotrexate toxicity.

Table 43-5: Staging Criteria for SLE

Stage 0	Patients with serologic evidence of SLE without clinical manifestations
0	Positive ANA without other manifestations
0a	Positive ANA plus false positive VDRL; or anticardiolipin antibodies; or antibodies to Ro, La, or RNP
0b	Positive ANA plus antibodies to Sm or anti-DNA antibodies.
Stage 1	
1	Serologic evidence of SLE plus at least one nonserologic criterion from the ACR criteria for the diagnosis of SLE, but without sufficient findings to fulfill criteria for a definite diagnosis of SLE or constitutional symptoms. (Note that any patient who fulfills the ACR criteria, or has >25 RBC/HPF or 1+ or greater protein by dipstick on urinalysis automatically is classified stage 3 or higher.)
1a	Criteria for stage 1 plus an elevated total protein [>8 g, or Hb <11.0 g/dL (without hemolytic anemia)].
1b	The above plus an ESR >25 mm/H Westergren
Stage 2	
2	Serologic evidence of SLE plus at least one nonserologic criteria for the diagnosis of SLE with recurrent fever, weight loss, or other significant constitutional symptoms.
2a	The above plus hypocomplementemia (C3 <80% of lowest normal value)
2b	The above criteria plus an elevated total protein [>8 g, or Hb <11.0 g/dL (without hemolytic anemia)]
Stage 3	
3	Any patient who fulfills the ACR criteria for the diagnosis of definite SLE, or patients with renal involvement who do not fulfill the ACR criteria for a definite diagnosis of SLE
3a	The criteria for stage 3 plus hypocomplementemia (C3 <80% of lowest normal value)
3b	The criteria for stage 3 plus an elevated total protein level [>8 g, or Hb <11.0 g/dL (without hemolytic anemia)]
Stage 4	
4	Definite SLE with renal involvement characterized by consistent finding of >5 RBC/HPF or >500 mg total protein in a 24-h urine specimen (normal serum creatinine and creatinine clearance). These patients would be expected to have either minimal glomerulitis or focal segmental nephritis on biopsy
4a	These criteria plus hypocomplementemia (C3 <80% of lowest normal value)
4b	These criteria plus an elevated total protein [>8 g, or Hb <11.0 g/dL (without q hemolytic anemia)]
Stage 5	
5	Definite SLE with membranous glomerulonephritis on biopsy and >2.0 g total protein in a 24-h urine specimen
5a	These criteria with clinical evidence of nephrotic syndrome including serum albumin <3.0 g/dL or cholesterol >300. (Note presence of persistent hypertension with diastolic blood pressure >90 mm Hg without treatment automatically indicates stage 7.)
5b	These criteria with pitting edema
Stage 6	
6	Definite SLE with renal biopsy demonstrated diffuse proliferative glomerulonephritis; or seizure, or other neurologic manifestations (e.g., cerebritis) sufficient to warrant hospitalization
6a	These criteria plus hypocomplementemia (C3 <80% of lowest normal value)
6b	These criteria plus an elevated total protein [>8 g, or Hb <11.0 g/dL (without hemolytic anemia)]
Stage 7	
7	Definite SLE with CrCl <100 mL/min/1.75 m2 or serum Cr >1.5
7a	The above plus hypocomplementemia (C3 <80% of lowest normal value) or diastolic blood pressure consistently over 90 mm Hg before therapy
7b	These criteria plus an elevated total protein [>8 g, or Hb <11.0 g/dL (without hemolytic anemia)]
Stage 8	
8	Definite SLE with CrCl <50 mL/min/1.75 m2 or serum Cr >2.0
8a	Plus hypocomplementemia (C3 <80% of lowest normal value) or diastolic blood pressure consistently over 90 mm Hg before therapy
8b	These criteria plus an elevated total protein [>8 g, or Hb >11.0 g/dL (without hemolytic anemia)]
Stage 9	
9	Definite SLE with CrCl <30 mL/min/1.75 m2 or serum Cr >3.0
9a	These criteria plus hypocomplementemia (C3 less than 80% of lowest normal value) or diastolic blood pressure consistently over 90 mm Hg prior to therapy
9b	These criteria plus an elevated total protein [>8 g, or Hb less than 11.0 g/dL (without hemolytic anemia)]
Stage 10	Definite SLE with end stage renal failure requiring dialysis; or chronic psychotic or neurologic state unresponsive to corticosteroid or immunosuppressive drug therapy

HPF, high-powered field.

Adapted from Lehman TJA, Mouradian JA. Systemic lupus erythematosus. In: Holliday MA, Barrat TM, Avner ED, eds. *Pediatric nephrology.* 3rd Ed. Baltimore, MD: Williams & Wilkins, 1994:849–870, with permission.

The major risks associated with the aggressive use of cytotoxic agents are bone-marrow suppression (often complicated

by infection), hemorrhagic cystitis, infertility, and the induction of neoplastic disease (early or late). Infectious complications can be minimized by careful evaluation before the administration of each dose of cyclophosphamide and a high index of suspicion for infection if the patient experiences difficulty during the period of maximal marrow suppression following each dose. Cystitis, infertility, and the induction of neoplastic disease are the remaining concerns. In my experience, none of these has occurred among children receiving intravenous cyclophosphamide with in-hospital hydration, during a 3-year course of therapy. One child with severe recalcitrant disease who received 62 grams of cyclophosphamide over an 8-year period developed a renal papillary-cell carcinoma, which was removed in situ. Presently, any similar patient would be treated with the combination of intravenous cyclophosphamide and intravenous methotrexate to limit the total dose of cyclophosphamide received. Children failing such regimens may be candidates for autologous stem cell transplantation (see below).

Studies of older patients with SLE indicate that the risk of sterility following cytotoxic drug therapy increases with increasing age. Premenarchal children may have some protection. In children with amenorrhea that is secondary to active SLE, menses often return during cyclophosphamide therapy. Several successful pregnancies have been reported following cyclophosphamide therapy in adolescents, and one pregnancy that originated during cyclophosphamide therapy (despite counseling) was successfully carried to term without difficulty. (No further cytotoxics were given after the pregnancy was discovered.) No definitive data about the risks of infertility or neoplasia are available, however, for children with SLE. Both have occurred in children who received cyclophosphamide as part of multidrug regimens for neoplastic disease. Families should be warned about these concerns before therapy is begun, and patients should be selected accordingly.

The ratio of risk to benefit for cytotoxic drugs in children and adolescents with SLE is minimized by appropriate patient selection. Progressively deteriorating creatinine clearance, prolonged hypertension (>6 months), and significant nonhemolytic anemia identify children who are at high risk of ultimate renal failure (101). These findings may occur without significant evidence of extra-renal disease activity, and such children should be aggressively treated. With corticosteroid therapy alone, many children progress inexorably to renal failure. Controlled studies at the Hospital for Special Surgery and at the National Institutes of Health (NIH) have indicated that routine use of intravenous cyclophosphamide prevents or retards this deterioration (31 ,111 ,113 ,242 ,246).

Methotrexate, cyclosporine, and intravenous γ globulin all have been used in small numbers of children with SLE (124 ,247 ,248). Sufficient data have not been obtained to judge their efficacy. Recently, autologous stem cell transplantation has received extensive attention for the treatment of rheumatic diseases including SLE and some teenagers are included in the reported series (127 ,128 ,249 ,250). There are some who question whether to good responses reported are a result of the stem-cell transplantation or the preparative immunosuppressive regimen alone (251).

Most investigators who have taken care of children with SLE over an extended period are aware of children who were inadvertently profoundly immunosuppressed as a result of unexpected sensitivity to a drug or drug interactions. Many of them went into remission of their disease, but few remained in remission for longer than 24 months. Whether the benefits of autologous stem-cell transplantation will endure is unclear. The early European BMT consortium results already describe patients with SLE who have relapsed following autologous stem-cell transplantation. It increasingly is recognized that SLE is a chronic, recurrent disease that may require prolonged therapy, even in the absence of active disease. However, for the patient with continued active disease in the face of intensive chemotherapy these regimens may be warranted. Significant improvements in the therapy of children with SLE will require careful collaborative studies.

Prognosis

The prognosis for children and adolescents with SLE has improved dramatically over the past 20 years (123). With improved anti-inflammatory therapy as well as improved pediatric care, 10-year survival rates now are approaching 90% (11 ,29). Nonetheless, significant numbers of children continue to progress to chronic renal failure and/or death (101) (Tables 43-6 and 43-7).

Often, children and adolescents with SLE do poorly because of the child's, and the family's, inability to cope with the chronic, relapsing nature of the disease. Success requires a sustained relationship among the child, family, and the treating facility. Institutions serving stable populations with good socioeconomic status and easy access to care consistently report superior survival to those serving disadvantaged populations (11 ,29 ,101 ,115 ,252). Poor understanding of the importance of medications for silent manifestations of SLE, such as hypertension, remains a familiar cause of morbidity. These preventable deaths have become increasingly frustrating, as our ability to control the manifestations of SLE has improved.

Table 43-6: Incidence of Adverse Outcomes in 72 Children with Systemic Lupus Erythematosus

Outcome	Incidence (%)
Renal failure	15
Severe central nervous system disease	11
Stroke	1
Chronic thrombocytopenia	7
Chronic active disease	56
Death	18

Table 43-7: Predictors of Poor Prognosis in Childhood Systemic Lupus Erythematosus

1. Persistent anemia: Hb <10 g for >6 mo
2. Persistent hypertension: diastolic BP >90 mm Hg for >6 mo
3. Persistent hematuria: >20 RBC/HPF for >6 mo
4. Pulmonary hypertension
5. Recurrent emergency admissions

HPF, high-powered field.

The quality of survival must be addressed in efforts to improve the outcome for children and adolescents with SLE. Long-term survival of a Cushingoid adolescent with aseptic necrosis who requires dialysis may not be satisfactory to the patient. Platt et al. (29) described three young adults who died more than 10 years following diagnosis; two of the three died after they had discontinued their medications against medical advice.

Although end-stage renal failure and dialysis have been associated with decreased SLE activity in some reports (253 ,254), both children and adolescents requiring chronic dialysis often fare poorly. In one series, nine of 16 children with SLE succumbed within 5 years of beginning dialysis (101).

For children and adolescents with SLE, a satisfactory outcome is measured in decades. Our goal should be to report 90% 50-year survival. Children without renal disease who have survived 5 years are at low risk. Children with renal disease of any type, however, remain at risk. Gradual progression to renal failure over 5 to 10 years or more, despite clinically inactive disease, has been reported in both children and adults with SLE (28 ,104 ,225). Health care professionals dealing with children and adolescents who have SLE must strive to aid patients and their families through a normally difficult period under even more difficult circumstances. Every effort must be made to guarantee the availability of appropriate services. Not only must medical therapy be aggressive, so should patient and family education to ensure their compliance. With the increasing presence of specialized pediatric centers for children with rheumatic diseases and growing numbers of collaborative studies to determine optimal therapy, survival measured in decades now should become the norm.

Summary

The information in this chapter can be summarized as follows:

- Children and adolescents represent both a special challenge and opportunity. Success in caring for this group requires awareness of the complex interactions among the child's illness, the needs of their family, and their own needs as developing individuals.
- Childhood-onset SLE has been recognized since the early 1900s. Although it frequently is described as a more severe disease than adult SLE, this may result from failure to properly diagnose many mild cases.
- No thorough studies of the epidemiology of SLE in childhood have been completed. It is estimated that the annual incidence is approximately 0.6 per 100,000, and that between 5,000 and 10,000 US children have SLE today. The incidence of SLE is much higher in females than in males and in nonwhites than in whites.
- The cause of SLE remains unknown, but the high frequency of immunologic abnormalities among family members of children with SLE suggests that a combination of genetic and environmental factors plays an important role. The presence of Ro/SSA in a large proportion of the mothers of young children with SLE may indicate predisposing genetic factors in the family. SLE also is more frequent in children who have defects of the immune system, suggesting that defective antigen processing may predispose to the development of SLE.
- The most common clinical manifestations of SLE are fever, malaise, and weight loss, but these are nonspecific manifestations of many chronic ailments. The typical butterfly rash is present only in about one third of children with SLE. Diagnosis is based on fulfillment of the ARA criteria, just as in adults.
- Renal disease occurs in two thirds of children with SLE in most reported series. Although the renal disease may be mild, severe DPGN remains a leading cause of morbidity in childhood SLE. Mild renal disease often can be controlled with corticosteroids, but active renal disease that does not respond fully to corticosteroids and DPGN with a falling creatinine clearance requires therapy with cytotoxic agents. Children with active SLE do poorly on dialysis.
- All of the CNS manifestations that are described in adults with SLE also occur in children. Behavioral disturbances, which may be ascribed to acting out by an adolescent with SLE, often represent CNS disease that may respond to increased therapy. Chorea also is seen more commonly among children with SLE.
- Pulmonary involvement in childhood SLE takes many forms, including pleurisy, pleural effusions, pulmonary fibrosis, and pulmonary hemorrhage. Diaphragmatic dysfunction is common and may be the underlying factor predisposing to recurrent episodes of pneumonia. Pulmonary hypertension often is a life-threatening complication. Abnormal pulmonary function may be present despite a normal chest radiograph.
- Musculoskeletal manifestations of SLE include arthritis and mild inflammatory myopathy, and they often are predominant at presentation. Both are responsive to corticosteroid therapy, however, and rarely contribute to long-term morbidity. The exception is avascular necrosis,

which may occur as a complication of SLE with or without corticosteroid therapy and ultimately requires ultimate joint replacement.

- Dermatologic involvement is common in childhood SLE but rarely is a significant problem except when the face is prominently disfigured, causing psychologic problems (Fig. 43-1). DLE is unusual in childhood.
- Cardiac manifestations of SLE include pericarditis and myocarditis, sometimes with recurrent effusions. These usually can be controlled with NSAIDs or low-dose corticosteroids. Valvular involvement is common and may predispose to bacterial endocarditis. Careful consideration should be given to antibiotic prophylaxis whenever bacteremia is expected. Premature myocardial infarctions have occurred in young adults, with significant atherosclerosis following prolonged corticosteroid therapy.
- GI manifestations of childhood SLE are varied. Nonspecific findings such as chronic abdominal pain and anorexia are frequent, and significant bowel infarction may occur. Pneumatosis intestinalis may result from recurrent microvascular insults.
- Infection is a major cause of morbidity and mortality in children and adolescents with SLE. Active SLE pre disposes to infection. Often, it is unclear whether a child's rapid deterioration is the result of infection or of active SLE. In this setting, increased doses of both corticosteroids and antibiotics may be necessary. Reticulo-endothelial system overload and functional asplenia may predispose to rapid progression of sepsis in children with active SLE.
- Hematologic manifestations are common in children and adolescents with SLE. Most are nonspecific. Thrombocytopenia is a frequent presenting complaint, particularly in young males, and menorrhagia also may be a significant problem in adolescent females. As in adults, the presence of anticardiolipin antibodies predisposes to clotting dysfunction and stroke in children.
- Laboratory manifestations of childhood SLE are identical to those of adults. One unique concern is awareness that a positive serologic result for syphilis in a child or adolescent is reported to the school district and warrants prompt investigation by public welfare authorities. Families should be warned about this possibility, and inquiries should be promptly diverted to the physician.
- Therapy for childhood-onset SLE is similar to that for adults. Because of the increased burdens of growth and development on renal function, however, it may be important to institute aggressive intervention earlier in children with DPGN. The goal must be to develop therapies that provide acceptable 50-year survival, not 5- or 10-year survival, for children and adolescents with SLE. The systematic administration of cytotoxic drugs may provide superior quality of life and long-term survival.

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Chapter 44
Drug-Induced Lupus

Robert L. Rubin

Historical Perspective

Within 1 year after the introduction of hydralazine to control malignant hypertension in 1952, the first report appeared of a late onset “collagen disease” resembling systemic lupus erythematosus (SLE) in 17 out of 211 hydralazine-treated patients (1). However, because the treatment regiment of these patients was combined with hexamethonium chloride, the first definitive association between hydralazine and lupus-like disease should be attributed to Dustan et al. at the Cleveland Clinic (2) in which 13 out of 139 patients became ill after receiving 400 to 800 mg hydralazine per day for an average of 12 months. In their follow-up paper in 1954, Perry and Schroeder (3) demonstrated that hexamethonium ion was not implicated in the lupus-like reaction of the 17 patients described by Morrow et al. (1) because patients fully recovered after discontinuation of only hydralazine. Although procainamide was introduced for the treatment of cardiac arrhythmia at about the same time, it was not until 1962 that Ladd reported a patient who developed lupus-like features after 6 months of procainamide therapy (4). During the next 3 years, another 11 cases of procainamide-induced lupus were reported (5). By 1966, a scattering of cases of lupus-like disease as a side effect of therapy with isonizid, diphenylhydantoin, sulphamethoxypyradazine, primidone, and tetracycline appeared. It is now clear that patients on a diverse array of drugs can develop autoantibodies and clinical features similar to those seen in patients with idiopathic SLE. Since this subject was reviewed 5 years ago, at least six additional drugs have been reported to induce lupus-like abnormalities, suggesting that the spectrum of drugs with capacity to induce lupus will continue to increase.

It is important to distinguish drug-induced lupus-like disease from the more common condition of drug-induced autoimmunity. When patients receiving drug therapy develop autoantibodies or other laboratory features of autoimmunity such as elevated immunoglobulin levels, but are clinically asymptomatic, the term “drug-induced autoimmunity” (DIA) should be used. In contrast a minority of drug-treated patients develop de novo clinical signs and symptoms similar to the spectrum of features associated with idiopathic SLE. This syndrome is referred to as drug-related lupus or drug-induced lupus (DIL). Although DIA may be a more homeostatically regulated form of DIL as suggested by the quantitative differences in their overt autoimmune abnormalities as discussed below, most asymptomatic drug-treated patients with persistently high levels of autoantibodies do not progress into developing lupus-like symptoms (6).

Drugs Implicated in Drug-Induced Lupus

Table 44-1 lists currently used drugs reported to be associated with a lupus-like syndrome. In all cases drugs that induce lupus also induce autoantibodies in a much higher frequency. Excluded from this list are drugs implicated only in the exacerbation of SLE or with the onset of chronic SLE prior to diagnosis as discussed below. Also excluded are drugs that induce non-multisystem cell, tissue, or organ abnormalities, such as cytopenias or cutaneous manifestations, although these may have an autoimmune etiology similar to DIL as discussed below. Also not shown are drugs that were initially reported to be associated with a lupus-like syndrome, but no confirmation has appeared despite many decades of prescriptions with countless patients. However, several infrequently used drugs are included in which only a single case of autoimmune-like disease has been reported to heighten vigilance for these possible lupus-inducing drugs. Eight drugs previously listed have been removed from this table because they are no longer in use. Macromolecular immunotherapeutics that have potential for induction of autoimmunity such as cytokines and tumor necrosis factor (TFN)-α inhibitors (reviewed in (7)) are included, although the mechanism underlying this phenomenon is probably different from classical DIL as discussed below.

Thus, 46 drugs currently in use have a propensity for inducing autoantibodies and occasionally a lupus-like syndrome. Table 44-1 divides these drugs into therapeutic classes and indicates their approximate risk levels based on the number of reports. Although listed separately, some drugs such as enalapril and lisinopril or simvastatin and atorvastatin are essentially the same chemical structure and could be considered a single agent. By far the highest risk drugs are procainamide and hydralazine, with approximately
20% incidence for procainamide and 5% to 8% for hydralazine during 1 year of therapy at currently used doses. The risk for developing lupus-like disease for the remainder of the drugs is much lower, considerably less than 1% of treated patients. Quinidine can be considered moderate risk, whereas sulfasalazine, chlorpromazine, penicillamine, methyldopa, carbamazepine, acebutalol, isoniazid, captopril, propylthiouracil, and minocycline are relatively low risk. The remaining 33 drugs should be considered very low risk based on the paucity of reports in the literature; for some, there is only one case report. Obviously, the perception of risk is not rigorous since it depends on dose and frequency of prescriptions as well as occasion to publish case reports and should not be equated with a fundamental, lupus-inducing propensity. New drugs are suspect as case reports accumulate; for example, amiodarone has been added to the list of drugs with lupus-inducing propensity because of three case reports over the past few years (8 ,9 ,10). Four patients have recently been described with antihistone antibodies, arthritis, and constitutional symptoms that appear to be a result of ticlopidine (11). Some drugs listed as very low risk may be falsely implicated or are currently of negligible risk, because customary treatment doses have been decreased, but most reports on drug-induced lupus are convincing because cessation of therapy usually results in prompt resolution of symptoms and eventually autoantibodies. It should be appreciated that criteria for reaching a diagnosis of drug-induced lupus-like disease are not as rigorous as those for diagnosis of SLE (see Diagnosis) (Table 44-7).

Table 44-1: Drugs Reported to Induce Lupus-Like Disease and Associated Autoantibodies

Agent*	Risk**
Antiarrhythmics	
Procainamide (Pronestyl)	High
Quinidine (Quinaglute)	Moderate
Disopyramide (Norpace)	Very low
Propafenone (Rythmol)	Very low
Amiodarone (Cordarone)	Very low
Antihypertensives	
Hydralazine (Apresoline)	High
Methyldopa (Aldomet)	Low
Captopril (Capoten)	Low
Acebutolol (Sectral)	Low
Enalapril (Vasotec)	Very low
Clonidine (Catapres)	Very low
Atenolol (Tenormin)	Very low
Labetalol (Normodyne)	Very low
Pindolol (Visken)	Very low
Minoxidil (Loniten)	Very low
Prazosin (Minipress)	Very low
Lisinopril (Privinivil)	Very low
Antipsychotics	
Chlorpromazine (Thorazine)	Low
Phenelzine (Nardil)	Very low
Chlorprothixene (Taractan)	Very low
Lithium carbonate (Eskalith)	Very low
Perphenazine (Trilafon)	Very low
Antibiotics	
Isoniazid (INH)	Low
Minocycline (Minocin)	Low
Nitrofurantoin (Macrodantin)	Very low
Anticonvulsants	
Carbamazepine (Tegretol)	Low
Phenytoin (Dilantin)	Very low
Trimethadione (Tridone)	Very low
Primidone (Mysoline)	Very low
Ethosuximide (Zarontin)	Very low
Antithyroidals	
Propylthiouracil (Propylthyracil)	Low
Anti-Inflammatories	
D-Penicillamine (Cuprimine)	Low
Sulfasalazine (Azulfidine)	Low
Phenylbutazone (Butazolidin)	Very low
TNF-α inhibitors† (Remicade)	Very low
Mesalamine (Asacol)	Very low
Zafirlukast (Accolate)	Very low
Diuretics	
Chlorthalidone (Hygroton)	Very low
Hydrochlorothiazide (DiuchlorH)	Very low
Antihyperlipidemics	
Lovastatin (Mevacor)	Very low
Simvastatin (Zocar)	Very low
Atorvastatin (Lipitor)	Very low
Miscellaneous	
Aminoglutethimide (Cytadren)	Very low
Interferon-α (Wellferon) -ß-γ	Very low
Timolol eye drops (Timoptic)	Very low
Ticlopidine (Ticlid)	Very low

*Commonly used brand name is enclosed in parentheses.
**Risk refers to likelihood for lupus-like disease, not autoantibody induction, which is usually much more common.
†Refers to a drug family possessing similar pharmacologic properties (see text)

As early as 1957, it was suggested that anticonvulsants can induce lupus-like features (12). However, manifestations of convulsive disorders may precede typical SLE by many years, and some anticonvulsants are associated with only renal or cutaneous disease (13). It is also difficult to identify the drug that may be responsible for DIL or DIA reactions, because many of these patients are using more than one drug including an additional anticonvulsant

medication. Dubois and Wallace (13) have doubted many of the reports suggesting a causative relationship between anticonvulsants and DIL, and some of the anticonvulsants in Table 44-1 may be unfairly implicated. However, DIL associated with the use of phenytoin (diphenylhydantoin) and carbamezepine is well documented.

As with anticonvulsant therapy, patients who develop DIL or DIA associated with antituberculous drugs are on more than one medication. Triple therapy with isoniazid (INH), paraaminosalicylic acid (PAS), and streptomycin was once standard practice. The best evidence, however, points to isoniazid as the drug most likely to cause DIA or DIL (14). The major manifestation of autoimmunity in patients on INH appears to be the development of low-titer antinuclear antibodies (ANA), and clinically diagnosed DIL is rare (13). Interestingly, antihistone antibodies in these patients are predominantly IgA, suggestive of the involvement of the mucosal immune system in INH-induced autoimmunity (15). Some reports have suggested that the incidence of SLE is increased after taking oral contraceptives, and that remissions follow cessation of their use (16 ,17). These findings could not be substantiated (13 ,18 ,19). Gold therapeutics, such as disodium aurothiomalate frequently appear on lists of drugs associated with SLE induction or exacerbation. However, although late-onset toxic reactions to gold requiring discontinuation of therapy occur in up to one third of treated patients, the vast majority of episodes occur during the first few months of therapy and are limited to skin reactions or buccal irritation; rash occurs in about one half the toxic gold reactions, and it is of a generalized or upper body distribution (20). Approximately 1% of treated patients develop leukopenia, thrombocytopenia, or proteinuria (20). These abnormalities are generally considered immune-mediated (21), but no report of gold-induced lupus has appeared despite over six decades of treatment experience. Gold toxicity does not appear to behave as a classical delayed-type hypersensitivity reaction in that only one of 30 patients with gold reactions had rapid recurrence of skin symptoms upon re-challenge with gold therapy (20).

Table 44-2: Prospective Studies of Drug-Induced Lupus

Drug	Approx Dose (gram/day)	Average Duration (yr)	ANA Incidence	Drug-Induced Lupus Incidence	Reference
Procainamide	2–4	1	75%	15%–20%	(22,23,24,25,26,27)
Hydralazine	0.1–0.3	3	15%–45%	5%–10%	(28,29,30,31,32,33)
Isoniazid	0.35	0.6	22%	<1/102*	(14)
Methyldopa	1–2	2	19%	<1/53*	(34)
Levodopa	1.5–8.0	1	11%	<1/80*	(35)
Estrogen/Progest	0.05/1**	1.3	<1/80*	<1/80*	(18)

*No patients developed symptoms; the denominator is the number of patients in the prospective study.

**mg/day.

A broad variety of therapeutic purposes are encompassed by lupus-inducing drugs including control of convulsion disorders, psychoses, hyperthyroidism, hypertension, fungal and bacterial infections, heart arrhythmias, edema, and even anti-inflammatory agents. Consequently, the structures of these drugs show wide disparity and this is reflected in their diverse biochemical action. Although some of these drugs are aromatic amines (procainamide, practolol, and sulfapyridine, a metabolite of sulfasalazine) or aromatic hydrazines (hydralazine, INH), there is no common denominator of a pharmacologic, therapeutic, or chemical nature that links the drugs with capacity to induce lupus-like disease. Nevertheless, the remarkable similarity in clinical features and laboratory findings in lupus induced by most of the drugs other than the biologics strongly suggests that the same mechanism underlies the process regardless of the inciting agent. How this process may be related to drug chemistry is discussed later (see Oxidative Drug Metabolism).

Prospective Studies

Relatively few prospective studies have been undertaken to establish the true incidences of induction of lupus and ANA by drugs (Table 44-2). Because clinicians' choice of drugs and their doses depend in part on the country in which they practice, there is considerable geographic variability on the incidence of DIL. Even within the United Kingdom the incidence of hydralazine-induced lupus was reported to be 6.7% after 3 years of treatment (32) and 4.3% after 13 years (36). Women treated with a daily dose of 200 mg hydralazine showed a 3-year incidence of DIL of 19.4% (32). Most patients develop symptoms between 6 months and 2 years treatment, but it is not uncommon for a patient to require more than 3 years of treatment with a total intake of more than 1 kg hydralazine before symptoms become manifest (33). With procainamide, the typical patient (i.e., the arithmetic median (37)) develops symptoms after 10 months of treatment. However, variations in therapeutic response to control ventricular arrhythmias, as

well as differences in drug clearance and metabolism (see Acetylator Phenotype), result in dose requirements that vary from 0.25 to 6 g procainamide/day. As a result, approximately 25% of patients do not develop symptoms until more than 2 years and some as long as 6 years of continuous treatment with procainamide (22 ,23 ,38 ,39). Discrepancies in the literature on the incidence of procainamide-induced lupus (23 ,24 ,25) and especially hydralazine-induced lupus (32 ,33 ,36) appear to be related to the use of lower doses of these drugs in recent years just to minimize the chances of lupus-like disease. Prospective studies of INH, α-methyldopa, L-dopa, and oral contraceptives failed to reveal a single case of lupus-like illness during the observation period. Thus, the incidence of lupus induced by these latter drugs must be less than 1% but is probably considerably lower.

Autoantibody induction is relatively common with approximately one fifth of patients treated with INH and methyldopa and one tenth of patients treated with L-dopa developing ANA during 6 months to 2 years of treatment. Studies of patients receiving procainamide therapy have shown that 75% of patients develop ANA within 1 year of treatment (25 ,26) and almost 100% develop ANA after 2 years (24 ,40). Most of these patients remain asymptomatic. The incidence of ANA positivity in patients who remain asymptomatic during approximately 3 years of treatment with hydralazine has been reported as low as 15% (31) to as high as 44% (28). Although formal prospective studies have not been performed, several other medications shown in Table 44-1 also have a high propensity for inducing ANA, anti-denatured DNA, and/or anticardiolipin antibodies, especially chlorpromazine in which prevalences of 15% to 40% have been reported (41 ,42 ,43) and acebutalol with reported ANA prevalences from 15% to 89% (44 ,45 ,46 ,47 ,48 ,49). It should be appreciated that the fine specificity and predominant isotype of the ANA in asymptomatic patients is generally not the same as in patients with symptomatic DIL (see anti-[(H2A-H2B)-DNA] Antibodies), and there is no evidence that patients who convert to ANA positivity are more likely to develop symptomatic disease.

Table 44-3: Reappearance of Lupus-Like Symptoms after Rechallenge with the Lupus-Inducing Drug

	First Drug-Induced Lupus Episode			Second Drug-Induced Lupus Episode		
Drug	Dose/Day	Time for Symptoms Manifestation	Washout Period	Dose/Day	Time for Symptoms Manifestation	Reference
Procainamide	4–5 g	20 months	8 days	2 g	36 hours	(50)
Procainamide	1 g	25 months	3 months	1 g	“promptly”	(51)
Hydralazine	0.25 g	12 months	few days	not stated	“immediately”	(3)
Hydralazine	0.5 g	10 months	6 weeks	0.15 g	1–2 days	(52)
Hydralazine	0.4 g	14 months	2–3 weeks	0.6 g	1–2 days	(53)
Isoniazid	0.3 g	14 months	2 weeks	0.3 g	1 day	(54)
Penicillamine	2 g	27 months	18 months	0.5 g	never during 12 mo	(55)
Chlorpromazine	0.4 g	13 months	6 weeks	0.2 g	1–2 days	(56)
Quinidine	0.5 g	3 weeks	1 week	0.5 g	4 days	(57)
Minocycline*	N.R.	22 months	5 weeks	N.R.	5 days	(144)

*Values are the average of 6 patients
N.R. = not reported.

Rechallenge Studies

Between 1954 and 1973, reports appeared on the rapid re-occurrence of symptoms of DIL upon re-introduction of hydralazine (3 ,29 ,33 ,52 ,53), procainamide (50 ,51), INH (54), penicillamine (55), and chlorpromazine (56). These studies contributed to the notion that DIL behaves like a hypersensitivity reaction or that DIL occurs in patients predisposed to this side effect. However, examination of these reports reveals that in most cases, re-introduction of the implicated drug occurred just a few weeks after therapy was discontinued because of DIL (Table 44-3).

Typically, these patients displayed symptom recurrence within 1 to 2 days after resuming therapy. It is now clear that although subjective symptoms of DIL may resolve in a few weeks, serological abnormalities, especially antihistone antibodies, persist for much longer. Thus, immune abnormalities in most of these patients probably had not normalized when rechallenged with the drug, suggesting that a combination of autoantibody and another, unknown factor dependent on the presence of the drug is required for the pathology of DIL. In perhaps the most thorough study

of this phenomenon in 11 patients with hydralazine-induced lupus after a “long” (although unstated) washout period, Perry (33) reported that three patients had symptom recurrence within 1 day, two within 14 days, two within 2 months, one after 7 months, and three never developed symptom recurrence during the second treatment period of 1 to 5 years. It remains debatable whether variability in symptom recurrence reflects differences in putative predisposing factors for development of DIL or differences in time for the hyperimmune state to normalize prior to re-introduction of the drug.

Drug-induced lupus does not behave like a classical drug hypersensitivity reaction in that patients with DIL generally lack drug-specific T cells or antibodies, and the target autoantigens are not directly altered by the inducing drug. Also, the time course for development of drug-induced lupus tends to be much slower than that of classical drug allergies, which are usually immediate (58), and re-introduction of a lupus-inducing drug is not generally associated with memory of prior exposure if systemic autoimmunity had been allowed to normalize. Finally, although drug hypersensitivity can be triggered by relatively low or transient doses of the inciting agent at least after sensitization, the probability of expressing drug-induced autoantibodies and symptomatic lupus increases as the dose and duration of exposure increases (24). This feature suggests a phenomenon that depends on at least one event of low probability, so that drug-induced lupus is more likely as time and dose increase. Circumstantial evidence strongly suggests that this event is metabolic transformation of the drug to a reactive product.

Other Late-Onset Toxic Drug Reactions

Drugs That May Exacerbate SLE

The report of Hoffman in 1945 describing a 19-year-old army recruit who developed cutaneous, hematologic, and renal disease with features of SLE after treatment with topical and oral sulfadiazine (59) is often cited as the first description of DIL. In actuality this patient had a hypersensitivity-like reaction to sulfadiazine coincident with exacerbation of preclinical SLE or with the onset of SLE. This and subsequent, similar reports (although only one involving an oral sulfa-drug (60)) helped to entrench the view that many cases of idiopathic SLE are “unmasked” during drug therapy in patients with a lupus diathesis (61). This idea is difficult to discount or prove. Various drugs have been noted to have a temporal relationship with the exacerbation of SLE or with the onset of chronic SLE prior to diagnosis (13). In the latter cases SLE remains after withdrawal of the implicated agent. In the most recent case-controlled study of this phenomenon 12% of SLE patients with drug allergies were considered to display disease exacerbation, predominately lupus rash (62 ,63). Because SLE patients are significantly more prone to develop drug allergies especially to antibiotics such as sulfonamides, penicillin/cephalosporin, and erythromycin (62), these agents should be avoided in patients with SLE (64). Two SLE patients who developed lupus flares after ciprofloxacin treatment required hospitalization (65), and severe clinical relapses temporally associated with therapy with sulfonamides have been reported in the older literature (66). Drugs that appear to exacerbate SLE can be classified as antibiotics, anticonvulsants, hormones, nonsteroidal anti-inflammatory drugs (NSAIDs), and dermatologic agents. Sulfonamides (67), tetracyclines (68), griseofulvin, (69 ,70 ,71), piroxicam (72), and benoxaprofen (73) are reported to be photosensitizers of varying frequency. Rash or dermatitis related to drugs typically has a history of rapid onset and behaves as a drug hypersensitivity-type reaction that may be triggered by exposure to ultraviolet light (74). The majority of adverse drug reactions in previously diagnosed SLE patients are of this category (62 ,63). Another, possibly related category of patients are those with acute or subacute cutaneous lupus erythematosus related to photo-active medications; these patients may have systemic disease and can fulfill criteria for a diagnosis of SLE (75). Table 44-1 includes some of these drugs also associated with typical DIL. Drug-induced aseptic meningitis in SLE patients as a result of therapy with ibuprofen (76) and other NSAIDs (e.g., sulindac (77), tolmetin (78), diclofenac (79)) is an important consideration for the physician involved in the care of SLE patients who present with signs of meningeal irritation. Hypersensitivity reactions that have been interpreted as initiating or aggravating factors in SLE are associated with hydralazine (80), sulfonamides (59 ,60 ,67 ,81 ,82), penicillin (62 ,67), paraaminosalacylic acid (83), hydrochlorothiazide (84), cimetidine (85), phenylbutazone, (86 ,87), mesantoin (12), and various NSAIDs (13 ,88).

Unknown or suspected environmental chemicals are also occasionally implicated as causal agents in SLE or other distinct autoimmune diseases such as eosinophilia-myalgia syndrome associated with L-tryptophan ingestion, toxic oil syndrome associated with ingestion of aniline-adulterated cooking oil, silicosis associated with inhaled silica or asbestos dust, and autoimmunity associated with vinyl chloride exposure. These syndromes have unique features (reviewed in (89)) and should not be confused with DIL.

Whether or not an environmental or pharmaceutical agent might aggravate or unmask incipient SLE should be considered a clinical problem distinct from DIL because, by definition, symptoms of DIL resolve after discontinuation of therapy, although in severe cases full recovery may require up to 1 year. (Although this view is not in accord with the influential study of Alarcon-Segovia et al. (80) in which 60% of patients with hydralazine-induced lupus had persistence of several symptoms 0.5 to 9 years after withdrawal of hydralazine, three fourths of these patients were reported to have pre-existing rhematologic problems.) If drugs or environmental agents are truly causative in initiating

or aggravating SLE, the mechanistic basis is probably different from that of DIL because the steady-state blood levels of bonafide lupus-inducing drugs must generally be sustained for many months to years (i.e., medications two to six times daily) for development of DIL. In contrast for most cases believed to be aggravated or unmasked, exposure is of very low level or infrequent when the suspected agent is environmental or of relatively short duration when a drug is implicated. The association between drugs and the exacerbation or onset of SLE resembles the lupus flares following exposure to sunlight, exercise, or pregnancy.

Some environmental agents are suspected of causing lupus-like disease based largely on studies in experimental animals. A lupus-like syndrome was induced in monkeys by alfalfa sprouts and L-canavanine (90), and several case reports of lupus-like disease associated with the ingestion of abnormally large amounts of alfalfa seeds or tablets (91 ,92) implicate L-canavanine as capable of inducing or exacerbating lupus if excessive quantities are ingested. The other principal example in this category is a scleroderma-like autoimmunity associated with heavy metals in rats and mice (93 ,94). The major target of mercury-induced autoantibodies is the nucleolar protein fibrillarin, a specific serologic marker for a subset of patients with scleroderma (94). Although these animal models are of considerable mechanistic interest, these agents have not been definitely implicated in autoimmune disease in humans.

Autoimmunity Associated with Biologics and Other Immune-Modulating Agents

Various immune modulating agents can paradoxically induce autoimmune features. Cyclosporine A has been reported to precipitate graft-versus-host disease (GVHD) after withdrawal from patients receiving autologous bone marrow transplants (95 ,96). As discussed above, gold therapeutics have been implicated in immune-mediated disease (21), although this falls outside the usual presenting symptoms and clinical progression of SLE (Chapter 32) or DIL.

Therapeutic biologics, such as interleukin (IL)-2, interferon (IFN)-α, ß and γ, and tumor necrosis factor α (TNF-α) inhibitors have been occasionally associated with a variety of musculoskeletal and/or skin manifestations, especially serologic features suggestive of lupus-like autoimmunity. Autoimmune-like features related to INF therapy and to TNF-α blockers have been particularly well documented. De novo induction of ANA and cell-specific autoantibodies have been observed in IFN-α–treated patients with chronic myelogenous leukemia, many of whom were reported to have clinical features of autoimmune disease (97), especially thyroid and autoimmune hematologic disorders (98). A wide range of clinical abnormalities including SLE, RA, Raynaud phenomenon, and Behcet disease have been seen in these patients, which required discontinuation of IFN-α treatment (98 ,99). However, features typical of DIL were rare. A recent report on the de novo appearance of myalgia and arthalgia in a patient with multiple sclerosis treated for 2.5 years with IFN-ß (100) may echo a DIL-like process.

The bulk of data on induction of autoimmunity by biologics is related to TNF-α inhibitors because of the widespread use of these drugs to treat RA. There are currently three forms of TNF-α inhibitors: etanercept (Enbrel), which is the p75 TNF-α receptor type II fused to the Fc region of IgG1; Adalimumab, D2E7 (Humira), which is a human IgG1 anti-TNF-α monoclonal antibody, and infliximab, cA2 (Remicade), which is a chimeric (mouse/human) anti-TNF-α monoclonal IgG4 antibody. The best documented autoimmune-related side effect of these agents is induction of ANA and anti-DNA: in RA 23% to 61% of patients developed ANA compared to 6% to 32% of controls, and anti-DNA developed in 8% to 49% of patients treated with TNF-α blocking agents compared to none of the controls (101 ,102 ,103 ,104 ,105) (Table 44-4). It should be noted that, where studied, anti-DNA antibodies were predominately of the IgM isotype (102 ,105 ,106) and, less frequently IgA anti-DNA (105); IgG anti-DNA, which is specifically associated with SLE (Chapter 23), was rarely seen in significant titer. In Crohn disease in which the baseline ANA was positive in only 7% of patients, 52% of the patients became ANA+ after five injections of infliximab (107). Interestingly, although 62% and 71% of patients with spondylarthropathies (SpA) developed ANA and (IgM) anti-DNA, respectively, after 1-year treatment with inflixamab, there was no significant induction of ANA or anti-DNA in SpA patients treated with etanercept for up to 2 years (Table 44-4); ANA and (IgM) anti-DNA largely disappeared within 1 to 3 years after discontinuing therapy with inflixamab (105).

The majority of patients who were considered to have systemic autoimmunity related to TNF-α blocking agents presented only with cutaneous manifestations; this is clearly outside the typical presentation of DIL (see Diagnostic Criteria). The literature describes over the past 15 years (excluding abstracts and highly atypical presentations such as nephritis or neuritis suggestive of a demyelinating process) approximately 25 cases of lupus-like autoimmunity that were temporally related to TNF-α blockers (Table 44-4). Based on a recent survey of more than 10,000 patients in France treated with TNF-α blockers, the prevalence of DIL-like disease related to infliximab or etanercept (excluding rash-only patients) was 0.1% (108), and this is probably an overestimate. These patients usually had constitutional symptoms (fever, weight loss, fatigue), either myalgias or arthalgias, or serositis of the lung or heart; notably, 23 of 25 patients also had substantial cutaneous symptoms rarely seen in DIL. Symptoms of lupus typically occurred approximately 6 months after initiation of treatment and usually resolved within 1 to 4 months after discontinuation, an unusually long period.

In many of the cases purported to be DIL as a result of TNF-α blockers, other explanations were not excluded. These patients already had a pre-existing autoimmune or inflammatory disease, so the possibility increases that new

symptoms attributed to DIL were merely a coincidence. Flares or new symptoms of a pre-existing autoimmune disease or the coincident development of another type of autoimmune disease are not unusual in patients with a rheumatic disease. Articular symptoms attributed to DIL are obviously especially problematical in patients with RA, and the wide range of disease manifestations together with the relapsing–remitting nature of autoimmunity adds to the ambiguity. This means that there is a greater burden of proof to establish a convincing case of DIL related to anti-inflammatory biologics. In particular a long-term follow-up should be required to establish that the patient's clinical status remains free of lupus-like symptoms after discontinuing therapy with a TNF-α blocker and withholding corticosteroids; this has rarely been demonstrated. Naturally, strong consideration should be given to stopping treatment with a TNF-α blocking agent if new symptoms appear, whether or not they reflect true DIL. On the other hand, there is no evidence that RA patients who develop positive ANA or anti-DNA during treatment with a TNF-α blocker are at increased risk for the development of DIL.

Table 44-4: Prevalence of Autoantibodies and Lupus-Like Symptoms in Patients Treated with TNF-α Blocking Agents

Biologic	Patient Diagnosis*	Autoantibody Induction (% of Patients)**	Symptomatic Disease†	Reference
Etanercept	RA		4	(111)
	RA		2	(112)
	RA		3/3000	(108)
	SpAc	10%|10%	0/20	(105)
Infliximab	RA		1	(112)
	RA		2	(113)
	RA		1	(114)
	RA		9/7700	(108)
	RA	45%|33%	0/42	(103)
	RA	53%|14% vs. 32%|0%	1/156	(102)
	RA	23–61|%8–16% vs. 6–8%|0%		(101,104)
	Crohn's	7%|→52%| (0→5 infusions)	2/125	(107)
	SpA	62%|71%	0/34	(105)
	RA	41%|49%	0/34	(105)

*RA, rheumatoid arthritis; SpA: spondylarthropathy
**ANA|anti-DNA treated vs. ANA|anti-DNA controls
†Number of patients with lupus-like symptoms/total number of treated patients in the study. Patients displaying only cutaneous manifestations were not included.

It is currently unknown how a cytokine inhibitor might induce autoimmunity, but extensive studies in murine lupus have demonstrated both beneficial and damaging effects of manipulating the cytokine milieu (109). SLE is associated with dysregulation of cellular immunity, and some studies have shown that TNF-α protects against lupus nephritis as autoimmunity emerges in NZB/NZW mice (110). It is possible that therapeutic manipulation of cytokine levels in individuals predisposed to autoimmunity may disrupt cytokine-mediated immune homeostasis, leading to autoantibody induction and disease. However, the fact that anti-TNF-α monoclonal antibody (infliximab) but not soluble TNF-α receptor (etanercept) induces autoantibodies (105) makes any explanation on the mechanistic basis of this phenomenon highly speculative.

Regardless of the explanation, DIL related to small molecule pharmaceuticals occurs by de novo induction of autoimmunity in the setting of a formerly normal immune system and would be expected to have a mechanistically different basis.

Drug-Induced Immune Hemolytic Anemia

Long-term therapy with some drugs is associated with development of hemolytic anemia because of antibodies bound to red blood cells (RBC) in vivo (direct Coombs test positivity). In the penicillin-type, antibody to the drug binds to RBC as a result of adsorption of the drug or its metabolite to the RBC membrane. In the methyldopa-type, the drug is not required for (and does not effect) antibody binding, and anti-RBC antibodies typically have specificity for rhesus locus or other intrinsic RBC antigens. These antibodies rarely produce frank hemolytic anemia, possibly because their isotype or low avidity does not support complement fixation. Hemolytic anemia is commonly associated with the stibophen-type of drug-induced antibodies (as is quinidine and quinine) in which immune complexes

consisting of the drug or drug metabolite bind to RBC presumably via Fc or complement receptors.

The mechanism underlying the penicillin-type of anti-RBC response is frequently used as the basis for models for autoantibody elicitation in DIL (see Mechanisms). Interestingly, the autoantibodies associated with DIL behave more like the methyldopa-type of immune response in that the likelihood for autoantibody appearance is dose-dependent, but the drug is not required for antibody binding to its target antigen. In fact, many of the drugs associated with Coombs positivity of this drug-independent type (methyldopa, L-dopa, mefanamic acid (Ponstel), procainamide, chlorpromazine, and streptomycin (115)) are also known to cause DIA or DIL (Table 44-1), although there is generally no correlation between positive Coombs test and ANA or DIL. However, patients with methyldopa-induced hemolytic anemia have been reported to have positive lupus erythematosus (LE) cells and ANA (47 ,116 ,117 ,118 ,119 ,120). As with the autoantibodies associated with DIL, Coombs positivity gradually disappears after cessation of therapy, and individuals with a history of methyldopa-induced anti-RBC do not display significantly increased propensity for induction of anti-RBC upon reinstitution of therapy with the same drug (121). The mechanism for induction of this type of anti-RBC is unknown. However, despite the drug independence of anti-RBC binding, a drug-altered RBC model is commonly invoked (115) and incorrectly applied to the origin of autoantibodies associated with DIA and DIL.

Epidemiology

The incidence of DIL had been estimated as 15,000 to 20,000 new cases annually in the United States (122). However, most of these cases were a result of procainamide, a drug that has now been largely replaced by other antiarrhythmics. Therefore, the incidence of DIL is currently probably considerably lower but is unknown. The incidence of DIL in other countries is also unknown, but has been estimated to be 10% that of idiopathic SLE (13). Lee et al. (123) reviewed the medical histories of 285 consecutive SLE case records and found that drugs were a possible causative factor in 12%. Fries and Holman identified 12 DIL cases in a population of 198 lupus patients (124). In a more recent retrospective study of 30 patients with elevated antimyeloperoxidase antibodies and vasculitis usually involving the kidney, lungs, and/or skin, 60% were exposed to a lupus-inducing drug for 1 to 10 years, usually hydralazine or propylthiouracil, suggesting that the majority of patients with high-titer antimyeloperoxidase antibodies may be drug-induced (125). The frequency of DIL may be underestimated because most cases are mild, and only a small proportion are correctly diagnosed or seen by a rheumatologist. Because drugs such as INH are more frequently administered in certain countries, studies in such countries could provide a more thorough epidemiologic picture of DIL.

The age of patients developing DIL reflects the age of the population undergoing treatment with the implicated drug. Because procainamide and hydralazine, the most common lupus-inducing drugs, tend to be administered to the older population displaying cardiac arrythmias or hypertension, respectively, DIL usually occurs in people of age 50 or older.

Two epidemiologic features distinguish DIL from SLE. First, the high female-to-male predominance seen in SLE (9:1 to 7:1) is not seen in DIL largely because the majority of patients treated with the major lupus-inducing drugs are men. Nevertheless, procainamide-(25 ,126) and hydralazine-induced lupus (32 ,36 ,127 ,128) appear to be disproportionately more common in females. In the study of Totoritis et al. (25), the female-to-male ratio of procainamide-treated patients who developed lupus-like symptoms was 0.52:1 compared to a ratio of 0.19:1 for those who remained asymptomatic. A similar twofold to fourfold predominance of women over men for development of hydralazine-induced lupus has been reported (32 ,36 ,128). In the study of Cameron and Ramsey (32), the overall incidence of hydralazine-induced lupus during a 4-year observation period was 11.6% in women and 2.8% in men. In this same study, women treated with a daily dose of 200 mg hydralazine had a 19.4% incidence of hydralazine-induced lupus over a 3-year period. This is in contrast to the development of ANA in patients treated with hydralazine over a 3-year period, during which no gender differences were noted (127). Secondly, unlike SLE, the frequency of hydralazine-induced lupus in blacks was reported to be fourfold (29) to sixfold (33) lower than in whites. African Americans seem to be protected from lupus induced by procainamide as well (6).

Clinical and Laboratory Features

Table 44-5 shows the clinical and laboratory features of procainamide- and hydralazine-induced lupus compared with SLE. Musculoskeletal complaints are commonly observed, with arthralgia heading the list for both drugs. Arthritis is a less common feature with lupus induced by procainamide (20%) than by hydralazine (50% to 100%), whereas serositis (pleuritis and/or pericarditis) and/or myalgia are more common presenting features of procainamide-induced lupus. By contrast, hydralazine-induced lupus is associated with a higher frequency of skin rashes. However, in any one patient, lupus induced by procainamide, hydralazine, or other drugs can not be distinguished by clinical features. The onset of symptoms can be slow or acute, although an interval of 1 to 2 months typically passes before the diagnosis is made (23 ,24 ,25 ,26 ,29 ,126 ,127).

Because arthralgia is such a common feature of patients in the age group at risk, the presence of other features, such as pleuritis, pleural effusion, fever, splenomegaly, skin rash,

pericarditis, and certain autoantibodies should alert the clinician to consider the diagnosis of DIL. Approximately 50% of patients have constitutional symptoms of fever, weight loss, and fatigue. The symptoms of DIL usually resolve within days to weeks after discontinuing the offending drug and, therefore, this maneuver provides a key (although retrospective) diagnostic tool.

Table 44-5: Prevalence of Clinical and Laboratory Abnormalities in Drug-Induced Lupus and SLE

Feature	Hydralazine-Induced Lupus*	Procainamide-Induced Lupus**	Systemic Lupus Erythematosus†
Symptom			
Arthralgia	80%	85%	80%
Arthritis	50%–100%	20%	80%
Pleuritis, pleural effusion	<5%	50%	44%
Fever, weight loss	40%–50%	45%	48%
Myalgia	<5%	35%	60%
Hepatosplenomegaly	15%	25%	5%–10%
Pericarditis	<5%	15%	20%
Rash	25%	<5%	71%
Glomerulonephritis	5%–10%	<5%	42%
CNS disease	<5%	<5%	32%
Sign			
ANA	>95%	>95%	97%
LE cell	>50%	80%	71%
Antihistoned	>95%	>95%	54%
Anti[(H2A-H2B)-DNA]‡	43%	96%	70%
Antidenatured DNA‡	50%–90%	50%	82%
Antinative DNA	<5%	<5%	28%–67%
Anticardiolipin	5%–15%	5%–20%	35%
Rheumatoid factor	20%	30%	25%–30%
Anemia	35%	20%	42%
Elevated ESR	60%	60%–80%	>50%
Leukopenia	5%–25%	15%	46%
+Coombs' test	<5%	25%	25%
Elevated gammaglobulins	10%–50%	25%	32%
Hypocomplementemia	<5%	<5%	51%

*Data compiled from Alarc-n-Segovia D, Wakim KG, Worthington JW, et al. Clinical and experimental studies on the hydralazine syndrome and its relationship to systemic lupus erythematosus. *Medicine* 1967;46:1–33; Hahn BH, Sharp GC, Irvin WS, et al. Immune response to hydralazine and nuclear antigens in hydralazine-induced lupus erythematosus. *Ann Intern Med* 1972;76:365–374; Cameron HA, Ramsay LE. The lupus syndrome induced by hydralazine: a common complication with low dose treatment. *Br Med J* 1984;289:410–412; and Russell GI, Bing RF, Jones JAG, et al. Hydralazine sensitivity: clinical features, autoantibody changes and HLA-DR phenotype. *Q J Med* 1987;65:845–852.
**Data compiled from Weinstein A. Drug-induced lupus erythematosus. *Prog Clin Immunol* 1980;4:1–21; Harmon CE, Portanova JP. Drug-induced lupus: Clinical and serological studies. *Clin Rheum Dis* 1982;8:121–135; Russell AS. Drug-induced autoimmune disease. *Clin Immunol Allergy* 1981;1:57–76; and Hess EV, Mongey A-B. Drug-related lupus. *Bull Rheum Dis* 1991;40:1–8.
†Derived in part from Wallace DJ. The clinical presentation of systemic lupus erythematosus. In: Wallace DJ, Hahn BH eds. *Dubois' lupus erythematosus.* 4th Ed. Philadelphia: Lea & Febiger, 1993;317–321.
‡From Burlingame RW, Rubin RL. Drug-induced anti-histone autoantibodies display two patterns of reactivity with substructures of chromatin. *J Clin Invest* 1991;88:680–690; Burlingame RW, Boey ML, Starkebaum G, et al. The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J Clin Invest* 1994;94:184–192; and Suzuki T, Burlingame RW, Casiano CA, et al. Antihistone antibodies in systemic lupus erythematosus: assay dependency and effects of ubiquitination and serum DNA. *J Rheumatol* 1994;21:1081–1091.
Each prevalence represents a consensus value ± 5 percentage points. Abnormalities occurring in fewer than 5% of patients are not listed.

In addition to classical DIL with associated antihistone antibodies and polyarthritis (136 ,139 ,140), Cohen, et al. (57) reported that quinidine was associated with a mild drug reaction characterized only by polyarthalgias within 7 days to 3 months after initiation of therapy. These patients were ANA and antihistone antibody negative, and showed prompt recurrence of symptoms upon rechallenge with quinidine.

Lupus-like disease associated with minocycline is also atypical. Patients frequently present with symmetrical polyarthritis and may have evidence of hepatitis (elevated liver transaminases) and pneumonitis (as a result of pulmonary lymphocytic infiltrates) (141 ,142 ,143 ,144), which are assumed to be autoimmune in nature. These patients may not have ANA but frequently have perinuclear antineutrophil cytoplasmic antibodies (pANCA) because of antimyeloperoxidase (145). Nevertheless, as with classical DIL, symptoms and signs resolve after discontinuation of minocyline. Autoimmune

hepatitis in addition to systemic autoimmunity suggestive of DIL has also been associated with atorvastatin (146) and with mesalamine (5-aminosalicylic acid) (147).

The frequency of serologic abnormalities in lupus induced by procainamide and hydralazine are essentially identical (Table 44-5). The immune response in this setting is characteristic and restricted. The most commonly observed abnormality is a positive ANA, which is largely a result of histone-reactive antibodies (148 ,149). These antibodies are presumably responsible for the positive LE-cells reported in the synovial fluid of two patients with procainamide-induced lupus (150), although recent studies could not produce LE-cells in the indirect LE-cell test using sera from patients with procainamide-induced lupus but only with DIL and SLE sera containing anti-H1 antibodies (151). Although generally of low titer, antibodies to denatured (but not native) DNA are also common in DIL. Less common laboratory features include rheumatoid factor (procainamide) (152), circulating immune complexes (153 ,154 ,155) and antineutrophil cytoplasmic antibodies (hydralazine (156 ,157), propylthiouracil (125 ,158) and minocycline (144 ,145), positive Coombs test (methyldopa (34), chlorpromazine (159), and procainamide (152)), complement activation (procainamide) (37 ,160 ,161), hypocomplementemia (quinidine) (57), and a positive lupus band test (LBT) (162). Phospholipid and cardiolipin antibodies, circulating anticoagulant activity, and biologic false-positive serologic test for syphilis (STS) results are discussed below. It should be appreciated that many of these laboratory abnormalities are not linked to symptomatic DIL. Thus, appearance of antibodies to RBC, denatured DNA, and total histones (but not a histone-DNA complex described below) is independent of lupus-like symptoms. Nevertheless, like the clinical features, these autoantibodies are truly drug-induced, and they gradually subside after drug therapy is discontinued.

Other laboratory features noted in a minority of patients include a mild anemia, leukopenia, and thrombocytopenia (37 ,122 ,152 ,163 ,164 ,165), a hypergammaglobulinemia that is not as frequent as in SLE (37 ,163 ,164 ,165 ,166 ,167 ,168 ,169), and an elevated erythrocyte sedimentation rate (ESR) (139 ,153 ,170) which commonly reverts toward normal as symptoms resolve (57 ,139 ,164). Pancytopenia has been reported in association with procainamide therapy (171 ,172), but it is unlikely that these patients had DIL. Agranulocytosis or severe neutropenia develops in about 0.6% of procainamide-treated patients (173), but this condition is serologically and clinically distinct from DIL (174). Hydralazine-induced lupus has been reported to be associated with acute neutrophilic dermatosis (Sweet syndrome) (175 ,176).

Although the spectrum of clinical abnormalities in DIL are indistinguishable from those observed in SLE, the severity of disease is usually milder in DIL. The number of symptoms and their intensities tend to increase with duration of therapy, but patients who have inadvertently remained on procainamide for up to 2 years after onset of polyarthralgias did not progress to fulminant status (5). Interestingly, patients with more severe clinical problems and higher autoantibody levels tended to be treated with procainamide for shorter duration than those with milder DIL (27), suggesting the existence of clinical subsets differing in susceptibility to DIL. Patients with established SLE have been treated with procainamide for long duration without exacerbation of symptoms (124). These observations are contrary to the expectations of the hypothesis that drugs unmask a predisposition to SLE (61), and help to establish the view that DIL is a fundamentally different disease from SLE.

Histone Autoantibodies

Histone-reactive antibodies were discovered to be commonly present in patients with procainamide-induced lupus based on binding in a histone-reconstituted ANA assay (148). Although patients with hydralazine-induced lupus (177) and asymptomatic drug-treated patients (178 ,179) also have ANA, they tend to be negative for antihistone antibodies by the histone reconstituted ANA assay. However, with the advent of solid phase immunoassays using pure histones, it was found that most patients with DIL and asymptomatic patients with ANA (i.e., DIA patients) induced by a variety of drugs including procainamide, hydralazine, chlorpromazine, acebutolol, and isoniazid also have antihistone antibodies (15 ,44 ,136 ,177 ,180). Formal demonstration that histone-reactive antibodies constituted the bulk of the ANA was provided by chromatin-absorption studies (149).

Histones consist of five dissimilar proteins, and the fine specificity of antihistone antibodies in SLE, DIL, and DIA has been examined in detail (see Chapter 24). Considerable disagreement on the characteristic antihistone antibody profile in DIL accumulated (detailed in (181) and in (182)). However, these reports generally indicated that antihistone antibodies react with only limited regions of the protein, especially their N- and/or C-terminal tails, possibly reflecting the solvent accessibility of these regions within the nucleosome. More importantly, the diagnostic value of antihistone antibodies is limited. A positive test for antihistone antibodies may help to confirm a suspicion of DIL, but the conventional assay for antihistone antibodies can not distinguish patients with DIL from patients who develop ANA yet remain asymptomatic (i.e., DIA). In fact recent studies suggest that Ig interaction with solid phase histone occurs by binding via the Fc region of immunoglobulin rather than through the classical, antigen-binding site of specific antibody (183 ,184).

Anti-[(H2A-H2B)-DNA] Antibodies

Histones exist in the cell not as individual proteins but as a huge macromolecular complex termed chromatin which is formed by highly organized histone-histone and histone-DNA interactions. The repeating unit of chromatin structure is the core particle of the nucleosome, which consists of a $(H3\text{-}H4\text{-}H2A\text{-}H2B)_2$ histone octamer wrapped with approximately two turns of DNA (Fig. 44-1).

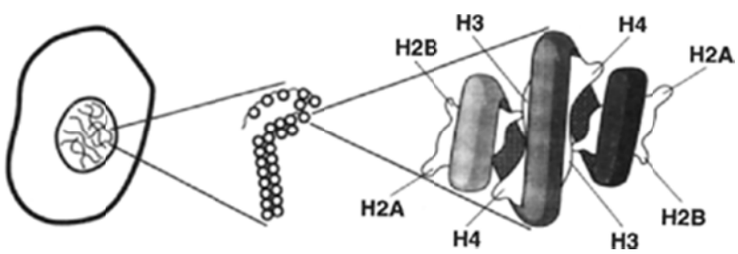

Figure 44-1. Organization of histones in chromatin. The core particle of the nucleosome (right) consists of 8 histone molecules arranged in a tripartite structure (two H2A-H2B dimers and one $(H3\text{-}H4)_2$ tetramer) around which is wrapped 146 base-pairs of DNA. For clarity, the core particle is depicted in an artificially loosened form; the actual structure is compact, and the DNA largely covers everything but the H2A-H2B side faces (190,191). A continuous strand of DNA connects each core particle to its adjacent core particles, forming the “beads-on-a-string” polynucleosomes fiber (center). After higher ordered supercoiling mediated in part by histone H1, the chromatin fiber becomes visible in the light microscope as a mitotic chromosome (left).

The pronounced antigenicity of the H2A-H2B complex, a subunit of the nucleosome, was first detected in SLE patients (192) and subsequently found to dominate the serology of patients with procainamide-induced lupus (6 ,126 ,193 ,194 ,195).These antibodies also react with the components H2A and H2B (126 ,196), but the magnitude of antibody binding to the H2A-H2B complex is often fivefold greater than to the individual histones. A systematic study of the antigenicity of nucleosome subunits revealed that the (H2A-H2B)-DNA complex contained the complete epitope for these antibodies and could largely account for the capacity of procainamide-induced lupus sera to bind to nucleosomes, chromatin, and nuclei (136).

IgG anti-[(H2A-H2B)-DNA] antibodies occur in over 90% of patients who develop procainamide-induced lupus (136 ,185) and have been detected in individual patients with lupus induced by penicillamine (185 ,186), INH (185 ,187), acebutalol (185), methlydopa (120), sulfasalazine (189), opthalmic timolol (189), and lisinopril (197). However, only approximately 50% of patients with quinidine- and 35% with hydralazine-induced lupus have detectable anti- [(H2A-H2B)-DNA] (Table 44-6). IgG anti-[(H2A-H2B)-DNA] has a sensitivity for procainamide-induced lupus of 84% at the time of diagnosis and a prognostic value of 70% 1 year before recognition of symptoms (27).

Table 44-6: Prevalence of IgG Anti-[(H2A-H2B)-DNA] in Lupus Induced by Various Drugs

Lupus-Inducing Drug	Patients with Elevated Anti-[(H2A-H2B)-DNA]	Reference
Procainamide	23/24	(136,185)
Quinidine	9/17	(136,185)
Hydralazine	6/14	(136)
Penicillamine	1/1	(185,186)
Isoniazid	1/1	(185,187)
Acebutalol	1/1	(185)
Methyldopa	2/2	(120,185)
Timolol	1/1	(188)
Sulfasalazine	2/2	(189)
Lisinopril	1/1	(197)

The use of an IgG-specific detecting reagent in testing for anti-[(H2A-H2B)-DNA] is important because procainamide-treated patients who remain asymptomatic commonly produce IgM and/or IgA antibodies of this specificity (27 ,196). There is no evidence that patients with these Ig classes of drug-induced antibodies (and who are ANA positive) have an increased risk for converting to IgG anti-[(H2A-H2B)-DNA] and symptomatic drug-induced lupus. Additionally, asymptomatic, procainamide-treated patients who develop DIA commonly have low levels of IgG antibodies to the DNA-free H2A-H2B complex (126 ,195 ,196 ,198), because of antibodies to the individual histones H2A and/or H2B. Therefore, a sensitive test for DIL that will exclude DIA patients requires employing the complete (H2A-H2B)-DNA complex.

Denatured DNA Autoantibodies

Denatured (single-stranded) DNA (dDNA) autoantibodies are found in up to 50% of DIL sera (31 ,129 ,130 ,163 ,168 ,185 ,193 ,196 ,199 ,200). These autoantibodies probably have multiple reactivities, which have also been identified as binding to the nucleoside guanosine (126 ,201), polyriboadenylic acid (31 ,196), the phospholipid cardiolipin (202), and unusual conformations of DNA, such as Z-DNA (196 ,203). Even antibodies directed to lymphocytes described in hydralazine- (204) and procainamide-induced

lupus (205 ,206) may be related to the dDNA (or possibly chromatin) reactivity, because lymphocyte membranes can bind DNA (207). Anti-dDNA antibodies bearing the 16/6 and 32/15 idiotypes have been reported in one third of procainamide-induced lupus patients (208), idiotypes which are also commonly expressed in idiopathic SLE. However, the clinical significance of anti-dDNA is doubtful, because these antibodies are commonly seen in asymptomatic (normal) people and in those with a wide variety of rheumatic and inflammatory conditions (209).

Antibodies to Drugs

Antibodies to the offending drugs have been reported in lupus induced by procainamide (210) and hydralazine (130). Other studies have been unable to detect drug-specific antibodies in patients treated with various lupus-inducing drugs (149 ,167 ,199 ,211). In a prospective study of patients treated with hydralazine, only 1 of 27 sera bound to the drug (31). The significance of antibodies to drugs is unclear, because they have been reported in varying amounts in asymptomatic and symptomatic patients. Additionally, it is unlikely that drug-binding antibodies represent cross reactions with anti-dDNA or antichromatin autoantibodies (211 ,212 ,213).

Phospholipid Antibodies

The observation that DIA patients can have antiphospholipid (cardiolipin) antibodies, circulating anticoagulants, or a biologic false-positive STS activity is noteworthy because much attention has focused on the clinical and pathogenic significance of these autoantibodies. The presence of the lupus anticoagulant (LAC) or of cardiolipin antibodies has been described in patients on hydralazine (214 ,215), procainamide, (216 ,217 ,218 ,219 ,220), chlorpromazine (43 ,159 ,221 ,222 ,223 ,224 ,225 ,226), quinidine, and quinine (226). In one study, up to 75% of patients treated with chlorpromazine for up to 2.5 years developed a lupus anticoagulant (159). Canoso and deOliveira (43) reported that 54 of 93 chlorpromazine-treated psychiatric patients had IgM LAC activity. Of the 54 LAC-positive patients, 31 also had IgM cardiolipin antibodies, 4 also had IgG cardiolipin antibodies, and 5 had cardiolipin antibodies alone. During a mean follow-up period of 5 years, thrombotic events occurred in three patients (one with LAC, two with IgM cardiolipin antibodies). In a Canadian study, Lillicrap et al. (227) evaluated 97 psychotic patients treated with chlorpromazine, fluphenazine, or promazine. Of these, 25% developed a positive ANA, 4% had elevated titers of antibodies to cardiolipin and phosphatidylserine, and 5% had elevated titers of antibodies to phosphatidylinositol. None of the patients developed features of SLE or evidence of thrombotic events. Drug-induced LAC in a group of 13 patients had a narrower range of specificities than LAC in SLE or in primary antiphopholipid syndrome, rarely reacting with phosphatidylserine or phosphatidylcholine (226). None of these patients had thrombosis, indicating that there is no increased risk of thrombotic events associated with chlorpromazine- or quinidine-induced LAC. The antibody isotype in drug-induced LAC is usually IgM (43 ,159 ,221) as was the associated ANAs (41). It is likely that these observations also apply to procainamide and hydralazine, because thrombosis appears to be a rare clinical event in lupus induced by these drugs as well (228). The clinician should be cautious, however, because recurrent thrombotic events have been reported in phenothiazine- (229) and occasional procainamide-induced (216 ,220) lupus patients. It has not been determined whether the antiphopholipid antibodies associated with DIL interact with β_2-glycoprotein I, a correlative feature of SLE patients with a history of thrombembolisms (230).

The origin of lupus anticoagulants and cardiolipin antibodies in patients on various drugs, especially those in the phenothiazine group, is unknown. The pharmacologic action of drugs such as procainamide and chlorpromazine (Table 44-2) depends in part on their membranotropic nature, intercalation into plasma membranes, and interaction with phospholipids or lipoproteins (225 ,231 ,232). Based on these features, it has been postulated that the binding of the drug lipid moieties exposes cryptic epitopes or creates neoantigens that then serve as the stimulus for the production of antiphospholipid antibodies (225). However, since antiphospholipid antibodies are also common in SLE patients (Chapter 27), induction of lupus anticoagulants in DIL probably does not involve a drug-altered antigen mechanism.

Other Autoantibodies

Rheumatoid factor (RF) has been reported in procainamide- induced lupus patients (163). Prospective studies suggest that RF is not drug-induced, and might merely reflect the increased prevalence of this autoantibody in the population treated with the drug (155 ,233). One study has reported antibodies in DIL sera directed against poly(adenosine diphosphate-ribose) (234). Nassberger et al. (156 ,157 ,235) first demonstrated neutrophil myeloperoxidase (MPO) and elastase antibodies in hydralazine-induced lupus patients, and this has been confirmed (236); these specificities as well as antiproteinase-3 antibodies were also reported in patients treated with propylthiouracil (125 ,158), and anti-MPO antibodies were observed in patients with lupus induced by sulfasalazine (237) and minocycline (145). These observations are of considerable interest, because these antibodies contribute to the p- and c-ANCA neutrophil staining patterns that is associated with vasculitis of the capillaries and Wegener granulomatosis, respectively (238). The occurrence of glomerulonephritis in some patients with hydralazine-induced lupus (Table 44-5) may be related to anti-MPO antibodies (125 ,235 ,236). Although anti-MPO antibodies occur in more than 50% of patients

with hydralazine-induced lupus, they are not found in lupus induced by procainamide (unpublished observations), consistent with the absence of kidney disease in procainamide- induced lupus. Antinuclear ribonucleoprotein (RNP) antibodies were reported after short-term (prophylactic) procainamide treatment (38). This observation has not been confirmed, and anti-RNP antibodies have not been observed in DIL (148 ,149 ,167 ,199). Slightly elevated antinative DNA antibodies were observed in four patients with mesalamine- induced lupus (147). Cold-reactive lymphocytotoxic antibodies (LCTA) have been reported in procainamide-induced lupus (205 ,206), but these reactivities were also detected in procainamide-treated patients at initiation of therapy and in asymptomatic procainamide-treated patients (31 ,149 ,155), and are unrelated to DIL. LCTA were also reported in four of seven patients with hydralazine-induced lupus, but also in 13 of 40 asymptomatic hydralazine-treated patients (28 ,204). Antibodies to high mobility group (HMG) proteins, especially HMG-14 and -17, were detected in approximately half the patients treated with procainamide, hydralazine, and quinidine, whether or not they expressed symptomatic DIL (239). This finding is of interest, because the HMG proteins bind to the core particle of the nucleosome especially on transcriptionally active chromatin, consistent with the central role of chromatin in the autoantibody response in drug-treated patients.

Nature of the Immune Response in Drug-Induced Lupus

Humoral Response

Numerous features of DIL point to the activation of the humoral immune system as its principal overt abnormality. As discussed above, the autoimmune response in DIL is largely restricted to antibodies reactive with ((H2A-H2B)-DNA) in native chromatin and to denatured histone and DNA components of nonnative chromatin. IgM, IgA, and IgG anti-[(H2A-H2B)-DNA] antibodies often appear to arise simultaneously during procainamide treatment, although patients who remain asymptomatic fail to develop IgG of these specificities (27). In a normal humoral immune response, recognition of a foreign antigen results in rapid clonal expansion of B cells, isotype switching to IgG, and somatic mutation to higher affinity antibodies. With drug-induced autoantibodies there appears to be a slow development of autoantibodies of the IgG, IgA, and IgM isotypes or the perpetuation of IgM autoantibodies for many years in asymptomatic, procainamide-treated patients (6 ,27), suggesting a weak adaptive immune response and/or evidence of homeostatic downregulation of autoimmunity. Nevertheless, the restriction in the immune response to chromatin-derived antigens suggests that B cells develop with immunoglobulin receptors for certain parts of chromatin, possibly the highly solvent exposed (H2A-H2B)-DNA region. In mouse models of SLE autoantibody secretion has been shown to require T cell help (see Chapters 9 and 18), and drug-induced autoantibodies are likely to have a similar requirement. In fact, initiation of autoimmunity by drugs may be at the T cell level (see Mechanisms) with activation of autoreactive B cells merely a manifestation of this process. This view is supported by the observation that serum autoantibodies have an apparent half-life of 2.5 to 5.0 months after withdrawal of procainamide (27), approximately five times longer than the stability of immunoglobulin in the circulation, suggesting persistent, autoreactive T cell help independent of the drug. Also, increased numbers of $CD4^+$ T cells of the $CD29^+$ activated phenotype along with soluble IL-2 receptor were detected in the pleural effusion of a patient with procainamide-induced lupus (169), consistent with the involvement of T cell help in DIL.

Cellular Immune Response

Studies of in vivo cellular immune abnormalities in procainamide- and hydralazine-treated patients have been minimal and conflicting. Forrester et al. (240) observed increased spontaneous IgM and especially IgG secretion by circulating B cells from procainamide-induced lupus patients compared to B cells from patients with DIA or from normals, but whether polyclonal B cell activation was being measured in patients with DIL or these were plasma cells secreting specific autoantibody was not distinguished. This result is consistent with older studies that failed to detect antibody-secreting cells in the circulation of procainamide-treated, asymptomatic patients (241 ,242). Although some or most of these patients would be expected to have serum ANAs (i.e., display DIA), whether these B cell assays are too insensitive or antibody-secreting cells are primarily noncirculating and reside in lymphoid organs is unclear. One study reported that B cells from procainamide-treated patients were hyper-responsive to pokeweed mitogen (242), although just the opposite effect (241) or no difference (243) was seen by others. Reports of B cell responses from procainamide-treated patients to procainamide in vitro have also been discrepant, with some detecting an increase (205 ,244) and others no effect (245 ,246). Only marginal or no capacity of hydralazine to activate peripheral blood lymphocytes from patients with hydralazine-induced lupus was observed (130), although another study reported that lymphocytes from 50% of asymptomatic hydralazine-treated patients showed a three- to fourfold stimulation index in the presence of hydralazine- albumin conjugates (31). Taken together, these older observations argue against the development of drug-specific T or B cells that would have been expected in a drug hypersensitivity response as discussed (247), consistent with other features of DIL such as its slow kinetics (delay of months to years from the onset of drug therapy to the development of autoantibodies and clinical symptoms), correlation with drug dose and the inconsistent recurrence of symptoms upon drug rechallenge.

Diagnostic Criteria for Drug-Induced Lupus

Specific criteria for the diagnosis of DIL have not been formally established. Although some of the criteria for the classification of SLE are applicable to DIL, the requirement for four manifestations as established by the American College of Rheumatology (ACR) (248) is overly rigid for DIL and is obviously not useful for distinguishing between drug-induced and idiopathic SLE. Patients with DIL often do not fulfill criteria for SLE, and a diagnosis of DIL can readily be missed if SLE diagnostic criteria are strictly applied. In particular, symptoms common to SLE such as malar or discoid rash, photosensitivity, oral ulcers, alopecia, and renal or neurologic disorders are very unusual in DIL. Patients frequently present with mild or few lupus-like symptoms that typically worsen the longer the patient is maintained on the implicated drug, so that patients could readily be underdiagnosed if SLE criteria are strictly applied.

Table 44-7 shows guidelines for a diagnosis of drug-induced lupus.

Elaboration of Diagnostic Guidelines

Treatment Duration

Drug-induced lupus usually occurs after several months or years of continuous therapy and should not be confused with the short-term toxic side effects that are often suffered by patients treated with pharmaceuticals. Time for manifestation of lupus-like symptoms varies greatly among drug treated patients—for procainamide, a median of 10 months was calculated, although one fourth of the 50 patients did not develop symptoms until 2 or more years of therapy (37); for hydralazine, the majority of patients require 6 months to 2 years of exposure (33), but it is not uncommon for a patient to be treated for more than 3 years before symptoms become manifested. This variation may be largely a result of differences in the steady state drug concentrations employed in order to maintain therapeutic control, but genetic factors may also be involved as discussed below.

Table 44-7: Guidelines for Diagnosis of Drug-Induced Lupus

1. Continuous treatment with a known lupus-inducing drug for at least 1 month and usually much longer.
2. Presenting symptoms:

   Common: arthralgias, myalgias, malaise, fever, serositis (pleuropericarditis, especially with procainamide), polyarthritis (especially with quinidine and minicycline).

   Rare: rash or other dermatologic problems, glomerulonephritis (primarily with hydralazine).
3. Unrelated symptoms suggestive of SLE: multisystem involvement especially neurologic, renal, and skin symptoms.
4. Laboratory profile

   Common: ANA which is a result of antihistone antibodies especially IgG anti-[(H2A-H2B)-DNA], leukopenia, thrombocytopenia and mild anemia, increased erythrocyte sedimentation rate;

   Absent or rare: antibodies to native DNA, Sm, RNP, SS-A/Ro, SS-B/La, hypocomplementemia.
5. Improvement and permanent resolution of symptoms generally within days or weeks after discontinuation of therapy. Serologic findings, especially autoantibody levels, often require months to resolve.

Symptoms

In some patients, symptoms gradually appear and worsen over the course of many months of treatment with the implicated drug, whereas in others, symptom onset is rapid. In one study of 21 patients, diagnosis of procainamide- induced lupus was based on one symptom in 20% of patients, two symptoms in 25%, and three or more symptoms in 55% of patients (126). Some suggestion of a drug-specific symptomatology in addition to the usual musculoskeletal and constitutional symptoms is suggested by the literature, with pleuritis and pericarditis common to procainamide-induced lupus, polyarthritis common to quinidine- and minocycline-induced lupus, glomerulonephritis, and rash reported in hydralazine-induced lupus and autoimmune hepatitis in lupus related to minocycline (141 ,142 ,144) and atorvastatin (146). Lung involvement in procainamide-induced lupus occurs in approximately 50% of patients and consists of pleuritis, pleural effusions and/or pulmonary infiltrates; pericardial effusions are also common. In most patients symptoms are mild, although indistinguishable from SLE and consist of fever, malaise, weight loss, polyarticular arthralgias, and symmetric myalgias.

Probable Exclusionary or Unrelated Symptoms

Central nervous system (CNS) disease is distinctly uncommon in drug-induced lupus, but because of possible independent neurotoxic effects of drugs or the occurrence of stroke, convulsions or dementia syndromes in the elderly commonly treated with these drugs, CNS disease should not be an exclusion criterion. Similarly, although serious kidney disease is hardly ever reported for most lupus-inducing drugs, glomerulonephritis has been associated with hydralazine-induced lupus (235 ,249 ,250 ,251 ,252) so should also not be a formal exclusion criterion. In the latter cases, it is difficult to distinguish hydralazine-induced glomerulonephritis from that related to the underlying hypertension for which hydralazine was administered. Mucocutaneous manifestations are also rare in DIL. Although a history of rheumatologic disease independent of the suspected drug tends to negate a diagnosis of DIL, obviously a patient can have two diseases. This situation is characteristic of patients

with various forms of arthritis who also develop DIL from penicillamine, sulfasalazine, minocycline, or TNF-α inhibitor therapy. In these difficult cases, serologic findings are especially informative.

Laboratory Abnormalities

Most patients with DIL have ANA, which are largely restricted to histone-containing antigens (148). IgG antibody to the (H2A-H2B)-DNA complex is an especially sensitive marker for lupus induced by a large variety of drugs except hydralazine (Table 44-6). This test is particularly useful for distinguishing asymptomatic drug-treated patients who develop benign ANA (i.e., DIA) from patients with symptomatic DIL because only the latter have IgG anti-[(H2A-H2B)-DNA] antibodies (27). Some patients with DIL also have mild leukopenia, thrombocytopenia, and/or anemia and elevated sedimentation rate, but rarely hypocomplementemia. Approximately 1% of procainamide-treated patients will present with neutropenia, but these patients do not have classical DIL (173 ,174). Although antihistone and anti-[(H2A-H2B)-DNA] antibodies are also common in SLE (137), these patients are rarely monospecific for this activity. Therefore, when a diagnosis of SLE or DIL can not be clearly distinguished on clinical grounds, the presence of antibodies to native DNA, Sm, RNP, SS-A/Ro, SS-B/La, or other nuclear antigens should be considered as evidence against a diagnosis of DIL.

Symptom Resolution

Resolution of symptoms and laboratory abnormalities by withdrawing the offending drug is a defining feature of DIL. This simple manipulation and follow-up is obviously a retrospective diagnosis but can be very reassuring. Although there is a strong temptation to treat patients suspected of DIL with anti-inflammatory agents (see Treatment), this maneuver may confound the diagnosis and should not be required for recovery from DIL. Although autoantibody activity also resolves after discontinuation of therapy, these abnormalities often take much longer than symptom resolution and can still be present 1 to 2 years after withdrawal of therapy. However, quantitative measurements of autoantibodies should show a systematic decline in activity once the causative agent is withdrawn.

Differential Diagnosis

One concern of clinicians is how to differentiate DIL from SLE, and from other systemic rheumatic diseases. The reasons for this concern are threefold. First, the elderly SLE patient often does not present with the classic features of SLE (e.g., butterfly rash, glomerulonephritis) (253 ,254). Indeed, the clinical features of SLE in the elderly and DIL have significant overlap. Second, the treatment of DIL is generally straightforward, requiring only withdrawal of the drug and short-term anti-inflammatory therapy, whereas the treatment of SLE can involve the prolonged use of corticosteroids or other immune modulators. Third, the outcome of DIL is better than SLE because these patients rarely, if ever, develop renal or neurologic disease. As discussed above, SLE is usually characterized by a much broader array of autoantibodies than is DIL, so the serologic profile can be very helpful in these difficult cases.

Because the clinical features of DIL are protean, a differential diagnosis should be considered (164). Viral syndromes and infectious diseases may present with arthralgia, fever, and pleuropericarditis. Dressler syndrome should be considered in a patient with a previous myocardial infarction. An additional ischemic myocardial event may present with fever and pericarditis. The postpericardiotomy syndrome, which may occur after cardiac surgery and is similar to Dressler syndrome, can be confused with DIL because these patients are often treated with antiarrhythmics such as procainamide. Other diagnoses to be considered in the appropriate setting are rheumatoid arthritis, polymyalgia rheumatica, underlying malignancy, adverse or hypersensitive drug reactions, and GVHD.

Treatment

Once the diagnosis of DIL has been established, the first step is discontinuation of the offending drug. Treatment with anti-inflammatory agents, including corticosteroids, may be indicated for those with severe manifestations of the disease such as pericarditis with tamponade, inflammatory pleural effusions, or debilitating polyarthritis. The judicious use of nonsteroidal anti-inflammatory agents and corticosteroids in the elderly is important because of potential side effects. The prolonged use of high doses of corticosteroids, or the use of chloroquine, hydroxychloroquine, or immunosuppressive agents is not indicated in the treatment of DIL. If DIL is associated with the rare feature of glomerulonephritis, as reported with hydralazine-induced lupus (235 ,249 ,250 ,251 ,252), corticosteroids can be used. Although some DIL patients have been restarted on procainamide or hydralazine without incident, this is not advisable if insufficient time (which may be as long as 1 year) has elapsed since the first episode of DIL (see Rechallenge Studies).

Clinicians are often consulted to consider the safety of drugs associated with DIL in the treatment of patients with idiopathic SLE. The use of hydralazine to treat hypertension in SLE patients has not been associated with exacerbations of the disease (13). Prockop (50) described an SLE patient treated for myotonia with 4 g procainamide per day for 15 months with no exacerbation of SLE despite the occurrence of lupus flares before and after the period of drug administration. The use of anticonvulsants to treat seizure disorders in SLE patients has not been associated with flares or acceleration of disease activity. INH has been given to SLE patients on corticosteroids without aggravating lupus (164). Despite the apparent safety of these drugs in the setting of SLE, the clinician should use the drugs

judiciously and carefully document the clinical and serologic status of the patient being considered for treatment.

Genetic Factors

HLA Phenotype

The immunogenetic factors that underlie DIL are of interest because only a small proportion of drug-treated patients develop symptomatic disease, and the immune response is restricted to a relatively narrow range of autoantigens. These observations implicate a role for the class II major histocompatibility complex (MHC) human leukocyte antigens (HLA), which are required for T cell–dependent antibody responses. In a recent study of 13 patients with minocyline-induced lupus, all the patients had either HLA-DR4 or HLA-DR2 of subtypes that share a structural similarity ($p < 0.01$ vs. normals) (145). A study of 25 hydralazine-induced lupus patients by Batchelor et al. (128 ,255) showed a 73% frequency of HLA-DR4 versus a frequency of 25% in asymptomatic patients, representing a relative risk of 8.1. Another study using some of these same patients found a 70% frequency of HLA-DR4 in hydralazine-induced lupus patients (36). HLA-DR4 has been reported in individual patients with penicillamine-induced lupus (256 ,257), hydralazine-induced Sweet syndrome (175) and atorvastatin-induced lupus (146). However, hydralazine-induced lupus patients from Australia (258) and a limited study of American procainamide-induced lupus patients (126) failed to find a significantly increased incidence of any HLA markers. Re-examination of the hydralazine-induced lupus patients in the English study for complement protein phenotypes demonstrated that 76% of these patients had one or more C4 null alleles compared to 43% of normal controls ($p < 0.01$) (259). The genes encoding the C4 complement proteins are situated between the HLA-B and HLA-DR loci, and the C4 null/DR4 haplotype displays linkage disequilibrium in Caucasians. Therefore, the reported association of hydralazine-induced lupus with HLA-DR4 is probably a result of the C4 null trait (260), and linkage disequilibrium between HLA-DR4 and C4 may not occur in the Australian study group.

In chlorpromazine-treated patients, HLA-B44 was a significant risk factor in the induction of ANA (relative risk = 3.6) (261) and LAC (relative risk = 2.1) (262), and HLA-DR7 was also weakly associated with chlorpromazine-induced LAC (262). Among procainamide-treated patients, there was a significant association between HLA-DQw7 and IgG antibodies to histones and H2A-H2B (198). These results may suggest that these class II MHC antigens have a propensity to present histone peptides to T cells. However, because most patients treated with hydralazine or procainamide develop ANA, it is unlikely that paticular HLA allotypes are necessary for autoantibody induction by these drugs. More controlled studies using DNA typing for MHC class II alleles rather than the less accurate serological typing are required to determine whether MHC genes are important in the development of DIL.

Complement

The complement genes are part of the MHC class III region, and C4A and C4B are encoded by separate loci. Individuals with genetic deficiencies in one, and especially both, C4 genes have greatly increased susceptibility to SLE (263) (see Chapters 6 and 13), presumably because of insufficient classical pathway activation, resulting in poor immune complex clearance. As mentioned above, 76% of hydralazine-induced lupus patients had one or more C4 null alleles compared to 43% of normal subjects (259), suggesting a congenital defect in immune complex clearance may also predispose to DIL. It has not been determined, however, whether patients treated with hydralazine who remain asymptomatic have a low frequency of C4 null alleles. Similarly, it is not clear whether the occasional hypocomplementemia associated with procainamide-induced lupus (264) may be related to C4 deficiency. In most patients with DIL, serum complement levels are within the normal range (154). Interestingly, the null alleles of either C4A or C4B were also significantly more frequent in patients with procainamide-induced lupus than in normals at $p = 0.05$ (198).

A small but significant reduction ($p < 0.01$) in the mean number of type 1 complement receptors for C3b on erythrocytes (CR1) was observed in patients with a prior history of hydralazine-induced lupus, and these individuals tended to have elevated circulating immune complexes (154). However, based on restriction fragment polymorphism analysis, there was no difference between symptomatic and asymptomatic hydralazine-treated patients in the frequency of the allele encoding the low expression CR1 phenotype (265), indicating that hydralazine-induced lupus patients may have acquired a deficiency (perhaps a result of persisting immune complexes) in CR1 expression. However, measurements of Fc-receptor-mediated immune clearance in patients treated with chlorpromazine, procainamide, penicillamine, or hydralazine with or without associated symptomatic disease demonstrated no differences and normal clearance function (153). These data suggest that, unlike in SLE, handling of circulating immune complexes by the reticuloendothelial system in DIL is not overloaded, possibly explaining the lack of kidney and CNS disease in DIL.

Acetylator Phenotype

Acetylator phenotype is the best described genetically determined predisposing factor in DIL, but its significance is often misunderstood. The studies of Perry et al. demonstrated that the level of hepatic acetyltransferase activity was inversely associated with the likelihood for development of DIL (30). The acetyl group of acetyl-CoA can be transferred to the amino group of many small molecules by the action of acetyltransferases. North American white and black populations can be almost evenly divided into slow or fast acetylators based on their acetyltransferase activity. The acetylator phenotype can be determined by administering

a tablet of dapsone, INH, or caffeine to a patient and analyzing the serum or urine for acetylated and unacetylated drug. A preferable method is to determine acetylator genotype by amplyifying lymphocyte genomic DNA by polymerase chain reaction followed by restriction nuclease digestion. Slow acetylators are homozygous for a recessive gene that controls hepatic acetyltransferase activity and have an approximately twofold higher serum level of unacetylated drugs at equivalent therapeutic doses.

Compared to rapid acetylators, autoantibodies and clinical symptoms develop more quickly and in higher frequency during the treatment of slow acetylators with hydralazine (30 ,128) and procainamide (24), although in a recent study seven out of nine patients (78%) with procainamide-induced lupus were rapid acetylators (6). Development of clinical symptoms can occur in up to 20% of rapid acetylators (36), but both the dose and duration of drug administration are generally higher in these patients (24). These studies generally support the hypothesis that the steady-state concentration of unacetylated procainamide and hydralazine and the duration of exposure are important elements in the development of DIL. Such observations have led to the use of N-acetyl procainamide (NAPA) in the successful control of cardiac arrhythmias while bringing about the remission of procainamide-induced lupus (266). NAPA did not induce lupus, ANA, or anti-dDNA (267 ,268). Furthermore, if the procainamide dose was adjusted so that all patients have the same steady state plasma concentration by increasing the dose for rapid acetylators, no difference was observed between slow and rapid acetylators in the time for development of ANA or DIL (23). Nevertheless, a recent study did not observe a tendency for the use of higher procainamide dose in rapid acetylators (6).

The importance of the free amino/hydrazino group in these drugs in the development of autoantibodies and lupus has been interpreted in two ways. A commonly held view is that these chemical moieties play a direct role in the induction of autoimmunity, and acetylation prevents this action. Alternatively, in vivo metabolism (other than acetylation) of the drug at this moiety generates the active, autoimmunity-inducing compound, and that N-acetylation blocks drug metabolism. With this view, the putative reactive metabolites, rather than the parent molecule, would interact with a key immune target, leading to induction of autoimmunity (see Oxidative Drug Metabolism). The association of the slow acetylator phenotype with symptomatic DIL can then be explained by a higher steady-state concentration of the metabolizable form of the drug. Although INH, like hydralazine, is a substrate for hepatic acetyltransferase, there is no difference in the development of ANA in fast and slow acetylators on INH therapy (269). The lack of an association between acetylator phenotype and induction of ANA by INH (269) or by captopril (270) as well as idiopathic SLE (271), indicates that the slow acetylator phenotype is not a general predisposing factor for the autoimmune state, nor is it genetically linked to a putative autoimmunity-inducing or autoimmunity-accelerating gene.

Gender and Race

As discussed under Epidemiology, lupus induction is three- to fourfold more likely in females than males and four- to sixfold more likely in whites compared to blacks.

Oxidative Drug Metabolism

The following features of drug-induced autoimmunity are difficult to explain by a direct action of the ingested, parent compound on some component of the immune system:

- Lupus-inducing drugs are highly diverse in chemical structure (Fig. 44-2) and pharmacologic action, yet the laboratory and clinical features of lupus induced by all the drugs are essentially the same.
- Except for their pharmacologic action, lupus-inducing drugs are largely inert at normal doses; nonspecific or generalized toxicity would preclude their use as therapeutic agents. DIL is an idiosyncratic drug reaction not predicted by any known property of the implicated drugs.
- Drugs reach a steady-state concentration within a few hours, but drug-induced autoimmunity and lupus require many months for manifestation.

The requirement for metabolic transformation of the ingested drug to a reactive compound would account for many of the features of DIL. In vivo metabolism of dissimilar drugs to a product with a common, reactive property could explain how compounds with widely different pharmacologic and chemical characteristics could produce the same adverse reaction. The low probability for a productive metabolic event could explain the long lag time for autoimmunity to unfold.

Incubation of procainamide with human or rat liver microsomes that contain the “mixed function oxidases” results in the formation of an unstable product, procainamide-hydroxylamine (PAHA) (272 ,273), and PAHA can be detected after the perfusion of rat liver with procainamide in a blood-free environment (274). Hydralazine and isoniazid are also susceptible to hepatic oxidative metabolism (275 ,276). PAHA enters RBCs, and oxyhemoglobin enhances its biologic activity (245 ,274), presumably by converting PAHA to nitro-procainamide (277 ,278). Hepatic metabolism of drugs to reactive products demonstrates that the chemistry exists for generation of potentially toxic compounds, but these products typically bind to microsomes or macromolecules near their site of formation and fail to exit the liver in reactive forms. Although rats treated with procainamide showed increased liver lipid peroxide levels and antioxidant activity (279), hepatotoxicity is generally not associated with drug-induced autoimmunity or lupus, making it unlikely that sufficient amounts of reactive metabolites of lupus-inducing drugs are generated in the liver to interact with resident lymphocytes or inflammatory cells. Although drug metabolites might bind to self-antigen in the liver, there is not

enough structural information in such small molecules to specifically interact with the chromatin-derived targets that characterize the autoimmune response in DIL.

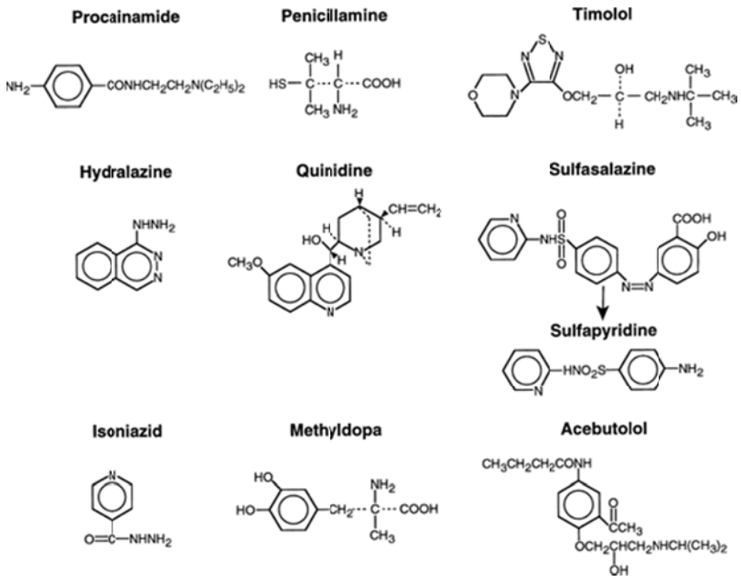

Figure 44-2. Structures of drugs reported to induce lupus and anti-[(H2A-H2B)-DNA] antibodies. Sulfasalazine undergoes hydrolysis in the intestine to 5-aminosalicylic acid and sulfapyridine, which is absorbed and the candidate agent for inducing lupus. However, cases of lupus induced by 5-aminosalicylic acid (mesalamine) have been reported (147). Although four drugs are aromatic amines (procainamide and sulfapyridine) or hydrazines (hydralazine and isoniazid), the chemical structures of drugs with capacity to induce lupus are highly diverse.

Phagocytic leukocytes including neutrophils, monocytes, macrophages, and skin Langerhans cells have drug metabolizing capacity because of the presence within these cells of various enzymes with promiscuous substrate properties such as myeloperoxidase, prostaglandin H synthase or, less commonly, the cytochrome P450s. Rubin et al. (280) showed that activated peripheral blood neutrophils have capacity to metabolize procainamide to PAHA in the extracellular milieu; the role of the respiratory burst and degranulation events associated with neutrophil and macrophage activation was confirmed (281) and analyzed in detail (282). Neutrophils are clearly the greatest drug-metabolizing engine outside the liver because of their preponderance in the circulation, apparently unlimited hematopoietic regenerative capacity, ability to freely circulate and populate essentially any organ or tissue including lymphoid tissue where autoimmunity presumably develops, and capacity to generate in response to stimulants a robust extracellular oxidizing machinery. It is this latter feature that is particularly important because generation of reactive drug metabolites outside a cell sets up a condition for delivering these agents some distance from their immediate site of formation.

Essentially all pharmacologic classes of lupus-inducing drugs (Table 44-1) but not their nonlupus-inducing analogues have been demonstrated to undergo oxidative metabolism by activated neutrophils including procainamide, hydralazine, phenytoin, quinidine, dapsone, propylthiouracil, penicillamine, chlorpromazine, INH, and carbamazepine (281 ,282 ,283 ,284 ,285 ,286 ,287 ,288 ,289 ,290 ,291 ,292 ,293 ,294). The general mechanism responsible for drug transformation is shown in Figure 44-3 . Activation of neutrophils by opsonized particles or certain soluble factors triggers the ectoenzyme NADPH oxidase to

produce superoxide anion (O_2^-) in the extracellular environment. O_2^- spontaneously dismutates to hydrogen peroxide (H_2O_2). Degranulation often follows, releasing myeloperoxidase (MPO). If a drug with an appropriate functional group is present, it will participate in electron transfer with the H_2O_2-MPO intermediate. Consequently, the functional group accepts an oxygen atom from H_2O_2, resulting in a new compound. Neutrophil-mediated drug metabolism by this mechanism requires the enzymatic action of MPO, as evidenced by the competitive inhibition of MPO activity by all lupus-inducing drugs tested and the correlation of this property with neutrophil-dependent drug cytotoxicity (Table 44-8) (293). The instability of these drug metabolites precludes their detection in vivo, but in vitro generated reactive oxidative intermediates of procainamide (277 ,278 ,295), hydralazine (290), chlorpromazine (291), and isoniazid (276) have been identified. Reactive intermediates of lupus-inducing drugs are strong candidates for triggering autoimmunity.

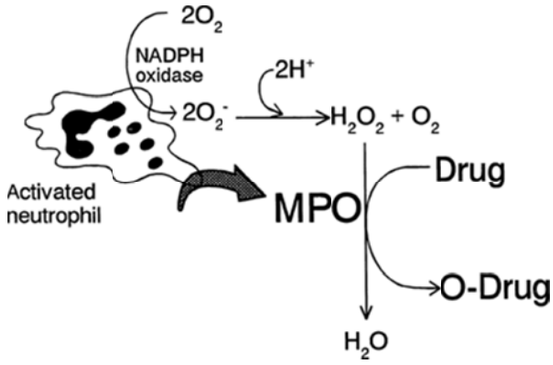

Figure 44-3. Mechanism for transformation of drugs by activated neutrophils (see text).

Table 44-8: Correlation Between Lupus-Inducing Propensity and Neutrophil-Dependent Drug Metabolism Mediated by Myeloperoxidase (MPO)*

Drug	Lupus-Inducing Incidence (%)	Neutrophil-Mediated Cytotoxicity (%)**	Inhibition of MPO Activity (%)**
Procainamide	15–20	76 ± 11	17 ± 5
N-Acetylprocainamide	0	-3 ± 3	3 ± 2
Hydralazine	5–10	37 ± 4	95 ± 5
Phthalazine	N.T.	-3 ± 1	2 ± 1
Quinidine	<1	26 ± 4	21 ± 7
Quinilone	N.T.	1 ± 1	0 ± 3
Chlorpromazine	<1	30 ± 7	29 ± 8
Promazine	N.T.	-5 ± 1	3 ± 5
Isoniazid	<1	24 ± 3	40 ± 5
Isonicotinamide	N.T.	1 ± 1	1 ± 3
Propylthiouracil	<1	63 ± 11	87 ± 1
Propyluracil	N.T.	-4 ± 2	0 ± 2

*Derived from Jiang X, Khursigara G, Rubin RL. Transformation of lupus-inducing drugs to cytotoxic products by activated neutrophils. *Science* 1994;266:810–813.

**Neutrophil-dependent drug cytotoxicity measures the capacity of the indicated drug in the presence of activated neutrophils to affect the viability of a target cell line and is expressed as the percent of cells killed by the drug metabolite. Inhibition of MPO activity measures the capacity of the drug to act as a competitive inhibitor of the enzymatic activity of MPO. Both in vitro assays were measured at a drug concentration of 10 μM. Drugs are grouped as pairs with the incidence of drug-induced lupus indicated for the upper drug, whereas the lower member being the an inactive analogue or metabolite.

N.T. = not tested.

Although it is difficult to prove that neutrophil-mediated bioactivation of drugs occurs in vivo, the frequent finding of anti-MPO antibodies in patients with lupus induced by hydralazine (125 ,235 ,236), propyylthiouracil (125 ,158), and minocycline (145) is consistent with an autoimmune reaction initiated by drug bioactivation mediated by neutrophil-derived MPO. Indirect evidence that neutrophil- mediated drug metabolism is required for induction of autoimmunity is suggested by the strong correlation between MPO-mediated oxidative drug transformation and propensity of the drug to induce lupus (Table 44-8). Nitro-procainamide, a further oxidation product of PAHA (277 ,278), has been detected in the urine of procainamide- treated patients (198 ,296). Urinary metabolites of hydralazine have also been described (233 ,297). Murine T cells sensitized to oxidative metabolites of procainamide (298), propylthiouracil, or gold(I) thiomalate (299 ,300)

displayed specific responses to lysates of phagocytic cells (which were derived from mice subjected to long-term treatment with the respective parent compound), but did not respond to the parent compound itself. These data suggest that lupus-inducing drugs undergo oxidative metabolism in vivo, implicating these products in the induction of autoimmunity. N-acetylation of hydralazine and procainamide competes with N-oxidation of these drugs, accounting for the lower probability for development of autoimmunity in people with the rapid acetylation phenotype.

Pathogenesis

Because symptoms and serologic features of DIL overlap with those of idiopathic SLE, it is presumed that similar pathogenic factors underlie both syndromes. However, immune complex formation and deposition in vital organs, one of the mechanisms believed to operate in SLE, have not been well-documented in DIL. Immune complexes have been reported in DIL (153 ,154 ,155), but their composition, correlation with disease activity, and pathogenic potential have not been evaluated. Evidence supporting the involvement of immune complexes in disease pathogenesis include the observations that complement breakdown products (e.g., C3d and C4d) were detected in DIL (37 ,161). C4d/C4 ratios were significantly elevated in five of six procainamide-induced lupus patients (37), and, in a prospective study, C4d gradually increased during the development of DIL and returned to normal 2.5 months after discontinuation of procainamide treatment (160). Immune complexes involving IgG anti-[(H2A-H2B)-DNA] and a chromatin-derived antigen would be a candidate mediator of complement activation and subsequent inflammatory reactions. This possibility is consistent with the finding that anti-[(H2A-H2B)-DNA] activity is predominantly IgG1 and IgG3 (301), immunoglobulin subclasses which are potent activators of the classical complement pathway when engaged by antigen. Detection of LE cells in procainamide-induced lupus (150) also suggests the presence of complement-fixing autoantibodies. However, in comparison to the complement fixing activity of autoantibodies in SLE, antihistone and antichromatin antibodies from patients with DIL displayed much lower capacity to activate the complement system using an indirect immunofluorescence assay involving cell nuclei (302 ,303 ,304 ,305). In addition to this weak complement-fixing activity, which may be related to the relative homogeneity of autoantibodies in DIL compared to SLE (305), overloading of the immune complex clearance machinery and pro-inflammatory signaling through complement and Fc receptors (306) may be necessary for the serious pathologic features that are typical of SLE (Chapter 12).

Hydralazine and INH inhibit C4, especially C4A, binding activity in vitro, and it has been suggested, therefore, that these drugs may interfere with immune complex clearance by inhibiting classical pathway activation (307 ,308). Although these effects were only observed at drug concentrations that were 10- to 100-fold higher than therapeutic plasma levels in humans, penicillamine inhibition of C4A binding may fall within therapeutically relevant concentrations (308). Of particular interest was the finding by Sim et al. (309) that PAHA inhibits C3 or C4 activity at concentrations 10-fold lower than that of procainamide. However, this effect still required concentrations of PAHA not pharmacologically obtainable, making it unlikely that an acquired C3 or C4 deficiency contributes to DIL.

Animal Models

Compared to human standards all strains of mice are slow acetylators (310) and, therefore, should be susceptible to autoantibody elicitation upon exposure to lupus-inducing drugs. On the other hand, drug clearance rates in mice are at least ten times faster than in humans (311), so at equivalent weight-adjusted oral doses, mice will have much lower steady-state blood levels of delivered drug. The measured elimination half-life for procainamide in 20 strains of mice varied from 23.5 to 54.7 minutes (312), compared to 180 to 300 minutes in humans (313). Use of excess drug concentrations in the drinking water is limited by the natural aversion of mice to higher oral doses.

Continuous oral administration of isoniazid or hydralazine for 6 to 8 months in C57BL/6 mice was reported by Cannat and Seligmann (314 ,315) to result in ANA induction in 34% to 46% of mice. TenVeen and Feltkamp (316 ,317) reported similar findings and extended positive results to procainamide and to two other mouse strains. Tannen and Weber (318 ,319) also were able to elicit ANA by procainamide in A/J mice, but, contrary to the earlier reports, observed suppression of spontaneous ANA appearance during 9 months of exposure of C57BL/6 mice to procainamide. Injection of procainamide three times per week for 30 weeks failed to elicit ANA in mice, rats, rabbits, or guinea pigs (320). Although provocative, the murine models in which a lupus-inducing drug is administered in the drinking water are impractical, because of the long duration required to develop even a partial response, the common spontaneous seroconversion of aging mice to ANA positivity and the inability to develop steady-state blood levels of the drug which are comparable to therapeutic doses in humans. Monoclonal antichromatin antibodies derived from mice receiving quinidine or penicillamine in their drinking water for 5 to 8 months were reported (321), but it is not certain that these autoantibodies were truly drug-induced. Uniform mean plasma levels of 2.1 ± 0.7 μg/ml (7.4 μM) procainamide, which is at the lower end of the human therapeutic level, were obtained by delivering the drug via implanted osmotic pumps, but the mice failed to develop autoantibodies during the 7 weeks of exposure that this protocol could be sustained (312 ,322).

Oral administration of propylthiouracil into mongrel cats for 2 months resulted in ANA, direct anti-RBC antibodies and lupus-like symptoms in approximately half the animals (323). Discontinuation of propylthiouracil or replacement with propyluracil caused resolution of symptoms and signs within 1 to 4 weeks (323), indicating that this was a bonafide drug-induced lupus-like syndrome. Induction of autoimmunity was drug dose–dependent, and, as in human DIL (see Rechallenge Studies), cats that had previously developed propylthiouracil-induced lupus followed by a 3-month washout period were not hyperresposive to challenge with propylthiouracil (i.e., they failed to redevelop disease at lower challenge doses) (324). Interestingly, the ANA was predominantly a result of antinative DNA antibodies; antihistone antibodies were not detected (324). Therefore, this syndrome has features of both drug-induced and idiopathic lupus and may be a unique feline animal model.

A lupus-like pathology including autoantibodies and glomerulonephritis has been produced in mice by injection of splenocytes treated with procainamide in vitro (325). For this effect to be manifested, splenocytes were activated by allogeneic stimulation and treated in vitro with procainamide prior to their adoptive transfer monthly for 6 months into syngeneic mice. A conalbumin-specific T cell clone treated with procainamide or hydralazine, but not their structural analogs, produced a similar in vivo pathology (326 ,327 ,328), suggesting that autoimmunity in this animal model was mediated by nonantigen specific activated T cells exposed to lupus-inducing drugs (see Mechanisms).

A number of lupus-inducing drugs (hydralazine, chlorpromazine, carbamazepine, phenylbutazone, and nitrofurantion) will cause significant enlargement of the draining popliteal lymph node when injected subcutaneously into the hind foot pad of mice (298 ,299 ,300 ,329). In this popliteal lymph node assay (PLNA) T cells apparently respond to drug-altered self proteins. Interestingly, procainamide, INH, and propylthiouracil were negative in this assay (298 ,329 ,330), unless oxidatively metabolized by rat liver microsomes (330) or peritoneal macrophage (298) or if the metabolite itself such as PAHA or propyluracil 2-sulfonate was injected (298). The requirement for a drug metabolite is in good agreement with the in vitro studies demonstrating neutrophil- or macrophage-mediated metabolism of the same drugs (see Oxidative Drug Metabolism). The possible significance of this animal model in the context of DIL is discussed under Mechanisms.

Injection of PAHA into the thymus of normal adult mice resulted in the delayed appearance and long-lasting production of IgG anti-[(H2A-H2B)-DNA] antibodies, similar to those found in patients with DIL (331). Transfer into naïve mice of autoreactive peripheral T cells derived from PAHA-injected animals elicited a similar autoantibody profile, indicating that autoreactive T cells that emigrated from the thymus to the periphery accounted for autoantibody production in this system (332). The cellular basis for autoantibody production in this mouse model is discussed below.

Mechanisms

Most of the older experimental studies on DIL explored the significance of the presumed capacity of lupus-inducing drugs to form stable complexes with self-macromolecules or to directly stimulate lymphocytes. The premise underlying much of this work was apparently based on previously described immune reactions to xenobiotics such as the penicillin-type of drug hypersensitivity (allergic) reaction mediated by antibodies or the delayed-type hypersensitivity reaction associated with allergic contact dermatitis mediated by T cells. Autoantibodies might develop if an immune response to the drug in the form of a hapten or to a self-antigen altered by the drug induces antibodies that cross-react with or cause spreading of the immune response to native self-macromolecules. For the most part these experiments have not been illuminating because nonpharmacologic concentrations of drugs or artificial drug-macromolecular complexes together with immunologic adjuvants were employed (reviewed in (182)). A more recent examination of the specificities of antibodies elicited in rabbits by immunization with drug-albumin complexes revealed no cross-reaction between denatured DNA or histones and antibodies to procainamide or its oxidative metabolites (211). However, several groups are pursuing variants of this hypothesis at the T cell level as discussed below.

Studies over the past decade attempting to determine which component(s) of the immune system are important in the initiation of drug-induced lupus and exactly how autoimmunity is triggered by drugs or drug metabolites are organized below into four general mechanistic hypotheses. The pros and cons of these ideas as well as the hypersensitivity mechanism are summarized in Table 44-9 .

- Drug Metabolites Act as Haptens for Drug-Specific T Cells.

  Immune responses require presentation of the antigen on the class II molecules of the MHC on antigen presenting cells, and most drugs are ignored by the immune system, in part because their molecular mass is too small to enter this machinery. However, if a drug or its metabolite can form a stable bond to self-molecules, it (as a hapten) or a combined epitope generated by the drug-self molecule complex may be recognized by antigen receptors on B and/or T cells. Such a phenomenon appears to be the basis of the immunoreactivity of many drugs in the PLNA reported by Gleichmann and others (298 ,299 ,300 ,329 ,330 ,333) (see Animal Models). Since monocytes, macrophages, and Langerhans cells can present antigens to T cells, they have received special attention as a potential source of both drug biotransformation producing drug conjugates as well as immune presentation of the conjugate to initiate an immune response (82). These professional antigen-presenting cells could also take up drug conjugates produced in their immediate microenvironment by other cells such as neutrophils. Oxidative metabolites of carbamazapine,

chlorpromazine, hydralazine, phenytoin, procainamide, and propylthiouracil have been demonstrated to form covalent bonds with cellular proteins. Alternatively, B cells within the microenvironment of a drug-specific T cell response might become activated by cytokine-mediated bystander mechanisms.

Table 44-9: Possible Mechanisms in Drug-Induced Lupus*

Mechanism	Pro	Con
Immune response to drug-modified self-macromolecule initiates autoimmunity	Precedent in allergic drug hypersensitivity and contact dermatitis	Antibodies to drug-altered antigens not cross-reactive with autoantibodies in DIL; Nature of the immune response in DIL dissimilar to that in drug hypersensitivity
Drug metabolite-specific T cell responses spread to autoantigens	Experimental elicitation of drug (metabolite)-specific T cells	Drug-specific T cells not associated with autoimmunity
Cytotoxic drug metabolites release self-macromolecules that initiate autoimmunity	Toxicity of many drug metabolites demonstrated in vitro	No evidence that drug-induced cytotoxicity enhances autoantibody production
Non-antigen-specific activation of lymphocytes by drug leads to autoimmunity	Prototypic lupus-inducing drugs can cause polyclonal T-cell activation, resulting in autoimmunity	Global inflammatory reactions dissimilar to specific autoimmune features of DIL
Drug metabolites disrupt immune tolerance machinery	Disruption of central T cell tolerance leads to autoantigen-specific T cells and DIL-like autoantibodies	No evidence for action of reactive drug metabolites in thymus

*see text for details

Unfortunately for this hypothesis, mice that developed enlarged lymph nodes in response to lupus-inducing drugs or their oxidative metabolites failed to develop autoreactive T cells or autoantibodies, indicating that drug-specific T cells do not typically lead to autoimmunity. Additionally, the autoantibodies that arise in people with DIL or drug-induced cytopenias are limited in specificity, a feature not consistent with a bystander activation scenario. However, if an incipient autoimmune response were independently underway and if the drug as a hapten becomes expressed on the MHC of autoreactive B cells, it is possible that drug-specific T cells could accelerate development of autoimmunity. Presentation of a drug on the MHC could occur after endocytosis and intracellular processing of the drug bound to the cognate self-antigen recognized by an autoreactive B cell or by binding directly to the MHC through a noncovalent association (334).

- Drug Metabolites Cause Cell Death.

Cytopenias associated with certain drugs (see Clinical and Laboratory Features) may be related to the capacity of various reactive drug metabolites to directly cause cell death in in vitro studies. This would be a nonimmune-mediated process. Demonstration that PAHA under certain in vitro conditions can directly kill a wide variety of cells at pharmacologically relevant concentrations (245 ,278 ,293 ,295) or enhance reactive oxygen species generation by murine macrophage (335) and human neutrophils (245) is consistent with this view. Apoptosis rather than necrotic cell death was mediated by sulphamethoxazole hydroxylamine (336) or by the nitrenium ion of clozapine (337). Cell death may be initiated by damage to the plasma or mitochondrial membrane or by covalent binding to critical intracellular molecules or may be a result of redox (reduction/oxidation) cycling with NADH/NADPH, depleting the cell of its energy stores as suggested by the correlation between cell-reducing potential and sensitivity to PAHA cytotoxicity (278). Cytotoxic drug metabolites generated by neutrophils in vitro have been demonstrated for amodiaquine, carbamazapine, chlorpromazine, clozapine, hydralazine, INH, procainamide, propylthiouracil, quinidine, vesnarinone (348) and sulfonamides at therapeutically feasible concentrations. Cytopenia could occur by killing of stem cells if reactive drug metabolites were produced by myeloperoxidase released from immature promyelocytes undergoing granulopoiesis or from activated neutrophils recirculating into the bone marrow (Fig. 44-3). Additionally, certain hematopoietic cell lineages sensitized by an immune-mediated mechanism because of drug binding to the cell surface could be destroyed by an otherwise subtoxic concentration of a reactive drug metabolite. Lymphocytes from patients with a history of agranulocytosis secondary to clozapine therapy were somewhat more sensitive to the cytotoxic effects of oxidative metabolites of clozapine than normals or patients who did not develop agranulocytosis (338 ,339), possibly related to differences in

drug bioinactivation by intracellular glutathione or cysteine (340).

Although it is possible that this type of direct cytotoxicity of drug metabolites could be an independent pathogenic mechanism, especially in certain susceptible populations, such a process can not explain the bulk of the immune abnormalities in DIL.

It is also formally possible that drug toxicity alters degradation, clearance or processing of self-materials by antigen-presenting cells, producing abnormal macromolecular forms or unusual peptides. These “cryptic” T cell autoepitopes may induce classical adaptive immune responses because immune tolerance to such unusual forms of self-materials was never established (see Chapter 20). Autoreactive B cells pre-existing in the immune repertoire could present such cryptic epitopes to T cells, resulting in B cell activation and autoantibody secretion. Alternatively, repetitive macromolecular structures released from dying cells could theoretically elicit T-independent autoimmune responses. This type of scenario has been proposed to account for autoantibodies associated with diverse medications, environmental agents, and viruses, but there remains little in the way of experimental support.

- Drugs Nonspecifically Activate Lymphocytes

A study of Adams et al. (245) suggested that PAHA at 2 μM had capacity to enhance pokeweed mitogen-mediated lymphocyte proliferation and increase the number of Ig plaque-forming cells, but these observations have not been verified. A more recent study reported an approximately twofold increase in poly(ADP-ribosylation) level in the lymphocyte Wil-2 cell line exposed to micromolar levels of procainamide or hydralazine (341). Mouse splenocytes exposed to procainamide or hydralazine while activated in vitro displayed an increased proliferative response to autologous antigen-presenting cells without the need for cognate antigen, killed autologous macrophage, and promoted B cell differentiation into antibody-secreting cells (342 ,343 ,344 ,345). Autoantibodies and glomerulonephritis were produced after adoptive transfer of such drug-treated cells into mice (325 ,326). The autoreactive nature of drug-treated cells was shown by Richardson et al. to be a result of decreased DNA methyltransferase activity in CD4+ T cells as a result of competitive inhibition by procainamide of DNA methyltransferase (346) or by hydralazine of the extracellular-regulated kinase (ERK) pathway resulting in lower expression of DNA methyltransferase (347). As a result, with each round of cell division undermethylation of deoxycytosine residues in CpG pairs occurs in the genomic DNA. Hypomethylation of promoter sequences is associated with enhanced gene transcription, and drug-treated T cells showed increased expression of lymphocyte function antigen-1 (328), an important adhesion molecule that helps stabilize the interaction between T cells and antigen-presenting cells, and enhanced CD70 expression (349), a costimulatory ligand for CD27 on T and B cells. Longer contact between T cells and antigen-presenting cells may promote T cell activation and/or stimulate IgG production in B cells.

One concern about these studies is that the immunopathologic features of this mouse model do not resemble DIL but are more like the global autoimmune characteristics of a graft-versus-host reaction when adoptively transferred semi-allogeneic T cells recognize histoincompatible MHC molecules in the host (350). This syndrome is similar to SLE, whereas DIL displays much more limited autoimmune features (Table 44-5). Additionally, since multiple exposures to large numbers of dividing lymphocytes were required to produce the in vivo phenomena, it is not clear what the natural counterpart of such a polyclonal T cell activation would be. Immune responses to infectious agents that might occur coincident with medication with a lupus-inducing drug would be expected to be associated with a much more limited, oligoclonal T cell activation. However, it is possible that in DIL autoreactive T cells developing through another mechanism become more aggressive because of such a drug-induced LFA-1 and/or CD70 overexpression process, thereby aggravating disease in DIL.

- Drug Metabolites Disrupt Immune Tolerance Machinery

Rather than stimulating mature T cells, drug metabolites when present during T cell development in the thymus may prevent the de novo acquisition of self-tolerance. T cells originate in the thymus, where they are selected to ensure that only cells that are nonresponsive to self are released into the periphery. The possibility that lupus-inducing drugs interfere in this process had been generally ignored, because it was widely assumed that there is no thymic function in the adult, but several studies have demonstrated that T cells are generated in the thymus at least up to the seventh decade of life in normals (351 ,352) and in patients with a history of DIL (352). Kretz-Rommel and Rubin showed that injection of PAHA into the thymus, but not into any part of the peripheral immune system, of normal adult mice resulted in the delayed appearance and long-lasting production of anti-[(H2A-H2B)-DNA] autoantibodies (331 ,332), similar to the autoantibodies that characterize patients with DIL. Observations in this animal model and in an in vitro model of T cell tolerance (353) indicated that PAHA did not reverse self-tolerance of the mature thymocyte and did not prevent deletion of high affinity autoreactive T cells in the thymus. Instead, PAHA apparently interfered with the establishment of tolerance to endogenous self-antigens that are normally presented by the MHC on thymic epithelial cells during the positive selection of thymocytes (354). As a result, mature T cells are produced that are capable of undergo spontaneous activation when

they encounter similar self-antigens in the periphery. These studies would imply that the restricted autoantibody production associated with DIL reflects a limited diversity in the selecting antigens encountered as T cells undergo positive selection in the thymus and that T cell tolerance to these antigens is compromised in the presence of reactive drug metabolites.

Currently, there is no evidence that reactive metabolites of procainamide can be produced in the thymus by circulating or resident phagocytic cells and that metabolites of other lupus-inducing drugs can also initiate autoimmunity by this mechanism. Ultimately, identification of the molecular target(s) in developing thymocytes that are compromised by reactive drug metabolites will be needed to make this a convincing story.

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Chapter 45

Infections in Systemic Lupus Erythematosus

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Infection is a major source of morbidity and mortality in patients with systemic lupus erythematosus (SLE). The literature includes anecdotal reports of sepsis from common and unusual organisms, as well as compilations of infections in both small and large series. Although the most frequent infections continue to be attributed to pyogenic organisms such as *Staphylococcus* sp. and *Escherichia coli,* opportunistic pathogens such as uncommon bacteria, deep-seated fungal organisms, viruses, and protozoans have been well described, including infection at multiple sites and multiple organisms at a single site. Features of SLE itself, including many forms of immunologic dysfunction, appear to play a role in an increased susceptibility to infection, which may be further affected by treatment modalities, such as corticosteroids and other immunosuppressive agents. At times, the actions of microbial agents on the host are difficult to differentiate from those of a lupus flare and, by themselves, may aggravate the disease. This chapter reviews infections in SLE, discusses differential diagnosis, and describes the infectious agents that most frequently are found in these patients.

SLE Mortality from Infection

In 1987, Hellmann et al. (1) reviewed mortality studies from a 40-year period. Among the 3,175 patients included in their report, there were 641 deaths, 170 (27%) of which resulted from infection. A fairly constant death rate caused by infection has persisted since the pre-antibiotic era of the 1930s to 1940s, when Klemperer et al. (2) attributed 40% of the deaths in their patients with SLE to infection, generally with pyogenic organisms. Even with advances in treatment of the underlying disease, a large U.S. multicenter study of 1,103 patients with SLE found that 33% of the 222 deaths were directly caused by infection, and infection was a contributing cause in an additional 10% (3). When clinical and demographic features such as age, socioeconomic status, and race were considered, the leading cause of death in all groups in a study by Reveille et al. of 389 patients from 1975 to 1985 in Birmingham, Alabama, was infection; 35 of 89 deaths (39%) were from this cause (4). In developing countries, such as Jamaica, infection has been the major cause of death among patients with SLE, being directly responsible for the fatal course in 20 of 55 patients (36%) at the University Hospital of the West Indies from 1972 to 1985 (5). Nossent (6) observed that 50% of the 22 fatalities in his series of 68 Caribbean patients with SLE who were treated between 1980 and 1990 also resulted from sepsis; 25% of those with fatal infections had only mildly active SLE at the time of death. Similarly, a 1993 report from Thailand documented infection as the cause of death in 23 of 77 patients with SLE from a cohort of 537, with opportunistic organisms and other common bacterial pathogens being implicated equally (7). In European and Asian series reported in the late 1990s, despite wide ranges in overall case fatality rates of 4.5% to 24%, the proportion of deaths attributed to infection was similar, ranging from 20.5% to 32.5% (8 ,9 ,10 ,11).

Rates of Infection in SLE

The incidence of infection in SLE is a reflection of overall morbidity, and it has considerable prognostic significance. In a series of 223 patients followed at Downstate Medical Center in New York from 1966 to 1976, 150 patients had 384 infections diagnosed over 655 patient-years, for an overall infection rate of 59 per 100 patient-years (12). The bacterial infection rate was 25 per 100 patient-years. The rate of nonspecific viral infections was 28 per 100 patient-years. Twenty-eight opportunistic infections were identified in 23 patients. Twelve had oral candidiasis, and 3 other patients with oral thrush also had evidence of systemic infection. Two of 11 patients with deep-seated fungal infections had multiple organisms at a single site, 2 had coincident bacterial infection at the same site, and 1 had a single fungal organism at two sites. In a Swedish epidemiologic study reported in 1985, Nived et al.(13) found an overall infection rate of 142 per 100 patient-years of SLE. More than one-half of these infections were of suspected viral origin, and approximately 40% were bacterial. A high incidence of mucocutaneous infections was noted, often caused by *S. aureus*.

The 1974 report of hospitalized SLE patients by Staples et al. (14) identified an overall culture-verified infection rate of 1.22 per 100 hospital days. The most common infection site was the urinary tract. Nearly one half of the patients who developed infections had multiple episodes, with recurrent sites frequently involving the opportunistic organism *Candida albicans*. A 1991 review of infections in hospitalized patients at the University of Toronto found an infection rate of 1.94 per 100 hospital days (15). Nearly one half of these infections were deemed to be major, requiring intravenous antibiotics; sites included pneumonia, septic arthritis, bacteremia, pyelonephritis, abdominal abscesses, endometritis, and esophageal candidiasis. In the U.S. multicenter lupus study, infections were responsible for 29% of all but obstetric hospitalizations (3), whereas 14% of the 1989 and 1990 hospitalizations in the Hopkins Lupus Cohort resulted from infection (16).

Factors Influencing Susceptibility of Patients to Infection

Many investigators have examined the risk factors that predispose to infection in SLE patients. Most agree that treatment with corticosteroids, as well as some manifestations of active SLE itself, play an important role. Staples et al. (14) found that the infection rate in hospitalized patients increased from 0.43 to 1.63 per 100 hospital days with an increase in steroid dose from zero to more than 50 mg/day. In the Downstate study, a fivefold increase in the frequency of all infections was found, ranging from 35 to 179 per 100 patient-years as average prednisone dose increased from zero to greater than 40 mg/day (12). The same trend was observed with bacterial infections (10/100 patient-years, increasing to 87/100 patient-years) and opportunistic infections (range, 142/100 patient-years). Recently Noel reported follow-up data in 87 SLE patients followed from 1960 to 1997. Thirty-five of 87 had at least one episode of infection. Risk factors for infection by univariate analysis were severe flare, glomerulonephritis, corticosteroids, cyclophosphamide, and plasmapheresis (17).

The physiologic and pharmacologic actions of corticosteroids predispose to infection by affecting host responses to micro-organisms. These include a decreased inflammatory response, decreased effector cell response in cell-mediated immunity, lysis of lymphoid follicles, and decreased immunoglobulin synthesis (18). Frenkel (19) reviewed the role of corticosteroids as predisposing factors in fungal diseases and concluded that inhibition of cellular host responses, particularly impaired proliferation of epithelioid and giant cells as well as decreased digestive capability of these cells and macrophages, was responsible. The role of steroids in the development of opportunistic infections was examined in a case-control study of 797 SLE patients hospitalized at Bellevue/NYU Medical Center; 26 patients with a total of 32 opportunistic infections were compared to 26 patients without opportunistic infections (20). Prednisone was a major risk factor for the development of opportunistic infection, with the most common organisms including *Salmonella, Candida, Strongyloides,* and *Aspergillus* sp. The mortality rate was highest among patients who had both opportunistic infection and active disease. The need to aggressively investigate the possibility of occult opportunistic infection was underscored by Hellmann et al. (1), who reviewed 44 lupus-related deaths at Moffitt Hospital in San Francisco between 1969 and 1986, where opportunistic infections occurred in 15 patients (35%) and caused death in 10. Antemortem diagnosis was made in only 3 of these 15 patients; *Candida* and *Pneumocystis* sp. were the most common organisms.

Prolonged corticosteroid administration further increases the risk of infection by causing chronic changes in tissues, such as skin atrophy, which in turn allow increased access of micro-organisms into the circulation. A regimen of alternate-day steroid administration is believed to decrease the pharmacologic risk of infection; this was confirmed in a 1993 Spanish study that showed a significant decrease in infection rate ($P > 0.001$) among patients receiving an alternate-day dose compared with those on daily steroids (21).

Other immunosuppressive agents, especially azathioprine and cyclophosphamide, also have been implicated as risk factors for infection in patients with SLE, frequently in anecdotal reports and in the setting of aggressive treatment for disease exacerbations (7 ,22 ,23 ,24 ,25 ,26 ,27). When corrected for steroid dose, however, azathioprine use in the Downstate population was not associated with an increased risk of bacterial, opportunistic, or nonspecific viral infections (12). A French study of cytolytic therapy in rheumatic disease failed to find an increased risk of infection, with the exception of herpes virus, which was associated with a 10% to 20% incidence of infection compared with 2% in patients not treated with immunosuppressive agents (28). Similarly, a prospective study of infections in hospitalized patients with SLE in Singapore did not show an association of cytotoxic therapy and infection (29). On the other hand, Aringer et al. (30) observed that 7 of 9 patients treated with plasmapheresis in addition to intravenous cyclophosphamide had serious bacterial or viral infections, compared to only 2 of 12 patients with serious infections in the cyclophosphamide alone group. Recent clinical trials suggest that infections are less frequent in patient treated with mycophenolate mofetil, as compared to cyclophosphamide (31).

Clinical Features and Immunologic Dysfunction in SLE as Risks for Infection

Even in the absence of corticosteroid treatment, infections are common in patients with SLE. Ropes (32) used steroids sparingly and rarely, if ever, gave immunosuppressive agents, yet 108 of 137 patients (79%) had serious infections during their disease course. These infections usually were

associated with disease exacerbations. In the Downstate study, new exacerbations of disease were associated with increased infection rates, but the correction for steroid dose eliminated this effect (12). This study also found that specific renal measures, most notably active urinary sediment, were a significant predictor of infection, whereas presence of the nephrotic syndrome and uremia were associated with an increased, but not statistically significant, incidence of bacterial and opportunistic infections. Among renal parameters, including blood urea nitrogen (BUN), creatinine clearance, urine sediment, and 24-hour protein excretion, Staples et al. (14) found BUN to have the strongest association with infection, suggesting that poor renal function is more important than active renal inflammation. Studies of hospitalized patients have shown that overall disease activity, measured by either the SLE Disease Activity Index (SLEDAI) or the Lupus Activity Index (LAI), correlates well with the incidence of infection (15 ,16).

Even among patients with SLE in clinical remission, an increased tendency to develop bacterial and opportunistic infections has been documented. Staples et al. (14) compared infection rates in SLE to those in patients with rheumatoid arthritis (RA) and idiopathic nephrotic syndrome, controlling for steroid dose. Infections were 10 times more frequent among patients with SLE than in the other two groups. In the population-based study by Nived et al., which included many mild cases, a significant increase in bacterial infections was observed among patients with SLE as compared to an age- and gender-matched cohort of patients with RA and to a similarly matched cohort of normal controls (13). The incidence of fungal and viral infections was similar in patients with SLE and controls.

Among patients with end-stage renal failure receiving chronic maintenance hemodialysis, the incidence and severity of infections continue to be influenced by underlying SLE. Jarrett et al. (33) found that 4 of 14 patients with SLE undergoing long-term dialysis at Northwestern University died of infection, compared with only 1 infectious death in 62 non-SLE patients on hemodialysis.

Many abnormalities, including immunoglobulin deficiency, acquired and inherited complement deficiencies, defects in chemotaxis, phagocytic activity, and delayed hypersensitivity, may account for this susceptibility to infection. As the prototype immune complex disease, activation and consumption of complement have been well characterized in SLE, and the role of specific complement components has been defined (34). For example, fixation of C3 to bacterial cell walls is essential for phagocytosis and subsequent digestion of micro-organisms. C3b, which is an activation product of C3, is critical to the opsonization of bacteria before phagocytosis. C3-deficient individuals therefore are at risk for recurrent and/or disseminated bacterial infections (35 ,36). Hereditary deficiencies in the production of various complement components also have been described in patients with SLE or lupus-like syndromes. Autosomal recessive defects in C1q, C1r, and C2 production and homozygous deficiencies of C2 have been associated with repeated skin and upper airway infections, recurrent *Haemophilus influenzae* septicemia, and bacterial meningitis (37 ,38 ,39). Recurrent pulmonary infections in a family with lupus-like disease has been linked to an autosomal recessive, human leukocyte antigen (HLA)-associated total deficiency of C4 (40). Granulocyte phagocytic function was found to be normal in these family members; however, a serum defect, which was corrected by the addition of purified C4, appears to have been responsible for the abnormal phagocytosis and intracellular killing. Deficiency of complement components C5 through C9 (i.e., the membrane attack complex) make individuals especially prone to infections with encapsulated organisms such as *Neisseria* sp., for which phagocytosis tends to be normal but bacterial lysis is impaired (41 ,42). Recently, homozygosity for variant alleles in the coding portion of the mannose-binding lectin (MBL) gene in patients with SLE has been shown to be associated with increased susceptibility to infections (odds ratio 8.6) (43). MBL is a serum protein structurally similar to C1q, which binds to antibodies and protein structures on bacteria and viruses. Homozygosity for MBL variant alleles was observed in 7.7% of SLE patients compared to 2.8% of controls by Garret et al. (43). Among homozygotes with SLE, the time interval from SLE diagnosis to the first infection was shorter, and the annual number of infections was four times higher than in patients homozygous or heterozygous for the normal allele (43).

Acquired functional defects in complement components that are associated with disease activity also have been described. Perez et al. (44) observed an increased incidence of infections in patients with SLE in whom they identified a serum inhibitor of C5-derived chemotactic activity. When levels of this inhibitor decreased as disease activity lessened, the incidence of infection also fell. A National Institutes of Health (NIH) report also correlated a decrease in serum generation of chemotactic factors with infection in 23 patients with SLE (45).

Other studies have found impaired in vitro antibacterial activity from alveolar macrophages obtained at bronchioalveolar lavage (46), decreased *S. aureus* intracellular destruction capability (47), impaired opsonic capability (48 ,49), and defective degradation of bacterial deoxyribonucleic acid (DNA) by phagocytes in patients with both discoid and systemic lupus (50), independent of steroid therapy.

Handling of micro-organisms also may be influenced by abnormalities of reticulo-endothelial function. Saturation of Fc receptors on liver and spleen cells by circulating immune complexes may prevent the clearance of opsonized bacteria (51). Fries et al. (52) demonstrated, however, that Fc receptors on the cell surface of monocytes from patients with SLE actually are increased in number, suggesting that a primary defect in Fc receptor function exists. Such a mechanism might explain the observations of overwhelming pneumococcal bacteremia (53 ,54) and the chronic *Salmonella* carrier state in some patients with

SLE, leading to *Salmonella* arthritis and osteomyelitis (55 ,56). Functional asplenia (i.e., the complete absence of uptake of intravenously administered, radiolabeled sulfur colloid unrelated to splenic size) has been identified in 7 to 10 of screened series of patients with SLE, and it has been implicated in the development of pneumococcal and salmonella septicemia (57).

Abnormalities of cellular immunity in patients with SLE appear to contribute to the risk of opportunistic infections. The incidence of herpes zoster is increased in patients with SLE compared to normal individuals; the rate among Japanese patients with SLE is especially high. Nagasawa et al. (58) found a 43% overall incidence of herpes zoster in their patients, with an annual incidence of 9%. Despite significantly higher antibody titers against herpes zoster than those seen in non-SLE subjects, only 30% of the patients with SLE showed positive delayed hypersensitivity skin reactions to varicella-zoster antigen. In contrast, 100% of normal individuals had a positive skin reaction.

Specific Types of Infection

Bacterial Infections

The most frequent sites for bacterial infections in patients with SLE are similar to those in individuals without lupus, including the urinary and respiratory tracts and the skin (12 ,13 ,14 ,59). The organisms most often cultured include *S. aureus, E. coli, Klebsiella* sp., and *Pseudomonas* sp. (13 ,29). In general, gram-positive cocci and gram-negative bacilli are most often implicated as an infectious cause of death (5 ,60). Bacteremia is common, especially in hospitalized patients with SLE (7 ,24); other sites of infection also have been well described and may account for some of the difficulty in distinguishing sepsis from exacerbations of SLE. For example, anecdotal cases of septic bacterial arthritis continue to be reported (52 ,55 ,60 ,61 ,62 ,63 ,64 ,65 ,66 ,67 ,68 ,69 ,70). Bacterial seeding resulting in septic discitis may put a patient at risk for serious neurologic deficit (71). Bursal abscesses also may mimic musculoskeletal problems such as sciatica (72 ,73). Hung reported 17 cases of CNS infection during a 20-year follow-up of 3,165 patients with SLE. Ten cases were a result of *Cryptococcus neoformans,* 4 *Listeria monocytogenes,* 2 *Streptococcus pneumoniae,* and 1 *Enterobacter aerogenes*. An active flare of lupus was present in 94% of the cases at the time of CNS infection (74).

Commonly diagnosed bacterial organisms, even among otherwise healthy individuals, appear to occur more frequently in unusual locations in patients with SLE. Purulent pericarditis is a rare phenomenon, yet it has been described in at least 14 patients with SLE, most often resulting from *Staphylococcus* sp. (75 ,76 ,77), and in one case which resulted in tamponade being caused by *N. gonorrhoeae* (60). Bacterial epiglottitis, facial cellulitis, and multiple soft-tissue abscesses have been described as well (78 ,79 ,80).

Opportunistic bacterial infections also occur with increased frequency in patients with SLE. Among the most common are *Salmonella* sp., especially *S. typhimurium* and *S. enteritidis* (55 ,56 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95). Occasionally *Salmonella* infection can coincide with the initial presentation of the disease, and aspects of the infection can mimic features of SLE (79 ,82). Hospitalized lupus versus nonlupus patients have an increased risk for *Salmonella* infections (80), which can manifest not only as diarrheal illnesses but also as gas-producing leg abscesses (84), septic arthritis (55 ,56 ,85) especially at sites of former avascular necrosis (95), osteomyelitis (85 ,86), and spondylodiscitis (87). Patients with glomerulonephritis may have an increased susceptibility to becoming *Salmonella* carriers (55).

Infection with *Listeria monocytogenes* is uncommon in healthy adults, occurring predominantly in young children, the aged, and immunocompromised individuals. In two series of seven and eight patients with SLE, listerial infection usually presented as meningitis or bacteremia without a known focus, and it most often was associated with active disease, high-dose prednisone with or without other immunosuppressive drugs, or renal failure (25 ,96). Despite aggressive antibiotic therapy, a fatal course is not uncommon (26).

Active tuberculosis can mimic the manifestations of SLE, thus delaying diagnosis and appropriate treatment. Feng and Tan (97) found tuberculosis in 16 of 311 patients with SLE (5%) who were followed in Singapore between 1963 and 1979. Presentation with miliary (98) and far-advanced pulmonary disease was common. Seven of the Singapore cases were fatal, five of which were attributed directly to mycobacterial infection. A recent study from Korea reported tuberculosis in 15 of 283 SLE patients, associated with a high incidence of extrapulmonary and miliary involvement (99). Mok et al. noted 91 episodes of TB among 76 patients in a cohort of 652 SLE patients; 39.6% of cases had extrapulmonary involvement. Isoniazid (INH) prophylaxis was given in 33 of the 76 patients. Those receiving INH prophylaxis had a slightly lower recurrence of active tuberculosis, but a higher rate of subsequent SLE flare (100). The role of INH prophylaxis remains controversial, especially in endemic areas. Gaitonde studied 95 patients with SLE, of whom 70 were able to continue INH for at least 2 years. The incidence of active tuberculosis in his cases decreased from 11 to 2%. There were no reported deaths as a result of active tuberculosis or toxic hepatitis (101).

Atypical mycobacterial infections have been reported rarely in patients with SLE and tend to be limited to the skin (102 ,103 ,104). The presentation may mimic lupus profundus, delaying appropriate diagnosis and treatment.

Other unusual bacterial infections reported in patients with SLE include five cases of Legionnaires disease (105 ,106 ,107 ,108), *Campylobacter endocarditis* (109), toxic shock syndrome (110), *Pseudomonas pseudomallei* meningitis (111), and a documented tick bite with high antibody levels against *Borrelia burgdorferi* that coincided with the initial presentation of lupus (112). A 1992 report suggested that genitourinary colonization with mycoplasma organisms is

common in women with SLE, documenting 22 of 49 urine specimens (63%) to be culture positive for *Ureaplasma urealyticum* or *Mycoplasma hominis* (113). It also should be remembered that althouugh false-positive serologic tests for syphilis are an expected feature of SLE, concomitant infection with *Treponema pallidum* may occur (114), sometimes with signs and symptoms that may mimic those of active SLE (115). Distinguishing false-positive from true-positive serologic tests for syphilis in SLE patients has been problematic in part because of interference with the presence of antiphospholipid antibodies; the fluorescent treponemal antibody absorption (FTA-ABS) test is not considered to be an accurate confirmatory test for syphilis in such patients. Murphy et al. have proposed the treponemal Western blot test as the gold standard, based on its combination of sensitivity and specificity (116).

Viral Infections

Herpes zoster is the most common specific viral infection that is diagnosed in patients with SLE. In Western countries, its reported incidence ranges from 3% to 21% (12 ,117 ,118 ,119 ,120), which is somewhat increased over that observed in the general population (22) but considerably less than the 43% incidence diagnosed among Japanese patients with SLE (58). Treatment with corticosteroids and cytotoxic agents significantly increases the risk of herpes zoster; however, 65% of zoster episodes in Kahl's 1994 series occurred during mild or inactive SLE (119). In each of two series, dissemination occurred in 11% (118 ,119). Two reports of CNS herpes zoster mimicking active lupus have appeared (120 ,121); in one, visual loss and an unusual pattern of retinitis developed. Endoretinal biopsy established the diagnosis of herpesvirus infection, and the patient was successfully treated with acyclovir (121). Herpes simplex also is common in SLE and is particularly associated with esophageal and perianal lesions (122 ,123). If untreated, disseminated infection may progress to hepatic necrosis, coma, and death (124). Even in the absence of clinical manifestations of infection, antibody titers indicative of herpesvirus-6 infection were found in 55% of 56 patients with SLE in a 1991 study (125).

Cytomegalovirus (CMV) is uncommon. The usual presentation is pulmonary infection, which may be fatal (126 ,127 ,128). CMV has been identified in active SLE in the absence of immunosuppressive therapy (129), and unusual presentations including cutaneous vasculitis have been reported (130).

The role of human papilloma virus (HPV), which has been implicated in causing clinical disease ranging from warts to malignancy, has been examined in SLE. HPV titers were elevated in 45% of patients with SLE (compared to 12% of controls), in a 1977 study (131). Although a high prevalence of cutaneous warts did not correlate with immunosuppressive treatment in a 1993 series (132), an increased risk of uterine cervical atypia in women with SLE receiving cytotoxic agents was observed (133), and this in turn has been linked to a predisposition to infection with HPV, which may induce oncogenic mutations (134).

The association of parvovirus B19 (HPV-B19) with SLE is controversial. Especially in the pediatric population, presentation of the two diseases may be strikingly similar. Moore et al. described 6 of 7 children with HPV-B19 infection who had malar rash (the classic “slapped cheek” erythema) and positive antinuclear antibodies in titers of 1:40 or greater; all had prolonged arthralgias and fatigue (135). Although the overall course was generally self-limited, some patients had symptoms for up to 120 weeks. Although it has been suggested that HPV-B19 infection may actually trigger SLE (136), Bengtsson et al. found no evidence that HPV-B19 infection was more prevalent among 99 SLE patients (88% of all new cases seen from 1981 to 1995 in a specific health care district in Sweden) compared to age- and sex-matched healthy controls (137). (See the differential diagnosis section in Chapter 51 for a discussion of infection with human immunodeficiency virus and SLE).

Fungal Infections

Candida infection is a common complication in patients with SLE, most often presenting with oral thrush (12). Extension to the esophagus with mucosal invasion occurs in association with steroid and cytotoxic therapy. Peripheral blood lymphocytes from patients who are receiving high doses of prednisone have been shown to have markedly depressed in vitro lymphocyte transformation to antigenic stimulation by *Candida* sp. (138). One half of the patients with esophageal moniliasis do not have oral lesions (139 ,140). Esophageal candidiasis may co-exist with herpes simplex (122).

The development of deep fungal infections in SLE patients is generally associated with corticosteroid and immunosuppressive therapy, and is most often a result of *Candida* sp. or cryptococcus (141), frequently with a fatal outcome. Reports of visceral candidiasis include pericarditis with tamponade (142) and hepatic involvement (143). Cryptococcal infection is not uncommon and usually produces a terminal meningitis, with or without pulmonary changes (144 ,145 ,146 ,147 ,148). An insidious onset of persistent headache often is the earliest manifestation. It may also present with hepatic (149) or cutaneous (150) involvement. Zygomycosis (formerly known as “mucormycosis”) is a severe infection that has been associated with CNS complications, thrombotic thrombocytopenic purpura, and a high mortality rate (151 ,152 ,153). Infection with *Aspergillus* sp. Most often involves the lungs (143 ,154 ,155), presenting with fever and cough in an immunosuppressed patient. The finding of hyphae in the sputum should be confirmed with a tissue diagnosis demonstrating budding hyphae; long-term therapy with amphotericin B and/or fluorocytosine or itraconazole may improve survival. Fatal aspergillus meningitis (156) and septicemia (22 ,157) also have been documented in patients with SLE. Coccidioidomycosis has

been reported in four patients as a complication of steroid-treated lupus (158 ,159 ,160 ,161). Other documented fungal infections in SLE include nocardia of subcutaneous tissues, pneumonitis, laryngitis, encephalitis, and meningitis (162 ,163 ,164 ,165 ,166 ,167 ,168 ,169), disseminated histoplasmosis (170), and maduromycosis (171).

Parasitic Infections

Hyperinfection with *Strongyloides stercoralis* may occur in immunosuppressed patients. The syndrome is characterized by profound malabsorption, diarrhea, electrolyte disturbance, gram-negative or opportunistic fungal sepsis, coma, and death. It can mimic an SLE flare, and eosinophilia may be absent as a result of steroid treatment (172 ,173). Visceral leishmaniasis has been reported in one SLE patient (174), as has paragonimiasis (175).

Protozoan Infections

Pneumocystis carinii pneumonia (PCP) has been reported in patients with SLE since the 1960s, almost exclusively in the setting of aggressive treatment of active disease with high-dose corticosteroids and cytotoxic drugs (1 ,12 ,14 ,27 ,126 ,176 ,177 ,178 ,179 ,180 ,181 ,182). Porges et al. found that an additional important risk factor for PCP was severe lymphopenia; four of six patients in their series had lymphocyte counts of less than 350/mL (27). This is consistent with a single reported case of PCP in an untreated patient with SLE and severe lymphopenia (183).

Toxoplasmosis has been found in neonates with SLE (178) and in both lymphopenic (184) and immunosuppressed (185) patients. It may be difficult to identify, because the symptoms of CNS infection mimic lupus cerebritis (186), whereas false-positive antibody titers can be seen in patients with SLE (187). Further, toxoplasma infection may enhance the production of autoantibodies, which may interfere with the standard dye test that is used for diagnosis (188).

Immunization and Antibiotics

See Chapter 61 .

A Practical Approach to Infection in SLE

Despite case series and anecdotal reports of unusual infections, each patient with SLE must be considered individually when infection is suspected. There is no “cookbook” approach to diagnosis and treatment; however, a number of important principles can provide guidance in determining a plan of action:

- Patients with SLE develop community-acquired infections just like individuals in the general population. Especially with classic or usual presentations, common bacterial and viral infections should be high on the list of differential diagnoses. Appropriate treatment of these common infections should help to avoid the risk of toxicity (often potentiated by renal or hematologic abnormalities resulting from active lupus) from unnecessary antimicrobial agents.
- Because the symptoms of SLE and of infection often are similar, a high index of suspicion is important; therefore, culture early and often. Advise the laboratory to hold specimens for fungal cultures, and freeze acute serum to match with convalescent serum in the event that antibody titers may be useful.
- Although corticosteroids and cytotoxic agents increase the risk of infection, it is equally true that sick patients with active lupus are far less likely to recover from serious infections. Once a patient has been placed on an appropriate regimen for the suspected or documented infection, treatment of the underlying SLE activity should proceed with vigor.
- In difficult or recalcitrant cases, especially with persistent fever or increasing organ dysfunction (e.g., hepatic abnormalities, pulmonary infiltrates), a tissue diagnosis may be essential. Percutaneous or endoscopic biopsy procedures may be sufficient, but open surgical procedures may be necessary. These may be guided by radiographic procedures (e.g., computed tomography, magnetic resonance imaging) or by nuclear imaging (e.g., bone scans for suspected osteomyelitis).

Summary

To summarize the major points of this chapter:

- Infections are a major source of morbidity and mortality in patients with SLE.
- Patients with SLE are susceptible to infection; treatment with corticosteroids increases this susceptibility in a dose-dependent fashion.
- The respiratory and urinary tracts are the most common sites of infection in outpatients.
- Patients on steroids are at a particularly increased risk for opportunistic infections. The most common organisms include herpes, Candida sp., Salmonella sp., Cryptococcus sp., and Toxoplasma sp.
- Presentations of SLE often are difficult to differentiate from those of infection. The most helpful clues to infection are the presence of shaking chills, leukocytosis (unless steroids are being given), and the absence of active SLE in multiple systems.

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Chapter 46

Serum and Plasma Protein Abnormalities and Other Clinical Laboratory Determinations in Systemic Lupus Erythematosus

Daniel J. Wallace

The serum protein electrophoresis is an inexpensive test that can reveal increased or decreased levels of proteins in lupus patients, as well as screen for a variety of manifestations of the disease. The overwhelming majority of papers on this subject were published 30 to 40 years ago and the reader is referred to Chapter 44 of the 6th edition for a critical review of them. Table 46-1 lists the most important proteins germane to systemic lupus erythematosus (SLE). This chapter summarizes important abnormalities of clinical relevance and also reviews sedimentation rate, C-reactive protein, and blood viscosity, which are not covered elsewhere.

Hypoalbuminemia

Low serum albumins were first detected in SLE by Colburn and Moore in 1943 (1), and are present in 30% to 50% of patients (2 ,3 ,4 ,5 ,6 ,7). The most common cause is chronic disease, and the catabolic rate of albumin is increased in active SLE (8). Other associations include nephritic syndrome, protein-losing enteropathies, and malnutrition seen in lupus.

α Globulins

α_1 and α_2 globulins are acute phase reactants and are elevated with inflammation. These glycoproteins are made in the liver and defend against cellular injury. They are increased in 8% to 33% of lupus patients at any visit (4 ,5 ,6 ,9 ,10). Specific components include:

- α_1 acid glycoprotein—also known as orosomucoid is elevated in most lupus patients at some time in the course of their disease (11 ,12 ,13 ,14 ,15).
- α_1 Feto protein—increased in most pregnant women with SLE without associations with neural tube defects (16).
- α_1 Antitrypsin—the dominant protease inhibitor in plasma, and not associated with any phenotype in SLE. Studies suggest normal or slightly increased levels (13 ,17 ,18 ,19 ,20 ,21).
- α_1 Antichymotrypsin—one study suggests increased levels (13).
- Lactoferrin and neutrophil elastase—increase in one study (22).
- Ceruloplasmin—both an acute phase reactant and carrier protein, it was increased by 20% to 40% in lupus patients in a single study (11).
- Haptoglobin—decreased with hemolysis (see Chapter 41).
- α_2 Macroglobulin-Ha-glycoprotein—a protease inhibitor that is elevated in SLE (18 ,23 ,24).

β Globulins

Most β globulins relate to serum lipids, which are dysfunctional in SLE and influenced by corticosteroids. See Chapter 19 for a review of this subject. Complement is discussed in Chapter 13 and coagulation abnormalities in Chapter 27 . Transferrin, a beta globulin carrier molecule, is normal to decreased in lupus patients (11 ,17) and β_2 macroglycoprotein has been associated with SLE (25).

β_2 microglobulin is a single-chain polypeptide (molecular weight, 11,800 daltons) that is found on the surface of most nucleated cells, especially T and B lymphocytes. A normal constituent of serum that is catabolized by the kidney, it is associated with the light chains of class I human leukocyte antibody (HLA) antigens. Its serum values increase slightly with age and are elevated with decreased glomerular filtration rates and various rheumatic diseases. In SLE sera, anti-β_2-microglobulin antibodies inhibit in vitro mitogenic stimulation and lymphocyte proliferation (26). Eight well-designed studies have evaluated its clinical

importance in SLE (27 ,28 ,29 ,30 ,31 ,32 ,33 ,34), and all came to similar conclusions. Overall, it has a 64% sensitivity and 87% specificity for assessing disease activity when compared to healthy controls. β_2 microglobulin levels are increased with active disease, nephropathy, low C3 complement levels, elevated sedimentation rates, and anti-DNA. Its highest levels are seen in lupus nephritis, although azotemia with inactive disease also can result in larger values.

Table 46-1: Relevant Plasma Proteins in Systemic Lupus Erythematosus

Albumin
Globulin
α_1 Globulins
  α_1 Acid glycoprotein
  α_1 Fetoprotein
  α_1Antitrypsin
  α_1 Antichymotrypsin
  Neutrophil elastase
  Lactoferrin
α_2 Globulins
  Ceruloplasmin
  Haptoglobin
  α_2 Macroglobulin-Ha-glycoprotein
β_2 Globulins
  Transferrin
  β_2 Macroglycoprotein
  β Lipoproteins
  β_2 Microglobulin
  Complement components
  Prothrombin, fibrinogen, plasminogen, and other clotting factors
λ Globulin
  Paraproteins
  Cryoglobulins
  IgG
  IgM
  IgA
  IgD
  IgE

γ Globulins

A broad polyclonal elevation of the γ globulin fraction is a hallmark of autoimmune reactions, and if present should alert the clinician that the patient has an immune problem. It is seen in 8% to 77% of lupus patients, in our clinical practice a polyclonal gammopathy is present in 30% (4 ,6 ,9 ,10 ,35 ,36). Marked acquired hypogammaglobulinemia is present in less than 1% with SLE and is associated with recurrent infections (37 ,38 ,39 ,40 ,41 ,42 ,43 ,44 ,45); it can stem from marrow suppression, medication, or inflammation.

Serum Immunoglobulins

Immunoglobulin G and Its Subclasses

Mean serum levels of immunoglobulin (Ig)G are increased in patients with SLE compared to those in healthy controls (46). Evidence that this increase is polyclonal stems from the observation that isolated IgG elevation occurs in only 9% of patients with SLE (47). The IgG level tends to be elevated at diagnosis but normalizes with therapy. At any time, IgG was increased in 23% of 39 patients followed serially (48).

Levy (49) studied the mean survival half-life for IgG in patients with SLE. It averaged 8.2 days, compared with an average of 28 days in normal controls. An average of 10.1% of total-body IgG was catabolized daily, compared with a mean of 3.9% in normals. Despite normal serum IgG concentrations in patients with SLE, their synthetic rates were as much as four to five times normal, revealing far greater IgG antibody production in SLE than suggested merely by serum concentration. In a long-term serial study at the National Institutes of Health (NIH), 18 patients developed low IgG levels during the course of their disease, but it was transient in 110. Also, four developed recurrent infections. Excessive T-cell suppressor and decreased B-cell activity characterized this subset (50). Ward et al. (51) were unable to correlate IgG levels with age, sex, race, or duration of disease.

Several centers have evaluated patients with SLE for IgG subclasses. Among 20 children, significantly increased IgG1 and IgG3 subclasses were present, along with decreased IgG4; 48 adults with SLE had decreased IgG2 and IgG4 levels (52). Low IgG3 and IgG4 levels correlated with an increased rate of infection (53). Decreased IgG2 levels were found among 15 patients with SLE in Isenberg et al. (54), compared with 20 controls. In another report, an increased IgG1 level was associated with a subgroup of patients having high-titer rheumatoid factor, antinuclear antibody, and low levels of anti–double-stranded DNA (55).

Immunoglobulin M

Elevated mean IgM levels were found by Alarcon-Segovia and Fishbein (46) in 481 serum samples from 106 patients compared with those from 106 controls. Schoenfeld et al. (48) noted that the IgM level often was decreased at diagnosis but normalized later. In 39 patients followed serially, the IgM level was elevated at any time in 18%. Three other large-scale studies found that very low IgM levels (>2 standard deviations [SD] below the mean) could be found in 20% of over 200 patients with SLE (56 ,57 ,58). Low IgM levels tended to correlate with disease duration but not with activity (51). Survival studies of IgM in SLE show a normal half-life (49).

A 7S β-M-globulin occurs in SLE, RA, and in the cord blood of apparently normal infants, but it is absent in

normal human adult sera (59). This fraction was found by Rothfield (60) in 8 of 53 patients with SLE, 4 of whom were males, and in 32% of 31 men with SLE by Kaufman et al. (61). Low molecular weight IgM as a monomeric subunit probably comprises approximately 15% of the total IgM seen in patients with SLE (62).

In the hyper-IgM syndrome, patients have low IgG, IgA, and IgE with recurrent infections. The X-linked form is caused by mutations in the gene for CD40 ligand, and this has been reported in SLE patients (63).

Immunoglobulin A

IgA deficiency is found in from 1 in 400 to 1 in 3,800 adults. It has been observed in 3 of 72, 3 of 96, 0 of 181, 5 of 96, and 7 of 102 patients with SLE in five reports (64 ,65 ,66 ,67 ,68), which suggests an increased incidence of this uncommon finding. Men and those with antibodies to Sm and La may have an increased incidence of IgA deficiency in SLE (61), as may Afro-Caribbeans (67 ,68). Although the serum IgA level usually is normal or slightly elevated in SLE (46 ,47 ,57), elevations of IgA were found in 30% of patients during the course of disease in one study (48). Saliva γ A or secretory IgA levels may be reduced in patients with SLE and frequent attacks of respiratory disease (68). Blacks with SLE may have a higher IgA2 level than whites (69).

Immunoglobulin E

Increases in IgE levels may correlate roughly with disease activity in lupus erythematosus (LE) (70 ,71 ,72 ,73 ,74). Elevations of serum IgE are also associated with IgE isotype antinuclear antibody in those without signs of allergy and in males. IgE production is contributed to by DNA hypomethylation and enhanced by estrogens (74 ,75 ,76). Four cases of hyper-IgE syndrome, one following carbamazepine administration, have been reported in patients with SLE (77 ,78 ,79 ,80). A hyperimmunization phenomenon might by contributory to high IgE levels. (Chapter 67 reviews the relationship between IgE, lupus, and allergies.)

Monoclonal Gammopathies, Paraproteinemia, and Paraproteinuria

When ordering a serum protein electrophoresis, 2% to 4% of lupus patients will demonstrate a monoclonal gammopathy of unknown significance (MGUS). A long-term follow-up of 1,384 patients with MGUS at the Mayo Clinic suggested that 12% evolve myeloma, lymphoma, leukemia, amyloid, or macroglobulinemia over 10 years (81). Individuals with lupus and Sjögren syndrome should have a protein electrophoresis done every 6 to 12 months, since they have an increased risk of developing one of these complications. Cryoglobulinemia is reviewed in Chapter XX.

Of 415 patients with SLE followed in Toronto, nine (2.2%) had evidence of paraproteinemia (82). The monoclonal proteins were IgG (in 6 patients), IgA (in 2), and IgM (in 1). None had myeloma, and no consistent patterns could be discerned. Porcel et al. (83) noted a monoclonal gammopathy in 4 of 120 patients with SLE (3.3%). Approximately 30 cases have appeared in the literature (84). Characterized as transient, stable, or increasing, they are almost always benign. Kappa/lambda ratios for serum IgG, IgA, and IgM in 40 patients with SLE were not different from those in healthy controls (85).

Unbound free urinary light chains are increased in lupus nephritis and represent quantitative markers of concurrent in vivo immunoglobulin synthesis and secretion (86 ,87 ,88). Tsai et al. (89) observed free κ chains in 36% and free λ chains in 43% of 23 patients with active lupus nephritis but in none of the patients with inactive lupus nephritis.

Sedimentation Rate

Elevation of the sedimentation rate has been noted in 54% to 94% of lupus patients (7 ,9 ,90) and significantly associated with fevers, fatigue, alopecia, myalgias, and greater disease activity when elevated (7). Among 163 Italian SLE patients, the mean Westergren sedimentation rate was 37.26 ± 14.21 (1 SD) (91). Sedimentation rates can be high, with no obvious clinical activity, and normal with active disease. They usually are helpful in following the subset of patients for whom its rise and fall reflect other clinical and laboratory parameters. A recent study correlated sedimentation rate with disease activity and damage accrual among 450 patients (92). Occasionally, very high sedimentation rates in the absence of other findings lead to the performance of tests that ultimately result in the diagnosis of SLE (93).

The rapid sedimentation rate is partially attributable to the tendency of red cells to clump and form rouleaux, often because of the associated abnormal antibodies in SLE. When the Wintrobe method is used, rapid falling occasionally occurred, and the final value often was limited by the hematocrit. The Westergren method is more precise. (An excellent review of the subject can be found in Bedell and Bush (94)).

C-Reactive Protein

Why might C-reactive protein be important in lupus?

C-reactive protein (CRP) is a serum component that binds to pneumococcal C-polysaccharide. It is composed of five identical nonglycosylated polypeptide units of 187 amino acid residues (pentraxin proteins) each that are noncovalently associated in a disk-like configuration. CRP is synthesized by hepatic parenchymal cells, weighs 120,000

daltons, and circulates in the γ globulin fraction. It can act as an opsonin or agglutinin, and it mediates phagocytic activities while inhibiting immune responses. A putative evolutionary homology with immunoglobulin, complement, and human leukocyte antigen has been suggested (95). It activates complement inhibits cytokine production and generates T-suppressor cells, binds to phospholipids on damaged cell membranes, reacts with chromatin, and small nuclear ribonucleoprotein (snRNP) particles exposed in the setting of tissue injury with apoptosis. The latter might facilitate their same, nonimmunologic disposal and marks them for killing by phagocytes and complement. In mice expressing a human CRP transgene, CRP (in the presence of IL-10) protects against SLE by increasing blood and mesangial clearance of immune complexes (96 ,97 ,98 ,99 ,100 ,101 ,102).

Clinical Studies in SLE

First described as being elevated in patients with SLE and infection by Hill (103) in 1951, other reports using older methodologies of ascertainment suggested that it was an accurate test for active SLE (104 ,105 ,106 ,107). Enthusiasm peaked in 1980 when an editorial in the journal *Arthritis and Rheumatism* suggested that it might be a good American Rheumatism Association classification criterion for SLE (108). Other studies, however, found the CRP level to be useful for neither SLE nor infection (109 ,110). It soon became apparent that older methods for determining the CRP level were not accurate when rheumatoid factor also was present. Using the more reliable radioimmunodiffusion assay, Rothschild et al. (110) noted it to be elevated in 56% of 52 patients with SLE, with or without infection. It vaguely correlated with clinical activity but not with any organ system involvement, except for leukopenia. Bertouch et al. (111) observed elevated CRP levels in 55 of 70 patients with SLE; it was very high in 13, none of whom had infection. Morrow et al. (112) noted that CRP was present in 59% of 27 patients with SLE, especially in those with active disease. Zein et al. (113) found it in some patients without obvious explanation and observed numerous disease exacerbations in patients without any CRP changes. In another survey, only 9% of 34 patients with SLE had an elevated CRP level (114).

Pepys et al. (115) reviewed the subject at length, following sedimentation rates and CRP binding in 429 measurements involving 124 patients with inactive, mildly active, and active SLE, and in SLE with infection. It is clear that the mean CRP level is not elevated, or is only slightly elevated, in the first three categories, but it is high with infection. Mean CRP levels were significantly greater than sedimentation rates with infection but significantly less with active disease. The CRP levels in individual patients with active disease or infection, however, ranged from absent to high. In other words, CRP was not useful in individual cases, although the mean values in patient groups were significantly elevated. The only positive conclusion that can be reached is that if the CRP level is very high (>60 mg/L), the chances of infection are greater which has been confirmed by others (116 ,117 ,118 ,119), although Middleton et al. (120 ,121) have disputed this CRP values are high in those with RA and the seronegative spondyloarthropathies, but they are only modestly elevated in those with systemic vasculitis. Williams et al. found high levels with renal involvement (122).

Nearly half with SLE have autoantibodies to CRP, especially if anti DNA is present (123 ,124). High sensitivity CRP is only a rough guide to disease activity (125).

In conclusion, CRP is a misunderstood test of disease activity that is neither sensitive nor specific in SLE. It may be of some value, however, for ruling in infection and may be of future importance as an immune suppressive adjunct in the disease's management.

Viscosity

Viscosity is an important determinant of blood flow. Plasma viscosity can be increased by elevations of high molecular weight globulins, such as fibrinogen and immunoglobulin. Four studies have shown that patients with SLE have slightly increased levels when compared to control groups (34 ,114 ,126 ,127 ,128 ,129). Rosenson et al. correlated blood viscosity with a higher SLICC/ACR Damage Index (130). Rarely, complexes of IgG, and especially IgM rheumatoid factor, produce high levels of plasma viscosity and a clinical syndrome resembling that found in Waldenström macroglobulinemia. This so-called hyperviscosity syndrome has been observed infrequently in SLE, and it is an indication for emergency plasmapheresis and steroid therapy (131 ,132 ,133).

Miscellaneous Laboratory Abnormalities, Connective Tissue Components, and Trace Metals

Connective Tissue Components

SLE is characterized by striking changes in the amorphous ground substance of tissues. Consisting largely of hyaluronic acid and chondroitin sulfuric acid, hexosamine constitutes approximately 40% of each of these mucopolysaccharides, and human serum contains definite amounts of bound hexosamine as glucose and galactosamine. Serum levels of hexosamine were increased in active disease in one report that followed 19 patients serially (134). Free and bound glycosaminoglycans, which consist mostly of slow sulfated chondroitin 4-sulfate, also are elevated with active disease (135). Additionally, serum immunoreactive prolyl hydroxylase is an acute-phase reactant in SLE, and its increased levels may reflect greater connective tissue disease metabolism (136). On the other hand, serum sulfhydryl and serum histidine levels decrease in active disease (114 ,137). Urinary sialylated saccharides, serum sialic acid, and serum amyloid A protein may act as acute-phase reactants in SLE as well, but is disappointing (138 ,139 ,140). Serum laminin P1 is one of

the glycoproteins of basement membranes is found in high amounts with active disease (141 ,142). Calprotectin (L1), which is a granulocytic and monocyte cytosolic protein released during activation of these cells, is said to be elevated in SLE (143 ,144).

Trace Metals

Zinc and selenium levels are normal in SLE (145).

Other Putative Markers of Disease Activity

A marker for adenocarcinoma, CA 19.9, also can be increased with inflammatory joint disease (146), as can the ovarian cancer marker CA 125 (147 ,148 ,149). Several studies have suggested that serum thrombomodulin, a marker for endothelial cell injury, correlates with vasculitis and SLE activity (150 ,151 ,152 ,153 ,154 ,155 ,156). Nerve growth factor may be elevated in SLE as well as in RA (157 ,158). Serum levels of a cytoplasmic enzyme in the pyrimidine salvage pathway, cytidine deaminase, and the enzyme for purine nucleosides, adenosine deaminase, are increased with active lupus as well (159 ,160 ,161 ,162 ,163). Endothelin-1 is a vasoconstrictor peptide that is present in active SLE in the plasma in high concentrations and may correlate with vascular injury and/or pulmonary hypertension (164 ,165), as are serum levels of the lysosomal enzyme ß-glucuronidase (166) and the serine exoproteinase dipeptidyl peptidase IV (167). Single reports have claimed that cathepsin D activity (168) and plasma neopterin (169 ,170 ,171), ferritin (172 ,173 ,174), soluble vascular cell adhesion molecule-1 (VCAM-1) (175), tyrosine kinase receptor (176), matrix metalloproteinase-3 or 9 (177 ,178 ,179), serum levels of HLA class I antigen (180), katacalcin (181), serum leucine aminopeptidase (182), serum 3-nitrotyrosine (183), and plasma thrombospondin levels (184) are increased in SLE. Serum procalcitonin is increased in early infections in SLE (185) as is serum and urine nitrate and citrulline (186).

Summary

- Hypoalbuminemia occurs in patients with SLE and active disease, particularly nephrosis. Following serum albumin levels serially is of prognostic value.
- Both α_1- and α_2-globulins include acute-phase reactants that are increased and carrier proteins that are decreased in active SLE.
- The IgG level is elevated with disease activity in patients who are not on steroids or immunosuppressive drugs. Its turnover is greatly increased. No IgG subclass is characteristic, and the IgM level is consistently decreased in 20% of patients.
- Sedimentation rates are elevated with active SLE; disease activity thus can be followed in a subset of patients. In some patients, the sedimentation rate does not correlate with disease activity. The CRP level usually is normal or slightly elevated in SLE; high levels should raise suspicions of infection.
- $ß_2$-Microglobulin levels generally are increased in active SLE, especially if renal disease is present.
- Although plasma viscosity is slightly increased in SLE, hyperviscosity syndrome is an extreme rarity.

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Chapter 47

Clinical Indices in the Assessment of Lupus

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Systemic lupus erythematosus (SLE) is a heterogeneous disease with waxing and waning manifestations. As such, the assessment of SLE is complicated and needs to encompass a variety of domains. In 1998, OMERACT (outcome measures in rheumatoid arthritis clinical trials) evaluated 21 different domains that were candidates for inclusion in clinical trials of SLE (1). They concluded that a minimum of four domains were necessary. Broadly the domains specific to lupus can be described as disease activity, damage, and quality of life. Disease activity is a measure of the reversible manifestations of the SLE, whereas damage represents irreversible changes. Quality of life represents the functional ability of the patient and includes a variety of domains including physical and social function. The fourth domain proposed by OMERACT is toxicity and adverse events. This chapter will focus on the development and characteristics of the instruments used to assess these different domains.

Disease Activity

More than 60 different instruments have been developed to assess disease activity in lupus. To date, there has been no agreement as to the “gold standard” in this field. The more commonly used measures, in order of date of publication, include the British Isles Lupus Assessment Group index (BILAG) (2), the Systemic Lupus Activity Measure (SLAM) (3), the Lupus Activity Index (LAI) (4), the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (5), the European Consensus Lupus Activity Measure (ECLAM) (6). A key feature of all of these instruments in addition to the focus of active SLE as opposed to damage is the attribution of the manifestations to SLE. Although they are all valid, reliable, responsive indices that have been shown to correlate with each other (7), there are significant differences that will be reviewed.

British Lupus Isles Assessment Group index (BILAG)

BILAG was developed using a nominal consensus approach by a group of investigators from five centers in the United Kingdom and was first published in 1988 (2). The instrument scores the activity in eight organ-based systems occurring in the preceding month. It is based on an intent to treat principle and uses the following ratings:

- A—Action (severe disease that would warrant increased prednisone at more than 20 mg a day or the addition of other immunosuppressants)
- B—Beware (less active disease that would be treated with low-dose prednisone, nonsteroidal anti-inflammatories or antimalarials)
- C—Contentment (mild stable disease with no change in therapy or simple analgesics)
- D—(system unaffected)

The organ-based systems include general features, mucocutaneous, neurologic, musculoskeletal, cardiorespiratory, vasculopathy, renal, and hematologic. Immunologic abnormalities are not included in this index. Although not originally derived to be a global score, a weighted system where an A = 9, B = 4, C = 1, and D = 0, was devised to yield a total score of all the organ systems ranging from 0 to 72 (8). This was later revised to A = 9, B = 3, C = 1, D = 0, and E = 0 (version 3).

The initial BILAG has undergone a series of revisions. BILAG (version 2) included modifications of some features of the original BILAG that were found to have poor face validity such as small fluctuations in blood pressure. Liang et al. evaluated this version and found good reliability both within and between raters and good correlation with other measures of disease activity (3).

Hay et al. validated BILAG (version 3) (9). Modifications to this version included clarification of ambiguous terms, the addition of a glossary, definition of a time scale, the standardization of the calculation of the BILAG score, and the change of D to Discount (previously unaffected) and the addition of a category E, never affected. The positive predictive value (ppv) of a BILAG A, that is the likelihood that a patient with an A score would receive prednisone at greater than or equal to 20 mg a day or additional immunosuppressant therapy was excellent at 80%. The only individual systems that did not have at least an 80% ppv were the nervous system (ppv 30%) and hematologic (ppv 50%).

Stoll et al. used a cross-sectional study of 133 patients to further evaluate the validity of the BILAG in relationship to patients' and doctors' global assessments and the SF20+ (10). The BILAG organ systems of general, neurologic, musculoskeletal, and vasculitis correlated with the patient and physician global assessments. The same systems excluding vasculitis, but including the cardiorespiratory system correlated with the SF20+. The hematologic and renal components demonstrated an association with laboratory parameters of sedimentation rate and C3 level. Only the mucocutaneous system failed to demonstrate any significant associations with these measures. Importantly, the individual organ systems did not demonstrate significant correlation with each other. Also, disease activity was often very discordant among systems stressing the benefits of using an organ-system–based disease activity index as opposed to a global activity index.

Also using BILAG version 3, Gordon et al. evaluated whether treatment differed for BILAG A scores as compared with BILAG B scores in routine clinical practice at two different institutions (11). Two hundred and fifty patients seen regularly over a 12-month period were assessed with a BILAG score at each visit. A new A flare was seen in 10.4% of patients with 92% of these patients having an increase in their lupus-related therapies, consistent with the principles upon which the BILAG was derived. A new B score preceded by a D or E scores was seen in 26% and a B score preceded by a C score was seen in 25.2%. During the 1-year period, 38.4% of the patients did not have an A or B score. Of the B flares, 32% received some increase in steroid and/or cytotoxic therapy, whereas 59% did not receive any additional therapy for a variety of reasons ranging from recently starting therapy to allowing the disease to settle spontaneously.

A computerized program called the British Lupus Integrated Prospective System (BLIPS), which automatically calculates the A-B-C-D or E score has been developed using the BILAG version modified in 2000 (12). This program also includes a glossary for BILAG, SLAM-R, SELENA-SLEDAI, the SLICC/ACR Damage Index and SF-36 Health Survey. Figure 47-1 shows the BILAG worksheet for this. This sophisticated software should facilitate clinical research in SLE.

Although BILAG was originally developed for adult lupus patients, Brunner et al. adapted it and assessed its use in children (13). In 2004, BILAG was validated for use in childhood lupus using a prospective cohort of 21 patients under age 18 followed for a 1-year period (14).

Over time, members of the British Isles Lupus Assessment Group have found certain aspects of the BILAG index unsatisfactory and modifications have been made. As a result BILAG 2004 was developed (15). This version has nine organ-based systems rather than eight adding lupus-related ophthalmologic problems, re-organizing and expanding the gastrointestinal and hepatic manifestations into its own category and removing the vasculitis category and distributing its components to the appropriate organ system. Furthermore, BILAG 2004 deletes some items from the current BILAG that are really more damage than activity such as avascular necrosis. Also, BILAG 2004 states that an A score that is improving becomes a B score, rather than a C as it is currently measured to try to more accurately reflect change in activity. In two real patient exercises, the instrument demonstrated good reliability and agreement in all organ systems except musculoskeletal. Further improvement of the glossary as well as additional validation studies are underway. It is anticipated that BILAG 2004 will be the BILAG index used in the near future.

In a draft statement of guidelines for the development of drugs for the treatment of SLE put forward by the Food and Drug Administration (FDA), it is recommended that BILAG be used in randomized clinical trials, because it is based on the principle of intent to treat and a change in the score should be clinically meaningful (16). For this reason, this instrument will gain more widespread use in lupus trials. As such, it is imperative that investigators be trained properly on this instrument as it is more complicated and does have a subjective component that if not used consistently between different sites and studies will interfere with its utility.

Systemic Lupus Activity Measure

The SLAM was developed at the Brigham and Women's Hospital and published in 1989 (3). Like the SLEDAI, it is a global score assessing overall disease activity occurring in the month preceding the assessment with a score ranging from 0 to 84. It consists of 24 clinical and 7 laboratory manifestations of lupus. The manifestations are graded as active or inactive, with activity scores varying from mild to moderate to severe. A revised version, SLAM-R, which drops the pneumonitis and “other” manifestations and rewords some of the definitions has been more commonly used (17 ,18). Like SLEDAI, SLAM has been shown to be a valid, reliable measure that is sensitive to change in both adults and children (3 ,13 ,19 ,20). There are a few important differences between SLEDAI and SLAM. SLE manifestations in SLAM are not weighted giving equal importance to features, such as fatigue and urinary sediment. It does, however, allow for severity to be taken into account such that a severe rash scores more than a mild rash. It also captures subjective features in SLE such as fatigue and myalgias, which are not part of SLEDAI. This may be a problem in studies of disease activity as the SLAM scores fibromyalgia, which frequently does not correlate with active lupus.

Also, because it is a subjective index, it would be very difficult to use the instrument in multicenter studies, where reproducibility of an instrument between sites is critical.

The Lupus Activity Index

The LAI was developed at Johns Hopkins University and the University of California, San Francisco (4). It is a global scale with five parts that assesses disease activity occurring

up to 14 days before the visit. Part one is the physician's global assessment (PGA) scored on a 0 to 3 point Visual Analogue Scale (VAS) where 3 represents the most severe disease. Part two is an assessment of fatigue, rash, arthritis, and serositis again on a 0 to 3 VAS. Part three scores the activity of the neurologic, renal, pulmonary, and hematologic organ systems of a 0 to 3 VAS. Part four scores medications with higher doses of prednisone and cytotoxic agents receiving higher scores. Part five scores three laboratory parameters of urinary sediment, anti-DNA levels, and complement levels. The LAI summary score is calculated as the mean of part one score plus the mean of the four values in part 2 plus the maximum value in part 3 plus the mean of the scores in part 4 plus the mean of the three laboratory values. The summary score ranges from 0 to 3. The modified LAI (modified to exclude part 1, the PGA) correlated well with the PGA and with SLEDAI (4). It has also been shown to be sensitive to change (20).

SLEDAI-2K: DATA COLLECTION SHEET

Study No.: _____ Patient Name: _____ Visit Date: _____ _____ _____
d m yr

(Enter weight in SLEDAI-2K Score column if descriptor is present at the time of the visit or in the preceding 10 days.)

SLEDAI 2K Weight	SCORE	Descriptor	Definition
8	_____	Seizure	Recent onset, exclude metabolic, infectious or drug causes.
8	_____	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Exclude uremia and drug causes
8	_____	Organic brain syndrome	Altered mental function with impaired orientation, memory, or other intellectual function, with rapid onset and fluctuating clinical features, inability to sustain attention to environment, plus at least 2 of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious, or drug causes
8	_____	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serous exudate or hemorrhages in the choroid or optic neuritis. Exclude hypertension, infection, or drug causes.
8	_____	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	_____	Lupus headache	Severe, persistent headache; may be migrainous but must be nonresponsive to narcotic analgesia.
8	_____	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis.
8	_____	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis.
4	_____	Arthritis	≥ 2 joints with pain and signs of inflammation (ie., tenderness, swelling or effusion).
4	_____	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis.
4	_____	Urinary casts	Heme-granular or red blood cell casts.
4	_____	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	_____	Proteinuria	>0.5 gram/24 hours
4	_____	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	_____	Rash	Inflammatory type rash.
2	_____	Alopecia	Abnormal, patchy or diffuse loss of hair.
2	_____	Mucosal ulcers	Oral or nasal ulcerations.
2	_____	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	_____	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram or echocardiogram confirmation.
2	_____	Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory
2	_____	Increased DNA binding	Increased DNA binding by Farr assay above normal range for testing laboratory.
1	_____	Fever	>38° C. Exclude infectious cause.
1	_____	Thrombocytopenia	<100,000 platelets / $x10^9$/L, exclude drug causes.
1	_____	Leukopenia	< 3,000 white blood cells / $x10^9$/L, exclude drug causes.
TOTAL SCORE	_____		

Figure 47-1. BILAG (version 3) worksheet. Most items of the worksheet are recorded (not scored), 1 = improving, 2 = same, 3 = worse, and 4 = new. Items not present are recorded (not scored) as zero. Laboratory values and blood pressure readings are entered as numeric values and a few items are recorded as Yes/No depending on whether they are present or absent. Scores of A, B and C for each system are determine based on which items have been present during the last 4 weeks and whether they are recorded as 1, 2, 3 or 4, yes or have abnormal numerical values. (ref 9, 12)

Systemic Lupus Erythematosus Disease Activity Index

The SLEDAI was developed at the University of Toronto in 1992 (5). It is a one-page weighted scale for 24 items. The weighting system was derived by multiple regression analysis from expert clinicians' judgment about the features' contribution to the overall disease activity. The manifestations felt to be most commonly contributing to disease activity are included and are scored based on presence or absence within 10 days of the evaluation and are more objective rather than subjective in nature. The score can range from 0 to 105 and is a global score reflecting all aspects of disease activity. The SLEDAI does include immunologic laboratory results. It has been shown to be a valid, reliable instrument that is sensitive to change (7 ,21 ,22). Its validity has also been demonstrated in the study of childhood SLE (13).

Since the publication of the original SLEDAI, several modifications of the SLEDAI have been made. These versions of the SLEDAI include the MEX-SLEDAI (23), the SELENA-SLEDAI (24), and SLEDAI-2K (25). MEX-SLEDAI was developed by Guzman et al. for use in countries where immunologic tests are not routinely available (23). The MEX-SLEDAI does not include complement levels, anti-DNA antibodies, visual disturbances, lupus headache, and pyuria. It does, however, include creatinine increases of more than 5 mg/dL, lymphopenia, fatigue, hemolysis, and peritonitis. In a prospective study of 39 patients seen for three consecutive visits, the MEX-SLEDAI, like the SLEDAI, was found to be reliable and showed good correlation with expert opinion and treatment changes. The MEX-SLEDAI was less expensive to administer. The MEX-SLEDAI was also evaluated in the LUpus in MInority populations: NAture vs. nurture (LUMINA) study and found to show good correlation with the SLEDAI-2K and the PGA (26) and again was less expensive than the SLEDAI-2K.

Another modification of the SLEDAI occurred for the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA) trial (24). The SELENA-SLEDAI modification has several different definitions in an attempt to improve clarification and attribution for the individual items. The seizure descriptor in the SELENA-SLEDAI specifically excludes seizures that are caused by old, irreversible CNS damage. The visual disturbances item was expanded to include scleritis and episcleritis, and the cranial nerve descriptor was expanded to include vertigo. The cerebrovascular accident item was clarified to exclude hypertensive causes. The pleurisy and pericarditis item definitions were expanded to ensure attribution of the manifestations to lupus by added the phrase “severe and classic” to the definitions. The SELENA-SLEDAI also modified the proteinuria item by adding new-onset or recent increase to the definition of >0.5 g in 24 hours to try to better capture changes in disease activity.

In 2002, Gladman et al. published their updated version of the SLEDAI called the SLEDAI-2K (27) (Fig. 47-2). The modifications in this version were done in part to capture persistent active disease manifestations and not just new or recurrent ones. As in the SELENA-SLEDAI, the authors wanted to score ongoing rashes, proteinuria, alopecia, and mucous membrane lesions. However, they did not concur with all the modifications of the SELENA-SLEDAI, such as the inclusion of scleritis and episcleritis with a score of 8 points and were concerned about the lack of a rigorous validation of the new instrument. Using their Toronto cohort representing 18,636 visits, only 22% differed in the SLEDAI-2K versus SLEDAI score. Furthermore, 212 patients were assessed on a five-point scale as either inactive, mild activity, activity improved from the previous visit, persistent activity, or flare by an independent rheumatologist who was blinded to the SLEDAI score. They found that both SLEDAI and SLEDAI-2K scores were significantly different between the activity categories and that change in disease activity was similar for both SLEDAI and SLEDAI-2K. Thus, SLEDAI-2K, like SLEDAI, was a valid measure for disease activity in SLE. The authors propose to use this version in clinical trials to avoid the appearance of improvement when a score in the older version would decrease for persistent activity.

Gladman et al. have devised an adjusted mean SLEDAI (AMS) to capture disease activity over a period of time as assessed by SLEDAI-2K (28). This is calculated as an area under the curve over time divided by the observed time interval. In a cohort of 575 patients, higher scores have been associated with increased mortality. In a follow-up study, the authors further evaluated AMS SLEDAI-2K relationship to outcomes in addition to survival (29). Coronary artery disease (CAD) and the presence of damage defined by a score of ≥1 on the SLICC/ACR Damage Index was positively associated with the AMS-SLEDAI-2K, as was age, disease duration, and use of steroid or immunosuppressive agents. CAD was also associated with gender. These findings suggest that longitudinal studies in lupus

should consider using AMS-SLEDAI-2K to evaluate the relationship of disease activity with outcomes.

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change *or* optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impairec performance level) *or* major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebrovascular accident ever (score 2 if >1)	1 (2)
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured glomerular filtration rate <50%	1
Proteinuria ≥3.5 grn/24 hours *or*	1
End-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hyperteasion (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	1
Cardiovascular	
Angina *or* coronary artery bypass	1
Myocardial infarction ever (score 2 if >1)	1 (2)
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	1
Pericarditis for 6 months, *or* pericardiectomy	1
Peripheral vascular	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue los ever (e.g., loss of digit or limb) (score 2 if >1 site)	1 (2)
Venous thrombosis with swelling, ulceration, *or* venous stasis	1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver, or gall bladder ever, for any cause (score 2 if >1 site)	1 (2)
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture *or* upper gastrointestinal tract surgery ever	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (score 2 if >1)	1 (2)
Osteomyelitis	1
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (excluding thrombosis) for >6 months	1
Premature gonadal failure	1
Diabetes (regardless of treatment)	1
Malignancy (exclude dysplasia) (score 2 if >1 site)	1 (2)

Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

Figure 47-2. The Systemic Lupus Erythematosus Disease Activity Index 2K. (From Bombardier C, Gladman DD, Urowitz MB, et al. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee for Prognosis Studies in SLE. *Arthritis Rheum* 1992;35:630–640, with permission.)

The SLEDAI is a disease activity that is brief, easy to administer and more objective in its scoring. It is relatively easy to train investigators to use this instrument. This theoretically should improve the correlation between observers. It does have limitations in that is rather heavily weighted for central nervous system manifestations, which are not that frequent. Furthermore, it is not as comprehensive as other indices as it does not score several life-threatening manifestations such as pulmonary hemorrhage, hemolytic anemia, and thrombotic thrombocytopenic purpura. Also, it does not take into account severity of manifestations. For example, a platelet count of 99,000/mm^3 scores the same 1 point as a platelet count of 5,000/mm^3. These benefits and deficiencies need to be taken into account when deciding which indices to use for any given study. In the draft of the guidance for industry for the development of drugs for lupus, it is proposed that if SLEDAI is used as an outcome measure of disease activity for a particular trial, that another disease activity measure also be used as what is considered a clinically meaningful change in a SLEDAI score is complicated (16).

The European Consensus Lupus Activity Measurement Index

The ECLAM was developed in 1992 from data on 704 actual SLE patients. Using the PGA as the gold standard of disease activity, univariate and multivariate analysis were performed to determine which features should be included and the weight to ascribe to each feature (6 ,30). Like the other instruments described, it is a global score. Nine clinical and three laboratory manifestations are scored based on their appearance within the preceding 1 month. Complement levels and sedimentation rates are included but autobody titers are not. The total score ranges from 0 to 10. Like SLEDAI, it does not provide any additional weight for some items such as more severe arthritis, but new or worsening features such as rashes and nephritis do get additional points. ECLAM has been shown to be a valid measure that is sensitive to change (6 ,20). Ward et al. found that ECLAM, along with LAI, was the instrument most sensitive to change as defined by the PGA. The correlation with LAI may be artificially elevated as the LAI included the PGA (20). ECLAM has also been shown to be sensitive to the change in disease activity for childhood SLE (31). A retrospective study of disease activity using ECLAM found good correlation between scores obtained from retrospective chart review compared with direct standard ascertainment of the score (32). Unlike retrospective studies of SLEDAI (33) and SLAM (34), there was no underestimation of disease activity. This suggests that, although not the ideal study design, ECLAM may be the best disease activity measure to use if scores need to be calculated retrospectively. However, retrospective assessment by any index will only be as good as the details in the clinical chart and may vary between centers.

Comparison of the Disease Indices

Although all the activity indices are valid measures, there are some differences that may make one instrument a more desirable tool to use in a particular situation. Table 47-1 summarizes these differences. For example, some instruments with a shorter review period may be better for studies with more frequent visits. Global scores, although convenient and perhaps quicker to calculate are problematic in that patients can have the same score whether they are improving, stable, or worsening. For instance, using SLEDAI, a rash can improve but still be present and will get the same score. Furthermore, one system can improve while another system worsens, which has been estimated to happen 10% of the time (35), and the score can be the same. Furthermore, global scores can have difficulty in differentiating patients with many mild features from patients with one or two severe features. Indices that are more subjective will require additional training for investigators to ensure some enhanced degree of uniformity to minimize the inherent error.

Sensitivity to Change

BILAG, SLEDAI, and SLAM were simultaneous assessed to compare the ability to assess changes in disease activity (7). All three were found to detect differences between patients, although in this study, SLEDAI was perhaps a little better determining changes between visits. Fortin et al. evaluated 96 patients monthly for 5 months using SLAM-R, SLEDAI, PGA, and a physician's transitional score of stable, improved, or the same at each visit (19). Both SLEDAI and SLAM-R were sensitive to change, but SLAM-R was more sensitive. Ward et al. evaluated 23 patients at 2-week intervals for up to 40 weeks with SLEDAI, ECLAM, BILAG, SLAM, and LAI at each visit (20). Using the PGA as the gold standard, all indices were sensitive to change, with LAI and ECLAM the most sensitive and SLEDAI the least sensitive. As evident, there is no consensus as to which disease activity measure is the most sensitive.

Disease Flare

Flare in SLE has been an important yet elusive concept. A variety of definitions have been proposed, but there is no uniform agreement of what constitutes a flare. Petri et al. have defined disease flare as an increase in PGA of 1.0 or greater (36). In a study of 185 patients with SLE, the incidence of flare was 0.65 flares per patient-year.

Table 47-1: Comparison of the Different Disease Activity Indices (68)

	SLEDAI	SLAM-R	LAI	ECLAM	BILAG
Number of Items	24	30	14	30	86
Number of organ systems	9	9	8	10	8
Review period	10 days	28 days	14 days	28 days	28 days
Scoring	Global score	Global score	Global score	Global score	Individual organ systems but also global score
Transitional scoring (better/worse/same)	No	No	No	Partial for the renal and mucocutaneous categories	Yes
Objective/subjective	Objective	Both-but subjective rating of mild/moderate or severe	Both	Both	Both
Weighted variable	Yes	Yes	No	Yes	No
Immunologic variables	Yes	No	Yes	Yes	No
Severity assessment	No	Yes	Yes	No	Yes

Modified from Griffiths B, Mosca M, Gordon C. Assessment of patients with systemic lupus erythematosus and the use of lupus disease activity indices. *Best Pract Res Clin Rheumatol* 2005;19(5):685–708.

Using BILAG with its intent to treat premise, Gordon et al. defined a severe flare as a new BILAG A in any organ system from a previous B, C, D, or E. A moderate flare is a new BILAG B in any organ system from a previous C, D, or E (11). An important feature of the BILAG scoring system is the ability to assess each system separately. Thus, a flare data can be analyzed as a new A or B in a particular system, or as any new A or B from no A or B at all.

Gladman et al. compared SLEDAI scores to a 5-point scale of no activity, mild activity but no change in treatment, mild activity but improvement, persistent activity, and flare in a cohort of lupus patients (37). Their results suggested that an increase in a SLEDAI score of more than 3 was a flare, that a decrease in score of more than 3 was improvement, a SLEDAI score that was within 3 points of the previous score was persistent disease, and a SLEDAI score of 0 was remission.

Criteria for mild/moderate and severe flares were developed for use in the SELENA trial (24). Criteria for a mild/moderate flare included a change in the SLEDAI of 3 points or more, new or worse discoid rash, photosensitivity, lupus profundus, cutaneous vasculitis or bullous lesions, nasopharyngeal ulcers, pleuritis, pericarditis, arthritis, or fever attributable to lupus, the need to increase prednisone to less than 0.5 mg/kg/day, and an increase in the PGA of 1.0 to 2.5 (on a 0 to 3 VAS). A severe flare according to the study was a change in the SLEDAI score to greater than 12, or the following manifestations only if they required a doubling of the prednisone or an dose of prednisone greater than 0.5 mg/kg/day: new or worse CNS lupus, vasculitis, nephritis, myositis, thrombocytopenia of less than 60,000 per mL or hemolytic anemia with a hemoglobin level of less than 7 or a decrease in the hemoglobin greater than 3. A severe flare was also defined by need for hospitalization because of lupus activity, institution of new cytotoxic therapy, institution of prednisone to greater than 0.5 mg/kg/day or an increase in the PGA to a level greater than 2.5 (on a 0 to 3 VAS).

Abrahamowicz et al. evaluated the relationship between SLAM-R and SLEDAI in 30 paper patients (17). They found that 70% of physicians would initiate treatment at a score of 10 for both instruments.

Using a Dutch activity index, TerBorg et al. defined criteria for major and minor flares (38). A major flare consisted of severe manifestation of SLE with specific definitions that did not improve after 1 week of 30 mg/day of prednisolone. A minor flare included an increase in the activity index by at least two points to a score of at least three and the need to start at least 10 mg/day of prednisolone or increase the current prednisolone by at least 5 mg/day or start an antimalarial or immunosuppressant drug.

As clinical trials in lupus rapidly develop, the concept of flare will be further refined and defined.

Response in Lupus

Just as flare in SLE is an important idea, so is response. A priori definitions of response are critically important in lupus clinical trials. A variety of response definitions have

been proposed. Because the BILAG index is a transitional index, a variety of endpoints could be used. One possible response criteria is the time to remission defined as the loss of all initial A and B scores. Another response could be the proportion of patients that have lost all A and B scores at a predetermined time point. Responder Index for Lupus Erythematosus (RIFLE) has been developed and evaluated for this purpose (39). With this instrument, patients are characterized as complete responders, partial responders, and nonresponders.

The ACR set up an ad hoc committee to address the definition of response (35). This committee looked at the six most commonly used disease activity indices, the BILAG, SLEDAI, SLAM-R, ECLAM, SELENA-SLEDAI, and RIFLE, to define clinically meaningful improvement, no change, and worsening. To accomplish this task, they gathered data on 310 patients for paper vignettes. Then 88 lupus experts rated 5 common paper patient cases and an additional random 10 cases as to whether the patients were better, the same, or worse than the previous visit. The results were used to construct performance characteristic curves comparing the expert opinion with each of the different indices' scores. Using a 70% agreement of the expert physicians as the score at which a meaningful change could be determined, the following changes in score represented improvement: BILAG = 7, SLEDAI = 6, SLAM-R = 4, ECLAM = 3, SELENA-SLEDAI = 7, and RIFLE = 4. For a clinically meaningful worsening, the BILAG, SLEDAI, and SELENA-SLEDAI would need to increase by 8, the SLAM-R by 6, the ECLAM by 4, and RIFLE by 3. These findings may be useful in the design of clinical trials. However, it is not recommended to use BILAG as a global score. Whether these findings will hold true for all levels of disease activity deserves further evaluation as larger changes may need to be seen for those with already high levels of disease activity, whereas those with milder disease may need smaller degrees of change for significant improvement or worsening.

Damage Index

Damage is an important outcome for measurement in SLE. Although it is inherent in activity indices that all scoring must be attributable to active lupus, the same does not hold true for damage, which could be a result of the illness, side effects of medications, or comorbid conditions. In 1996, the Systemic Lupus International Collaborating Clinics developed the SLICC/ACR Damage Index (DI) (Fig. 47-3) (40). The DI was found to have reliability and validity when used by 10 physicians from five countries in the assessment of 10 actual patients with SLE (41). It has also been shown to have good agreement with prospective and retrospective measurement (42). The index is assessed from the onset of lupus and is irrespective of current disease activity, therapy, and disability in the patient. The index includes descriptors in 12 organ systems ranging from SLE-associated complications, such as renal with proteinuria, reduced glomerular filtration, and dialysis to those potentially related to complications of therapy, such as diabetes and premature gonadal failure to comorbid conditions associated with SLE, such as cardiovascular complications of angina and myocardial infarction. For the purposes of the SLICC/ACR DI, damage is considered only if present for at least 6 months. Some items such as cerebrovascular accidents and avascular necrosis can be scored twice if there is more than one occurrence. Also, once an item is scored, it will remain positive even if the manifestation resolves, such as could be seen with proteinuria, because the concept of damage argues against a score improving.

Damage is common in patients with lupus. In a study of 200 lupus patients from five centers, 61% were noted to have damage within 7 years of disease onset with a mean of 3.8 years (43). Furthermore, in a study of 1,297 patients from eight centers, the DI has been shown to increase over time (44). In a follow-up study from their own cohort, Gladman et al. also found that damage increased over time, and that a substantial portion of that increase was attributable to corticosteroid therapy (45).

The DI score has been associated with an increased mortality for those with high scores early in the course of the disease. Ninety-nine of the 1,297 patients had died and the DI was significantly higher early in those patients compared with those who survived (44). Two other studies have also found higher DI scores to be associated with an increased mortality (46 ,47).

Although there are few long-term longitudinal studies looking at what factors are associated with higher DI scores, intuitively, disease activity would be expected to correlate with damage. Gilboe et al. found that baseline disease activity did predict an increase in the DI score at 2 years (48). In a study of pediatric lupus, higher disease activity over time, as assessed by SLEDAI, was associated with more damage (49). More recently, in an adult lupus cohort of 141 patients followed prospectively for 5 years with serial DI and BILAG scores, damage and death were strongly associated with higher cumulative disease activity and a higher average number of A flares (50). Although there was no relationship between the SLEDAI and SLICC/DI score in the Toronto cohort (51), the adjusted mean in the SLEDAI, which assesses disease activity over time, did correlate with the DI (29).

In summary, the SLICC/ACR DI may be helpful in clinical practice to identify those with a poor prognosis. It may also be a useful instrument for stratification in trials of lupus, given its prognostic implications. Caution is advised, however, when using the DI in short-term clinical trials of up to 12 months' duration. If patients with long disease duration are included, damage may develop that reflects disease activity and therapy that predates the onset of the trial.

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change or optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g., memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level) or major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebrovascular accident ever (score 2 if >1)	1 (2)
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured glomerular filtration rate <50%	1
Proteinuria ≥3.5 gm/24 hours	1
or	
End-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	1
Cardiovascular	
Angina or coronary artery bypass	1
Myocardial infarcton ever (score 2 if >1)	1 (2)
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic murmur, or systolic murmur >3/6)	1
Pericarditis for 6 months, or pericardiectomy	1
Peripheral vascular	
Claudication for 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss ever (e.g., loss of digit or limb) (score 2 if >1 site)	1 (2)
Venous thrombosis with swelling, ulceration, or venous stasis	1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver, or gall bladder ever, for any cause (score 2 if >1 site)	1 (2)
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture or upper gastrointestinal tract surgery ever	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture or vertebral collapse (excluding avascular necrosis)	1
Avascular necrosis (score 2 if >1)	1 (2)
Osteomyelitis	1
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (excluding thrombosis) for >6 months	1
Premature gonadal failure	1
Diabetes (regardless of treatment)	1
Malignancy (exclude dysplasia) (score 2 if >1 site)	1 (2)

Damage (nonrevensible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinicl assessment and present for at least 6 months unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice.

Figure 47-3. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for SLE. (From

Gladman DD, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for Systemic Lupus Erythematosus. *Arthritis Rheum* 1996;39:363–369, with permission.)

Health Status

Health status refers to measurements of quality of life from a patient's perspective. This is a third important component in the assessment of patients with SLE. Gladman et al. compared five different health status instruments in lupus, the Health Assessment Questionnaire (HAQ), 1) Functional Ability Index, 2) the Fatigue Severity Scale (FSS), 3) the Disability Days Measure (DDM), 4) the Center for Epidemiological Studies Depression scale (CES-D) and 5) the Medical Outcomes Study Short Form Health Survey (MOS-SF-20) (52). They found that none of the instruments correlated with SLEDAI and that the MOS short form correlated with the other health status instruments, making it a valid, broad single instrument to use. Numerous other studies have shown poor correlation of the health status measures with damage and most aspects of disease activity demonstrating that these instruments capture an otherwise potentially unappreciated part of evaluating lupus (51 ,53 ,54). These findings are consistent with the discordant perspectives on disease activity that physicians and patients can have (55 ,56). Furthermore, recent work has demonstrated that psychosocial factors play an important role in determining health status (57 ,58 ,59).

Some studies, however, have shown some degree of correlation with disease activity and health related quality of life. In a cross-sectional analysis, Fortin et al. found that SLAM-R, but not SLEDAI, correlated with most subscales of the SF-36 (60). This may in part be related to the more subjective nature of SLAM-R and its inclusion of fibromyalgia symptoms. In a longitudinal analysis, both disease activity instruments were associated with changes in the SF-36. Sutcliffe et al. found that higher disease activity correlated with worse physical and emotional function, pain, and general health (61). Khanna et al. used the World Health Organization Quality of Life-Bref (WHOQOL-Bref) and Mex-SLEDAI to evaluate the relationship between disease activity and health status in the Indian subcontinent. The WHOQOL-Bref includes an environment domain, which the authors wanted to capture. They found that physical and psychological QOL are impaired with active lupus, whereas social and environmental QOL do not correlate with disease activity (62). To evaluate a cross-country comparison, Panopalis et al. evaluated the Short Form-36 General Health Survey (SF-36) scores done annually over 4 years in 231 patients from Canada, 269 from the United States, and 215 from the United Kingdom. They found that the physical and mental well-being components did not differ significantly between countries (63). Whether the equivocal results on the relationship with disease activity and quality of life measures will be resolved with more uniform use of activity and health status indices remains to be seen. Larger studies including cohorts from diverse geographic regions are also needed for comparisons to better understand lupus variability.

As illustrated above, a variety of general health status measures applicable to numerous different illnesses have been used in SLE. Although not specific to lupus, the SF-36 (see Appendix III) is an instrument that has been validated, is available in a number of languages, and is one of the most commonly used in lupus (64). It is a 36-item self-administered questionnaire that measures eight domains: physical function-role limitation, physical problems-role limitations, emotional problems, social function, mental health, general health perception, vitality, and pain. Scores range from 0 to 100, with higher scores representing better health. The SF-36 was chosen as the health status measure of choice at the SLICC workshop in 1995 (65). A study has shown that patients receiving LJP 394 reported stable or improved health-related quality of life measured by the SF-36 survey with active treatment following renal flares, compared with deterioration in placebo-treated patients. Bearing in mind the significant influence of psychosocial factors on health-related quality of life, it remains uncertain how much benefit in SF-36 scores can be expected in clinical trials designed to treat active inflammatory disease.

Although the SF-36 remains the most commonly used health status measure, other measures do exist and new ones are being developed that are specifically designed for SLE. This is felt to be necessary as the general health status measures are not comprehensive enough for lupus and focus more on physical rather than mental function. It is also felt that a disease specific QOL instrument may be more sensitive to change and therefore more useful in lupus trials. Recently, Leong et al. published their preliminary validation of a SLE-specific quality-of-life instrument (SLEQOL) (66). It contains 40 items scored from 0 to 7 for a maximum score of 280. As with other studies, there was poor correlation with disease activity and damage. There was only poor to fair correlation with the SF-36, which the authors attributed to fact that these instruments cover different domains. The SLEQOL was more sensitive to change than the SF-36. The authors currently recommend using both instruments in studies, becaise they can complement each other; the SLEQOL specifically addresses lupus issues, whereas the SF-36 captures comorbid conditions and provides generalizability to conditions other than lupus.

A Dutch SLE-specific QOL measure called the SLE Symptom Checklist (SSC) has been developed that assesses 38 disease- and treatment-related symptoms (67). Reliability and reproducibility has been shown in a preliminary study of 115 stable lupus patients. It has been translated into English and further larger-scale studies are pending.

In future clinical trials, it may be advisable to include a generic health survey, such as the SF-36, and a disease specific quality of life measure, such as the SLEQOL, as secondary outcome measures. In the guidance document put forward by the FDA for the development of drugs in lupus, the measure of health-related quality of life should not worsen with a specific therapy, further emphasizing the importance of these measures in clinical trials. A drug that significantly improves the quality of life in the treated

group compared with those on placebo, in addition to controlling clinical manifestations of the disease, will be of great value to patients and physicians.

Summary

SLE is a complex illness. Studies of lupus pose many challenges given the heterogeneity of the disease. Although biomarkers for the illness are in development, no current blood tests exist that capture disease activity or correlate with damage. This further stresses the need for reliable, responsive activity and outcome indices for SLE in order to further the understanding of pathogenesis and improve treatment. A variety of disease activity and damage indices and health status measures have been described in this chapter. All have been shown to be valid, reliable, and sensitive to change in lupus. Although none of the indices were developed in particular for randomized clinical trials, the current culture of potential new therapies in lupus will enhanced their use and expand their importance. In a draft version of guidance for the development of new therapies for lupus by the FDA, the acceptance of claims for SLE will be based on one of four categories: (1) reduction in overall disease activity, (2) treatment of a organ specific manifestation of lupus such as nephritis, (3) complete clinical response/remission, and (4) reduction in flares (16). Currently, BILAG is the favored disease activity index as changes in scores are more interpretable, because they are based on the principle of intent to change therapy. If other indices are used, then at least two different ones should be chosen, because what is a meaningful change in the score remains controversial. Inclusion of HRQL indices and a damage index are also necessary components of a study. Just as these indices have evolved, they will continue to change as their strengths and weakness are further brought to light in the testing of promising new interventions.

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Chapter 48

Clinical Application of Serologic Abnormalities in Systemic Lupus Erythematosus

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One hallmark of systemic lupus erythematosus (SLE) is the wide array of serologic abnormalities, including a polyclonal increase in serum gamma globulins, the presence of antinuclear antibodies (ANAs) and various serum organ-specific and nonorgan-specific autoantibodies, circulating immune complexes, and serum complement changes. The presence of some of these abnormalities is important in corroborating the clinical diagnosis of SLE, whereas others are useful in monitoring disease activity. Each abnormality is discussed in a separate chapter. This chapter focuses on the clinical application of selected serologic abnormalities in establishing the diagnosis, in assessing disease activity, and in predicting specific organ-system involvement and overall prognosis of the patient. Only serologic tests that are generally available in most clinical laboratories as well as promising tests are discussed.

Serologic Tests

Diagnosis of Systemic Lupus Erythematosus

When the diagnosis of SLE is suspected or made on clinical grounds, the following serologic tests are considered to be helpful in corroborating the diagnosis (Table 48-1): fluorescent ANA test, ANA panel, serum-complement level, and Venereal Disease Research Laboratories (VDRL) or other comparable serologic test for syphilis. In certain situations, other serologic tests also are applicable, such as the Coombs test in a patient presenting with hemolytic anemia, lupus anticoagulant test, and anticardiolipin antibody test in a patient with a history of thrombosis or multiple fetal loss.

Virtually all patients with active and untreated SLE test positive for ANA. Nevertheless, ANA is prevalent in other rheumatic and nonrheumatic disorders as well, including some conditions that may mimic the clinical picture of SLE. ANA also is found in healthy children and adults (1). Thus, by itself, a positive ANA has a low diagnostic specificity for the disease, but its value increases when the patient meets the clinical criteria for SLE. The indirect immunofluorescent test is the most commonly used method for detecting ANA, and the choice of substrate in this test is important. Sections of rodent liver or kidney and tissue culture cell lines (Hep-2 or KB cells) are used in most clinical laboratories. Certain types of ANA, such as anti-Ro/SSa and anticentromere antibodies, can be detected with these cell lines but not with rodent tissues (2). A positive serum should be titered to give a semiquantitative value to the antibody level. The fluorescent staining pattern also should be included, but in the presence of multiple types of ANA, the staining pattern may change as the serum is titered.

Test for ANA is a useful screening test when there is a high index of suspicion of SLE or other systemic rheumatic conditions. However, it should be noted that ANA can be seen in a number of nonrheumatic conditions as well as in some healthy individuals (3 ,4 ,5). A study of the clinical utility of ANA testing in a large teaching hospital revealed a high sensitivity of a positive ANA for SLE; however, the predictive value was low for SLE and for other systemic rheumatic diseases (6). Malleson et al. (7) concluded in a study of a pediatric population that a positive ANA test is a poor predictor of SLE or mixed connective-tissue disease (MCTD). Many children with a positive ANA in their study did not have a rheumatic disease. The clinician should recognize the significant limitations of a positive ANA when the patient in question does not have clinical features suggestive of SLE or other connective-tissue disease. A study of diagnostic accuracy for SLE in a community setting revealed that many patients with a positive test for ANA are incorrectly given a diagnosis of SLE (8).

The ANA panel that is available in clinical laboratories includes ANA of defined specificity: anti-double-stranded DNA (anti-dsDNA), anti-Sm, anti-U1 RNP, anti-Ro/SSA, and anti-La/SSB. Some laboratories include antinucleoprotein, anticentromere, antihistone, and/or anti-single-stranded DNA (anti-ssDNA) in their panel. When the fluorescent ANA is positive in a patient who is suspected of having SLE, an ANA panel should be obtained. Anti-dsDNA and anti-Sm antibodies are considered to be highly diagnostic,

and their presence almost confirms the clinical diagnosis. The other types of ANA in the panel have lesser value as diagnostic markers for SLE, except in special situations such as the presence of anti-Ro/SSa antibody in a patient with subacute cutaneous LE (SCLE) (individual ANA types are discussed later) or ANA-negative lupus.

Table 48-1: Serologic Tests Useful in the Diagnosis of Systemic Lupus Erythematosus

1. Fluorescent antinuclear antibody (ANA)
2. ANA panel: anti-ds DNA*, anti-Sm, anti-U1RNP, anti-Ro/SSA, anti-La/SSB
3. Serum complement level
4. VDRL**
5. Anticardiolipin antibodies
6. Lupus anticoagulant
7. Coombs test

*dsDNA, double-stranded DNA
**VDRL, Venereal Disease Research Laboratories.

The serum complement level generally is measured as concentration of C3 or C4, or as CH850 or CH8100 hemolytic units. Although more commonly used in assessing disease activity, the presence of both hypocomplementemia and high titers of anti-dsDNA in a patient who is suspected of having SLE almost confirms diagnosis of the disease (9). Additionally, a genetic deficiency of C1q, C2, or C4 may present clinically with an LE-like syndrome, and the combination of a low or absent CH_{50} and normal C3 level should raise the possibility of this diagnosis (10). In patients with incomplete lupus erythematosus, i.e., those presenting with less than four of the American College of Rheumatology (ACR) classification criteria, the presence of low C4 was predictive of subsequent evolution into SLE (11).

A biologic false-positive test for syphilis is one of the four immunologic abnormalities that are included in the ACR criteria for the classification of SLE. Other antiphospholipid antibodies including the lupus anticoagulant and anticardiolipin antibodies are helpful in delineating a subset of patients who are prone to develop recurrent arterial or venous thrombosis and/or fetal loss (antiphospholipid syndrome).

Monitoring Disease Activity in Systemic Lupus Erythematosus

Serologic tests are widely used for assessing disease activity and predicting exacerbations (Table 48-2). Determinations of the serum titer of anti-dsDNA and of the complement level are the most common and, probably, the most useful serologic tests that are readily available to the clinician. Although applicable to most patients, both tests have important limitations. Anti-dsDNA antibodies and hypocomplementemia do not occur in all patients, and their correlation with disease activity is not absolute. A few patients can have persistently elevated anti-dsDNA antibody titers without developing evidence of clinical disease, even when followed for several months (12 ,13). Serial measurement of the serum titer of anti-Sm and anti-Ro/SSA antibodies can be useful, particularly in those who test negative for anti-dsDNA antibodies.

Table 48-2: Serologic Tests for Assessing Disease Activity in SLE

1. Anti-double-stranded DNA antibodies
2. Serum complement level: C3, C4, CH_{50}
3. Anti-Sm and other specific types of antinuclear antibody
4. Split products of complement
5. Serum cryoglobulins
6. Serum level of soluble cytokine receptors, adhesion molecules, thrombomodulin

In analyzing reports about the predictive value of various serologic tests in SLE, several points should be stressed. The selection of patients varies widely, and the clinical criteria that are used to define active SLE are not uniform. The effect of previous or current drug therapy frequently is not addressed. Most studies are cross-sectional, comparing groups of patients, and only a few are well-designed, long-term, prospective studies. Conclusions often are derived from a single serum determination rather than from multiple specimens over a period of time. Different test systems are used by various investigators to measure a given serologic parameter. Thus, comparison of the results of various studies is not always feasible or appropriate. The use of uniform activity indices (e.g., Systemic Lupus Activity Measurement [SLAM] and Systemic Lupus Erythematosus Disease Activity Index [SLEDAI] indices) in future prospective studies will help to correct these deficiencies.

Serologic abnormalities do not always occur before or during disease exacerbations, especially in clinically mild flare-ups. In a well-designed prospective cohort study of 185 patients with SLE, Petri et al. (14) demonstrated that lupus flare is quantifiable and that the incidence of a disease flare was approximately 0.65 per patient-year of follow-up. Most of the flares were minor, manifesting as fatigue, other constitutional symptoms, mucocutaneous lesions, and musculoskeletal complaints. Only 10% flares were associated with the new appearance of anti-dsDNA antibodies, and only 17% with an increase in antibody titer. Depression of C3 and C4 were observed in 44% and 41% of the disease flares, respectively.

We, and others, have found the concentration of serum cryoglobulins to be a useful parameter that correlates with disease activity, especially in those with nephritis (15 ,16 ,17 ,18). Although technically simple, it is labor-intensive. Measurement of cryoglobulins requires careful handling of

the specimen for proper interpretation of the results. Venous blood is allowed to clot at 37°C immediately after venipuncture. Following incubation at 4°C for 48 hours, the specimen is centrifuged in the cold and the precipitate saved. The precipitate is washed carefully with a low-ionic phosphate buffer, and the protein concentration is measured by standard methods.

The role of circulating immune complexes in monitoring disease activity in SLE remains unproven and has been abandoned (19 ,20). The lack of a widely accepted standardized test system, the heterogeneity of immune complexes in SLE sera (18 ,19), and the imprecise and inconsistent correlation with disease activity limit the application of circulating immune complexes in following the clinical course of the disease in individual patients. Of the numerous serologic tests for circulating immune complexes, the C1q solid-phase binding assay has been the most frequently used method in patients with SLE (20 ,21 ,22 ,23 ,24 ,25 ,26 ,27). Serologic tests with promising value are the measurements of products of complement activation and of soluble cytokine receptors (28 ,29).

Clinical Significance of the Anti-DNA Antibody

Diagnostic Value

Antibodies to DNA are classified according to their reactivity to native or dsDNA or to denatured or ssDNA. The presence of anti-dsDNA is highly characteristic of idiopathic SLE and rarely is seen in other rheumatic conditions, including drug-induced LE (30 ,31). One of the immunologic criteria for the classification of SLE by the ACR is the presence of anti-dsDNA. In contrast, anti-ssDNA antibodies, although prevalent in SLE, are less specific and can be seen in many other disorders, including rheumatic and nonrheumatic conditions (32). Thus, in the clinical laboratory, anti-dsDNA, but not anti-ssDNA antibodies, are tested routinely in the ANA panel.

In a large cohort prospective study, Weinstein et al. (9) found that high titers of anti-dsDNA and a low-serum C3 level are sensitive, and that each test had a high predictive value (94%) for the diagnosis of SLE when applied to a patient population in which the diagnosis was clinically suspected. Moreover, the predictive value was even higher when both serologic abnormalities were present in an individual patient.

Clinical Tests for Anti-DsDNA

The most commonly available tests for anti-dsDNA antibodies in the clinical laboratory are radioimmunoassay using either the Farr or the Millipore-filter binding technique, enzyme-linked immunosorbent assay (ELISA), and the *Crithidia luciliae* immunofluorescence test. The radioimmunoassay is a sensitive technique, and approximately 60% to 70% of patients with SLE test positive for anti-dsDNA by use of this method (30 ,31). False-positive results occasionally are seen with this test because of the contamination of the DNA substrate with single-stranded forms. False-positive results have been reported with commercial kits (31). The ELISA test for anti-dsDNA is technically easy to perform, and is the least labor intensive. The serum titer can be readily measured and, more important, both high- and low-avidity anti-dsDNA antibodies can be detected. False-positive ELISA results can be seen when impure DNA is used as a substrate (33). The immunofluorescence test uses fixed smears of *C. luciliae,* which is a nonpathogenic hemoflagellate containing a circular cytoplasmic organelle (called a kinetoplast) that consists of dsDNA. Serum anti-dsDNA, but not anti-ssDNA antibodies, bind to the kinetoplast. My own group has used this method to measure not only the titer of the antibody but also the immunoglobulin class and complement-fixing property of anti-dsDNA (34).

Qualitative properties of anti-dsDNA antibodies, including avidity, immunoglobulin class, and complement-fixing property, may affect the pathogenicity of the antibodies.

Because the available clinical tests for anti-dsDNA preferentially measure antibodies of different properties, some controversy has arisen as to which test yields the most useful information in assessing disease activity.

Ward et al. (35) compared the ELISA, *C. luciliae* immunofluorescence test, and filter-binding radioimmunoassay in patients with SLE who were followed over a period of time. They found that in most patients, the changes in anti-dsDNA antibody levels measured over time parallel each other, and that the anti-dsDNA titer as measured by each assay is inversely correlated to the serum C3 concentration. Data from this study indicate that the repertoire of anti-dsDNA antibodies detected in an individual patient remains relatively constant over time, confirming the observation that high- and low-avidity anti-DNA antibodies do not move independently in an individual patient but rise and fall in a parallel pattern (36).

Smeenk et al. (37) compared four methods for measuring anti-dsDNA antibodies in a defined population of patients with SLE. They found good correlation between the Farr assay, a commercial radioimmunoassay kit, polyethylene glycol test, and *C. luciliae* immunofluorescent test. As a diagnostic test, however, they recommend the Farr assay and *C. luciliae* as having the highest specificity for SLE. Werle et al. (38) suggest that ELISA is a sensitive test that is well suited to screening for anti-dsDNA. However, positive sera should be confirmed by either *C. luciliae* immunofluorescent test or by Farr assay, because they found positive ELISA in some patients with chronic liver disease, infections, and connective-tissue diseases other than SLE. Bootsma et al. (39) found the Farr assay have the highest specificity and sensitivity for the diagnosis of SLE when compared to the ELISA and *C. luciliae* test. Moreover, SLE sera with IgM-class anti-dsDNA as measured by the ELISA were positive when tested by the Farr

assay. In contrast, patients with other medical conditions who have IgM-class anti-dsDNA by the ELISA test were negative when tested with the Farr assay.

IgA anti-dsDNA antibodies have been reported to be associated with disease activity and a clinical subset of SLE patients with cutaneous vasculitis, acral necrosis, and erythema (40). In contrast, the presence and the serum level of IgM anti-dsDNA were not associated with disease activity or to particular clinical features of SLE (39). In a prospective study of 72 patients over a period of 19.6 months, Bootsma et al. (41) found that rises in the serum titer of the IgM anti-dsDNA, in contrast to rises of IgG anti-dsDNA, were not useful in predicting clinical relapses of SLE.

Telomeres are repetitive sequences of DNA at the end of eukaryotic chromosomes. Wallace et al. (42) evaluated an ELISA test for antitelomere antibodies as a diagnostic test for SLE. Antitelomere antibodies were found to be more sensitive than the Farr assay for anti-dsDNA (71% vs. 50%) in a cohort of SLE patients. When compared to commercially available ELISA test kits using calf thymus DNA, *C. luciliae* immunofluorescent test and Farr assay, Salonen et al. (43) found IgG antitelomere antibodies to have high sensitivity (60%) and specificity (91%) for SLE. Moreover, antitelomere antibodies correlated with lupus nephritis. Prospective studies of patients evaluated for SLE and other rheumatic conditions are needed to confirm the high sensitivity and specificity as well as its application in assessing lupus disease activity.

In summary, the most commonly available tests for anti-dsDNA antibodies yield comparable results over time in individual patients; however, as a diagnostic test, the Farr assay and *C. luciliae* are preferred (44). In clinical practice, any of these tests can be used to follow the antibody titer sequentially in most patients with SLE. The ELISA test and *C. luciliae* immunofluorescent test are commonly used for this purpose. If a patient with clinically active lupus has repeatedly low serum levels of anti-dsDNA antibodies with one test, use of a different test system should be considered.

Assessment of Disease Activity

A number of studies mostly retrospective, but a few prospective have examined the value of anti-dsDNA antibodies in predicting disease exacerbations and response to drug therapy. Table 48-3 summarizes the results of selected studies (21 ,38 ,45 ,46 ,47 ,48 ,49 ,50 ,51).

Retrospective Studies

Davis et al. (46) described a fairly good correlation between disease activity and anti-dsDNA antibodies as measured by a Millipore radioassay. Several patients in clinical remission, however, had a mild to moderate elevation of anti-dsDNA antibody levels. Swaak et al. (49) found a sharp drop in anti-dsDNA antibody titer, usually preceded by a rise that correlated with lupus nephritis and other major organ involvement. In contrast, a continuously high antibody titer was not predictive of disease flare. Isenberg et al. (47) reported that severe lupus nephritis, but not central nervous system (CNS) and other extrarenal involvement, correlated with anti-dsDNA antibodies, especially with antipoly (dT) antibodies. Measurement of antibodies to different synthetic polynucleotides did not significantly add to the routine determination of anti-dsDNA antibodies. Lloyd and Schur (24) observed that anti-dsDNA antibody as measured by the *C. luciliae* test correlates only fairly with disease activity. A rising antibody titer correlated with 75% of renal, 60% of extrarenal, and 30% to 50% of combined renal and extrarenal flares. In a study of patients with lupus nephritis who were treated with azathioprine and steroids, Adler et al. (45), using the Farr assay, found a persistently elevated anti-dsDNA to be predictive of a poor renal outcome.

Prospective Studies

A few long-term prospective studies have evaluated the clinical significance of anti-dsDNA antibodies both alone and in combination with serum complement level and other serologic parameters. Minter et al. (48) studied 70 patients longitudinally over 3 years. Only slightly more than one half of the active-disease episodes were associated with both a low CH_{50} and a high anti-dsDNA level as measured by the Farr assay. Many patients with clinically inactive disease had isolated elevated anti-dsDNA iters or low CH_{50} level. Active lupus nephritis was associated with complement-fixing anti-dsDNA or a low CH_{50}. In contrast, most episodes of CNS disease occurred without significant changes in CH_{50} level and anti-dsDNA antibody titer. Overall, the CH_{50} parameter correlated better than the anti-dsDNA antibodies with disease activity.

In a longitudinal study of 143 patients with SLE, Swaak et al. (50) found that a progressive rise followed by a sharp drop in anti-dsDNA titer as measured by the Farr assay preceded all 33 major disease flares. A drop in the serum C4 level followed by a decrease in the serum C1q and C3 levels occurred 20 to 25 weeks before the onset of lupus nephritis.

Ter Borg et al. (51) reported that 89% of all disease flares that occurred in 72 patients with SLE studied serially were preceded by a rise in anti-dsDNA titer by 8 to 10 weeks. The anti-dsDNA antibody titer was more sensitive than serum C3 or C4 levels in predicting exacerbations. The Farr assay was superior to the *C. luciliae* or ELISA test for the determination of anti-dsDNA antibodies. Based on these observations, a randomized, controlled trial was undertaken to determine if increasing the daily dose of prednisone after a rise in anti-dsDNA is detected can prevent relapses in SLE (52). A 25% increase in serum anti-dsDNA titer by the Farr method was considered to be significant. The cumulative risk of a major or minor relapse was significantly reduced in patients who received an additional dose of prednisolone.

Table 48-3: Association of Anti-Double-Stranded DNA and Disease Activity

Source	No. of Patients	Method Used	Results and Comments
Retrospective studies			
Davis, 1977 (46)	23	Radioassay	Good correlation between disease activity but positive tests were seen in several patients in remission.
Swaak, 1979 (49)	78	Farr assay	Sharp drop in antibody titer especially if combined with low C3 and C1q; predictive of nephritis or major organ flare. Persistently high titer was not predictive.
Isenberg, 1988 (47)	39	ELISA	Severe lupus nephritis but not extrarenal involvement correlated with anti-double-stranded (ds)DNA and especially with antipoly (dT).
Lloyd, 1981 (24)	27 with 47 flares	*C. luciliae*	Only fair association with disease activity. Rising titer of anti-dsDNA coincided with 75% of renal 60% of extrarenal, and 30% to 50% of combined renal and extrarenal.
Adler, 1975 (45)	21 diffuse lupus	Farr assay	Persistently high anti-dsDNA despite drug therapy correlated nephritis with poor renal outcome. An initial high titer had no prognostic value.
Esdaile, 1996 (55)	202 with 83 flares	Farr assay	Fluctuation in anti-dsDNA, C3, C4, and C1q binding were poor predictors of disease flares.
Prospective studies			
Swaak, 1986 (49)	143, with 33 flares	Farr assay	All 33 flares proceeded by a progressive rise and sharp drop of anti-dsDNA titer; 20 to 25 weeks prior to onset of nephritis; serum C4 decreased followed by C1q and C3.
ter Borg, 1990 (51)	72, with 27 flares	Farr assay, ELISA, *C. luciliae*	24 flares (89%) preceded by rise in anti-dsDNA by 8 to 10 weeks; anti-dsDNA was more predictive than C3 or C4. Farr assay was the most sensitive test.
Minter, 1979 (48)	40	Radioassay filter	Half of active episodes associated with low CH_{50} and high anti-dsDNA; isolated high anti-dsDNA or low CH_{50} seen in inactive disease; most central nervous system episodes occurred with normal CH_{50} and anti-dsDNA. Low CH_{50} correlated better than high anti-dsDNA.
Bootsma, 1995 (52)	156	Farr assay	A rise in anti-dsDNA titer was treated with an increase in prednisone dose. This reduced the risk of a clinical relapse.
Zonana-Nacach, 1995 (245)	53	Farr assay	Odds ratio of three for flare in asymptomatic patients with high anti-dsDNA and odds ratio of two for low C3.
Bootsma, 1997 (41)	34 with 18 flares	ELISA and Farr	Rise in IgG anti-dsDNA but not IgM anti-dsDNA was assay predictive of disease flare. ELISA, enzyme-linked immunosorbent assay.
Ho, 2001 (53)	53	ELISA, *C. luciliae*, IF test	Previous increase in anti-dsDNA occurred before flare As measured by SLEDAI and modified lupus activity I index; anti-dsDNA titer decreased during renal flares
Linnik, 2005 (54)	487 with nephritis	Farr assay	Changes in anti-dsDNA correlated with renal flares; inversely related with serum C3

In a prospective study of 53 SLE patients for up to a year, Ho et al. (53) reported that a previous increase in anti-dsDNA, measured by ELISA and *C. luciliae* assays, occurred before disease flares when measured by SLEDAI and a modified lupus activity index. On the other hand, at the time of the lupus flares including those patients with renal flares, the serum titer of anti-dsDNA often decreased. The authors suggested that decrease may represent deposition of immune complexes during the disease flare.

A multicenter study of 487 SLE patients with a history of lupus nephritis and a positive test for anti-dsDNA, found that changes in the titer of anti-dsDNA measured by a Farr assay, correlated with a risk of renal flare and was inversely correlated with serum C3 levels (54).

Other investigators have found that anti-dsDNA and other serologic tests are not very useful in predicting disease flares. Esdaile et al. (55) analyzed retrospectively clinical and laboratory data from 202 SLE patients who were followed prospectively for a median period of 86.5 months. Using a modified SLE disease activity index, they concluded that fluctuations in the laboratory test values including anti-dsDNA, C3, C4, and C1q binding assay for immune complexes are poor predictors of disease exacerbations in SLE. For the four serologic tests, the sensitivity approximated 50% and the sensitivity was less than 75% (56). In this study, anti-dsDNA and other laboratory data were obtained every 3 months. In contrast, in the prospective studies that found anti-dsDNA to be predictive of disease flares (51 ,52), laboratory values were obtained more frequently every 4 to 6 weeks.

Guidelines for Immunologic Laboratory Testing

The ACR guidelines on anti-dsDNA antibody (57) can be summarized as follows: A positive test for anti-dsDNA offers strong support for the diagnosis of SLE but a negative test result does not exclude the diagnosis. Anti-dsDNA antibodies do correlate with overall disease activity in SLE, however, the correlation is at best modest and test results must be interpreted in the clinical context. Anti-dsDNA antibodies correlate with lupus nephritis to a limited extent. High serum antibody titers have a stronger correlation with disease activity. In longitudinal assessment of patients, the presence of anti-dsDNA alone does not predict a disease flare. Increasing titers of anti-dsDNA in an individual patient may antedate or be associated with disease activity.

Summary

The quantitative determination of anti-dsDNA antibodies does not adequately predict disease flares in every patient. This is not unexpected considering the heterogeneity of the clinical disease and the anti-dsDNA antibodies. Several investigators have proposed that the qualitative properties of the anti-dsDNA antibodies, such as the complement-fixing property, avidity, dissociation constant, and immunoglobulin class, are more important determinants than the total antibody content in regard to pathogenicity and correlation with disease activity (56 ,57 ,58 ,59 ,60 ,61 ,62). Data from these studies are not readily available to the practicing clinician. Meanwhile, the anti-dsDNA antibody titer continues to be used widely as a serologic parameter for assessing disease activity. Combined with serum complement and other renal laboratory parameters, it is valuable in patients with lupus nephritis. In my experience, it is especially useful if the patient in question had a high anti-dsDNA and low serum complement in past exacerbations of the disease. Data from the few prospective studies suggest that anti-dsDNA should be measured at frequent intervals, every 4 to 6 weeks, and an increasing antibody titer may be predictive of disease flares. Considering the cost of laboratory testing, additional longitudinal studies are needed to confirm this finding.

Clinical Significance of the Anti-Sm Antibody

Anti-Sm antibody is present in only 30% of patients with SLE, but it has considerable diagnostic value as it is rarely found in other rheumatic diseases, such as MCTD, systemic sclerosis, and rheumatoid arthritis (RA) (63 ,64). Anti-Sm is included in the ACR criteria for the classification of SLE, and as an immunologic parameter, it carries the same weight as anti-dsDNA, positive LE-cell test, and false-positive serologic test for syphilis.

The anti-Sm antibody usually is measured in the clinical laboratory, by immunodiffusion, counterimmunoelectrophoresis (CIE), ELISA, and hemagglutination methods. The ELISA test, using highly purified antigens, is the most sensitive but less specific than the immunodiffusion and CIE (63 ,65). The lower specificity of the former is partly a result of the difficulty in preparing highly purified Sm antigen (66). The ELISA test is superior to other methods, however, in measuring the serum titer of the antibody. The use of recombinant Sm antigen and Sm polypeptides in ELISA is promising, but additional studies are needed to evaluate their clinical application (67 ,68 ,69).

When SLE was compared to healthy controls, anti-Sm has a weighted mean sensitivity of 24% and a sensitivity of 98%. On the other hand when SLE was compared to other rheumatic conditions, anti-Sm had a mean sensitivity of 30% and a specificity of 96% (70).

Prevalence

Studies have shown that the prevalence of anti-Sm antibody in SLE varies among the different ethnic groups. In the United States, Arnett et al. (65) found anti-Sm and anti-RNP antibodies to be more common in African Americans (25% and 40%, respectively) than in whites (10% and 24%, respectively). Antibodies to Ro/SSA and La/SSB, however, occurred with equal frequencies in the two racial groups. The higher prevalence of anti-Sm and anti-U1RNP in African Americans has been confirmed by others (71 ,72 ,73). The frequency of anti-Sm antibody in SLE appears to be lower in France than in the United States (74). Anti-Sm was present in 12% (by immunodiffusion) and in 17% (by immunoblotting) of French patients with SLE; in contrast, the prevalence among French West Indies patients was five times higher: 39% by immunodiffusion and 50% by immunoblotting. In a smaller study, Field et al. (66) found a higher frequency of the antibody among patients with SLE originally from West Africa, the Caribbean Islands, and Asia than among local whites in England. The prevalence of anti-Sm antibodies in a large number of European patients

with SLE (93% whites) was 10.3% (74). The prevalence of anti-Sm in Thailand was 44% (75); in Mexico, 39.2% (76); in India, 13.7% (77) and in Malaysia 15% (78).

The selection of SLE patients and of controls, the antigen that is used, and the laboratory procedure are variable, so prevalence of anti-Sm antibodies may not be comparable among the cited studies.

Association with Organ Involvement

Whether the presence of anti-Sm antibodies defines a clinical subset of patients with SLE or carries a prognostic value in SLE remains uncertain. Winfield (79) found a higher frequency of anti-Sm antibodies among patients with SLE and CNS dysfunction. Winn et al. (16) reported anti-Sm antibodies to be associated with milder CNS and renal disease.

Other investigators, however, could not confirm these associations (80 ,81). Gripenberg et al. (82) found Raynaud phenomenon to be more prevalent in patients with high titers of IgG anti-Sm antibodies. Yasuma et al. (74) found a positive correlation among serositis, interstitial pulmonary fibrosis, and IgG anti-Sm antibodies in a large cohort of Japanese patients. Huynh et al. (84) reported an increased frequency of anti-Sm antibodies in SLE patients with peripheral neuropathy. Alba et al. (85) found anti-Sm, anti-dsDNA and lupus anticoagulant to be associated with a higher risk of lupus nephritis among young Black patients.

The discrepancies in these results may be a result of variation in prevalence among various ethnic groups and differences in the sensitivity of the test system. More important, conclusions were often based on a single serum specimen rather than on a sequential determination of the anti-Sm antibody.

Antibody Titer and Disease Activity

Few longitudinal studies on the usefulness of anti-Sm antibody titers in monitoring disease activity in patients with SLE have been performed. A prospective study of 14 patients with SLE and anti-Sm antibodies, over a period of 7 to 30 months, showed fluctuations in serum titer. A fourfold rise in titer predicted disease flare in 50% of patients (but in only 28% of episodes) and correlated with exacerbation of the disease in 60%. The rise in titer occurred within 2 to 12 weeks preceding a major disease flare (nephritis and CNS disease), but not in milder flares (arthritis, rash, or serositis) (83). Another study of 17 patients with SLE who were followed for 6 to 120 months showed a correlation between anti-Sm antibody and disease activity (82).

Immunologic Testing Guidelines

The ACR recently published guidelines on the utility of anti-Sm antibody tests in laboratory testing in rheumatic diseases (86). The guidelines were developed based on an extensive and critical literature review. The presence of anti-Sm antibody is highly specific for SLE and it is valuable serologic marker for diagnosis. It should be tested in all patients who are suspected on clinical grounds of having SLE. It is not useful in the diagnosis of other systemic rheumatic diseases. Anti-Sm antibodies did not correlate with presence of lupus nephritis and were not useful in predicting renal flares. Anti-Sm do not appear to predict disease flares, however, there were few published prospective studies.

Further studies are needed, to evaluate the value of anti-Sm antibody titer in monitoring disease activity, to determine whether it adds to the measurement of anti-dsDNA antibodies and other serologic tests, and to ascertain whether it may be more useful in African Americans and other ethnic groups in whom the anti-Sm antibodies are more prevalent.

Significance of Anti-U1RNP Antibody in Systemic Lupus Erythematosus

Prevalence and Diagnostic Significance

Arnett et al. (65) found that the prevalence of anti-U1RNP antibodies measured by the immunodiffusion and CIE tests, is higher in black patients (40%) than in white patients with SLE (23%). Using immunoprecipitation and autoradiography, Williamson et al. (72) found a higher frequency of anti-Sm (34% vs. 15%) but not anti-U1RNP (36% vs. 27%) in black compared to white SLE patients. The ELISA test is a sensitive test for anti-U1RNP, showing a prevalence of the antibody as high as 55% in patients with SLE (63). Anti-U1RNP antibodies were found in 20.1% of a large number of European patients with SLE who were collected from 14 different countries (74). A high frequency of anti-U1RNP antibodies in SLE has been reported in Japanese patients (24%) (87) and in Malaysian patients (36%) (78).

Unlike anti-Sm antibodies, anti-U1RNP antibodies are not considered specific for SLE. They can be seen in patients with other systemic rheumatic conditions such as MCTD, RA, Sjögren syndrome, systemic sclerosis, and polymyositis.

Clinical Association of Anti-U1RNP Antibodies

The presence of high titers of anti-U1RNP antibodies is associated with MCTD, a clinical entity that is characterized by overlapping features of SLE, scleroderma, and polymyositis (30 ,31). Some investigators have proposed that in MCTD, anti-U1RNP should occur in the absence of other autoantibodies such as anti-Sm and anti-dsDNA (88), and that the occurrence of multiple types of ANA in a patient is more indicative of SLE. The issue of whether MCTD is a distinct rheumatic disease or merely a syndrome that may occur during the course of SLE or systemic sclerosis remains controversial. Some patients with MCTD eventually

evolve into a more distinct rheumatic disease, such as definite systemic sclerosis (89 ,90). A study of the isotype of anti-U1RNP antibodies in Greek patients revealed a predominance of IgM antibodies in SLE. In contrast, IgG anti-U1RNP without IgM antibodies were found in MCTD (91).

In 1972, Reichlin and Mattioli (92) found that anti-U1RNP antibody is prevalent in SLE and is associated with a more benign disease. A cross-sectional study of 49 patients with SLE revealed that those with anti-U1RNP and anti-Sm antibodies have a higher frequency of scleroderma-associated features, such as Raynaud phenomenon, sclerodactyly, interstitial changes in the chest radiograph, and nailfold capillary abnormalities (93 ,94). Vasculitis, deforming, nonerosive, Jaccoud-type arthropathy of the hands and Raynaud phenomenon also have been reported to be associated with anti-U1RNP in SLE by other investigators (76 ,78 ,95).

A prospective study of a large cohort of SLE patients, predominantly Caucasian, found no association between the presence of anti-U1RNP or anti-Sm antibodies with specific major organ damage including renal, CNS, cardiac, or pulmonary (96). In contrast, both anti-U1RNP and anti-dsDNA were reported to be significant predictors of lupus nephritis among Hispanic and African-American SLE patients (97).

Serum Antibody Titer

A few reports of longitudinal measurements of the serum titer of anti-U1RNP among patients with SLE have appeared. Nishikai et al. (98) showed that in some, but not all, patients with SLE, the anti-U1RNP titer appeared to fluctuate with disease activity. A prospective study of 71 patients with SLE and 40 separate clinical exacerbations showed that the measurement of antibodies to 70 kd and A polypeptides of the U1RNP complex was not useful in monitoring disease activity or predicting disease exacerbations (99). The presence of anti-U1RNP and/or anti-Sm did not appear to affect survivorship in SLE (100).

A prospective study of patients with anti-U1RNP antibodies showed that most patients with a persistently high serum titer evolve into a clinical picture of MCTD (101). A 10-year follow-up of a group of patients with rheumatic disease and anti-U1RNP antibodies showed clinical features of MCTD and a high frequency of erosive and deforming arthritis (102). A large proportion of patients with “undifferentiated connective tissue disease” who tested positive for anti-U1RNP subsequently developed MCTD (103).

ACR guidelines on immunologic laboratory testing assert that anti-U1RNP is not specific for SLE, but supports a diagnosis of MCTD. Anti-U1RNP does not support the diagnosis of SLE, RA, or systemic sclerosis. Testing for anti-U1RNP is not useful in assessing for lupus nephritis. However, further prospective studies are needed to evaluate the association between anti-U1RNP and other SLE features (86).

Summary

Anti-U1RNP and anti-Sm antibodies commonly are found together in the sera of patients with SLE. Anti-U1RNP antibodies are not considered to be diagnostic of SLE; when present alone in a patient with systemic rheumatic disease (especially in high titer); the possibility of MCTD should be considered. Although the serum titer of anti-U1RNP may fluctuate in some patients, the determination of anti-dsDNA and complement is more useful in monitoring disease activity in patients with SLE, especially in those with nephritis.

Anti-Ro/SSA and Anti-La/SSB Antibodies in SLE

Diagnostic Specificity

Anti-Ro/SSA antibodies are most commonly found in primary Sjögren syndrome and SLE, although they also occur in other systemic rheumatic diseases, including systemic sclerosis, RA, and polymyositis (30). In the clinical laboratory, anti-Ro/SSA antibodies are detected by immunodiffusion in agarose gels or by CIE. They are present in 30% to 40% of patients with SLE and in 40% to 70% of patients with primary Sjögren syndrome (31).

Immunoblotting, RNA precipitation, and ELISA have been developed to measure anti-Ro/SSA and anti-La/SSB antibodies. A comparative study of these methods (104 ,105) has shown that although the RNA precipitation has the highest sensitivity and specificity, the most convenient and practical clinical test is CIE for anti-Ro/SSA and immunoblotting for anti-La/SSB. ELISA tests using affinity-purified antigens and recombinant protein have become available (106 ,107). An indirect immunofluorescent test using transfected HEp-2 cells that over express the human 60kDa Ro/SSA antigen is a highly sensitive screening test (108 ,109). Other investigators recommend the use of a combination of two or more commercially available test systems for anti-Ro/SSA to improve sensitivity and specificity (110).

Like anti-Ro/SSA antibodies, anti-La/SSB antibodies are found in patients with primary Sjögren syndrome and SLE. Precipitating anti-La/SSB antibodies are found in 12% of unselected patients with SLE (111).

Using various test systems, anti-Ro/SSA antibodies have been reported to occur in low titers in 15% of healthy individuals, especially those who are HLA-DR3 positive, and anti-La/SSB have been detected in 7.5% of normal subjects (63 ,112 ,113 ,114).

Disease Associations

Although anti-Ro/SSA antibodies do not have a high diagnostic specificity for SLE, their presence is associated with a number of clinical conditions. These include SCLE, neonatal lupus syndrome, homozygous C2 and C4 deficiency with

SLE-like disease, ANA-negative SLE, photosensitivity in SLE, and interstitial pneumonitis.

SCLE is a distinct clinical subset of SLE. It is characterized by recurrent, erythematous, photosensitive, nonscarring skin lesions in a characteristic distribution involving the face, trunk, and arms, and by mild systemic disease. Anti-Ro/SSA antibodies are found in 63% to 90% of patients with SCLE (115 ,116 ,117). The major anti-Ro/SSA response in SCLE is directed against the native 60-kD Ro protein (118).

Neonatal lupus syndrome is an uncommon condition in infants born of SLE mothers. It is characterized by photosensitive, annular, discoid, or erythematous skin lesions of the face and trunk, which appear at or before 2 months of age and disappear by 6 to 12 months of age. Congenital heart block with or without structural cardiac defects is seen in 50 of patients. Almost all afflicted infants and their mothers have anti-Ro/SSA and/or anti-La/SSB antibodies (119 ,120 ,121). Buyon et al. (122) found that women with both antibodies, especially if the anti-Ro/SSA antibodies identify the 52-kd component, have an increased risk of giving birth to an infant with neonatal lupus syndrome. Unfortunately, most of the commercially available tests for anti-Ro/SSA antibodies do not distinguish between antibodies to the 52-kd and the 60-kd components. The frequency of anti-Ro/SSA antibodies is increased in mothers of male children with SLE and in mothers of children with SLE that develops before the age of 10 years (123).

Homozygous C2 deficiency is characterized by a lupus-like illness with photosensitive cutaneous lesions reminiscent of those of SCLE and arthralgia, but rare CNS and renal involvement. Anti-Ro/SSA antibodies are present in 50% to 75% of these patients (116 ,124 ,125). A genetic deficiency of C4, which may manifest clinically as SLE or a lupus-like syndrome, also is associated with anti-Ro/SSA antibodies (125). In one study, one of four patients with a genetic deficiency of C1q had anti-Ro/SSA antibodies (125).

ANA-negative SLE, first described by Fessel (126) and by Gladman et al. (127), refers to patients with clinical features that are compatible with those of SLE, except that their sera test negative for ANA by immunofluorescence using sections of rodent liver or kidney. In a study of 66 patients, Madison et al. (128) had SCLE. Precipitating anti-Ro/SSA antibodies were found in 41 patients, and anti-ssDNA antibodies were present in 18. Moreover, 66% of patients actually had a positive fluorescent ANA when KB epithelial tissue culture cells rather than mouse kidney sections were used as substrate. The Ro/SSA antigen appears to have a variable species distribution with significant amounts in certain cells, including a concentration in mouse, rat, and rabbit tissues (1).

Blomberg et al. (129) found that 25% of a group of patients suspected of having systemic rheumatic disease with anti-Ro/SSA antibodies by an ELISA test but with a negative standard ANA test had SLE or cutaneous SLE. Further characterization of the antibody revealed specificity for the Ro 52-kd protein (130). Using a sensitive ELISA test, Reichlin (131) found anti-Ro/SSA in all 66 patients with ANA negative SLE. Anti-La/SSB was detected in 46% and anti-U1RNP was present in 35%.

The presence of anti-Ro/SSA antibodies has been reported to correlate positively with photosensitivity in white patients with SLE (132). In contrast, among African Americans, anti-Ro/SSA antibodies appear to be inversely associated with photosensitivity (133). A probable relationship between anti-Ro/SSA antibodies and interstitial pneumonitis as well as “shrinking lung syndrome” in SLE have been described (134 ,135), and deforming arthropathy in SLE has been reported to be associated with anti-Ro/SSA (especially the 52-kd component) and with anti-La/SSB antibodies (136). Anti-Ro/SSA and anti-La/SSB were strongly associated with sicca symptoms in a large cohort of SLE patients (137 ,138).

SLE patients with anti-La/SSB antibodies usually have anti-Ro/SSA antibodies concomitantly, and they tend to be older at diagnosis (111 ,139 ,140 ,141). Although lupus nephritis is positively associated with anti-dsDNA, it is inversely related with anti-La/SSB antibodies (139).

Hamilton and associates (140) suggested two serologic genetic subsets of SLE in whites, but not in African Americans, with different ages of onset. White patients with SLE and anti-Ro/SSA antibodies alone differ from those with both anti-Ro/SSA and anti-La/SSB antibodies. Those in the former group have a lower titer of anti-Ro/SSA antibodies, a younger age of onset, a higher frequency of anti-dsDNA and significant renal disease, and they are strongly associated with DR2 and DQw1. In contrast, those in the latter group are associated with an older age of onset, sicca complex, less renal involvement, and HLA-B8, Dr3, Drw52, and DQW2. A study of SLE patients in Taiwan revealed no correlation between anti-Ro/SSA and anti-La/SSB and the occurrence of lupus nephritis (142).

Cavazzana et al. (143) reported that 24% of patients with “undifferentiated connective tissue disease” who test positive for anti-Ro/SSA antibodies progressed within a short period of time to either SLE or primary Sjogren syndrome.

Serial Measurement of Antibody Titer

Scopelitis et al. (144) reported fluctuating titers of anti-Ro/SSA antibodies in patients with SLE that appeared to correlate with disease activity and anti-dsDNA antibody levels. Moreover, some episodes of acute exacerbation were characterized by a rising titer in anti-Ro/SSA antibody in the absence of detectable anti-dsDNA antibodies. A longitudinal study of anti-Ro/SSA and anti-La/SSB in a lupus mother who gave birth to an infant with congenital heart block revealed fluctuations of serum antibody titers that were unrelated to disease activity or to immunosuppressive therapy (145). Additionally, frequent measurements of these autoantibodies during pregnancy did not predict occurrence of congenital heart block. A 2-year prospective study of anti-Ro/SSA antibody by CIE method in SLE

showed fluctuation in the serum antibody titer, however, there was no correlation with lupus disease activity (146). On the other hand, Wahren et al. (147), using a sensitive ELISA test in a small number of SLE patients, found correlation between disease activity and serum titer of anti-Ro/SSA and anti-La/SSB antibodies.

A retrospective longitudinal study of 130 SLE patients revealed that anti-ENA antibodies including anti-Ro/SSA and anti-La/SSB fluctuates frequently over time. For patients who tested negative at baseline, the frequency of positive seroconversion was 56% for anti-La/SSB and 15% for anti-Ro/SSA (148).

Summary

Anti-Ro/SSA antibodies are strongly correlated with the clinical subsets of SCLE, ANA-negative SLE, and lupus-like syndrome associated with a genetic deficiency of complement. Infants of SLE mothers with anti-Ro/SSA and anti-La/SSB antibodies have an increased risk of neonatal lupus syndrome, thus, pregnant patients with SLE should be tested for these antibodies. A sensitive ELISA test for anti-Ro/SSA antibodies is useful in the diagnosis of ANA negative SLE. Prospective studies are needed to evaluate the value of anti-Ro/SSA and anti-La/SSB antibodies in monitoring disease activity.

Anti-Histone Antibodies in Systemic Lupus Erythematosus

Prevalence and Diagnostic Specificity

Antihistone antibodies comprise a heterogeneous group of antibodies that are reactive with various subfractions or complexes. Although found mainly in patients with SLE, drug-induced LE, or RA, these antibodies have been described in those with other rheumatic conditions, malignancy, and liver disease (see Chapter 22). In SLE, these antibodies are directed against H1, H2B, H3, and H2A-H2B complex (149), although other specificities can occur. All isotypes of antihistone antibodies are common in SLE (150 ,151).

Several methods have been devised to measure antihistone antibodies, including ELISA, immunoblotting, complement fixation, and immunofluorescence (152). Depending on the method, substrate, and patient selection, the prevalence of antihistone antibodies in SLE has been reported to be from 21% to 90% (152).

Antihistone antibodies have limited diagnostic specificity for idiopathic SLE. The presence of these antibodies does not appear to be any more significant than that of anti-dsDNA or anti-Sm antibodies in corroborating the clinical diagnosis of the disease. Wallace et al. (153) found that antibodies to histone (H2A-H2B) DNA complex in the absence of anti-dsDNA antibodies are found more commonly in MCTD and scleroderma-related conditions than in SLE.

Clinical Association

Several published studies on the relationship between the presence of antihistone antibodies and the clinical features of SLE have reported inconsistent results. In a small number of patients with lupus, Fishbein et al. (154) found a significantly lower prevalence of CNS involvement among those with antihistone antibodies. Fritzler et al. (155) confirmed the lower frequency of neuropsychiatric disease and the lower prevalence of nephritis, alopecia, anemia, and hypocomplementemia in patients with SLE and antihistone antibodies, suggesting a milder form of the disease. In contrast, antihistone antibodies have been reported to be associated with diffuse proliferative lupus nephritis (156) and to with lupus arthritis (137). Other investigators have failed to find any positive or negative correlation with specific clinical manifestations of the disease (150 ,157 ,158).

Similarly, the available data on the association between antihistone antibodies and disease activity are few and inconclusive. Fishbein et al. (154) found a significant drop in the serum antibody titer within a month after the initiation of steroid therapy for active SLE. Gioud et al. (157) reported a higher frequency of antihistone antibodies in patients with active disease (87%) than in those who were in remission (18%). A serial study in a small number of patients showed a correlation with disease activity (159). In untreated patients with lupus nephritis, antibodies to H2B correlated with renal, histologic, and clinical activity of the disease (159). Other investigators, however, have found no correlation among antihistone antibodies, disease activity, or activity index in the renal biopsy (151 ,155 ,158 ,160 ,161 ,162).

The discrepancy in the results of the above studies may be a result of several factors including variation in patient selection, the test system used, histone preparation, and study design.

Association with Anti-DNA Antibodies

Antihistone antibodies have been shown to correlate with the presence of anti-DNA antibodies (156 ,157 ,158) and circulating immune complexes (159). Subiza et al. (163) have established that some of the antihistone activity that is measured in SLE sera results from complexes of dsDNA anti-dsDNA, which bind to the histone substrate used in the assay. Stockl et al. (164) found that glomerular deposits of histones may bind to fixed anionic sites in the glomerular capillary wall, acting as a planted antigen that can induce immune complex formation in situ. Deposits of histones and DNA have also been identified at the basement membrane of nonlesional skin in SLE patients (165).

Histone Binding with SLE IgG

The binding of IgG isolated from SLE sera to histone has been reported to be noncognate, i.e., it is not dependent on true antigen-specific Fab recognition. In contrast, the

binding of SLE IgG with dsDNA, Sm, and other nuclear antigens was cognate, i.e., true antigen-antibody reaction (166). Further studies have revealed that histones bind an “anomalous” monomeric IgG that is present in the sera of patients with SLE as well as drug-induced SLE. The binding was sensitive to pepsin digestion of the IgG and appeared to be mediated Fc binding (167). The nature of this “anomalous” IgG remains to be elucidated, however, these observations may in part account for the discrepancies in the results of studies of antihistone antibodies in SLE and other diseases.

Association with Lupus Erythematosus Cell Test

Schett et al. (168) have identified serum antibodies to H1 as the major group of antinuclear antibodies responsible for the LE cell phenomenon in SLE. They also found that SLE patients who have antihistone-1 antibodies had marked immune response to other histones and other nuclear proteins. Moreover, these patients tended to have more severe organ involvement with nephritis and CNS disease (169).

Summary

Antihistone antibodies are of limited value in corroborating the clinical diagnosis of SLE. Serial determinations of these antibodies do not add significantly to the measurement of anti-dsDNA and other serologic parameters for assessing disease activity in patients with SLE.

Further studies on the binding of histone with SLE IgG, circulating immune complexes are needed to understand fully the significance of antihistone antibodies including pathogenicity and assessment of disease activity.

Antinucleosome Antibodies in SLE

Using purified nucleosomes in an ELISA test, Bruns et al. (170) found IgG antinucleosome antibodies in 56% of 136 SLE patients and in only 3% of 309 patients with other diagnosis. When applied to the diagnosis, IgG antinucleosome antibodies had a specificity of 97% and a sensitivity of 56%. Other investigators have reported sensitivities ranging from 30% to 90% (171 ,172 ,173 ,174).

IgG antinucleosome antibodies may be of diagnostic value for SLE in patients who test negative for anti-dsDNA antibodies. In a large series of consecutive patients suspected of autoimmune diseases. IgG antinucleosome antibodies were found in 88% of 197 SLE patients with anti-dsDNA and in 51% of 43 patients with negative anti-dsDNA. However, in this study, IgG antinucleosome antibodies were also found in patients with systemic sclerosis (21%) and in mixed connective tissue disease (7%) (175). Simon et al. (174) reported a high prevalence (70%) of IgG antinucleosome antibodies in mixed connective tissue disease and was not useful in differentiating MCTD from SLE.

When compared with conventional ANA and anti-dsDNA, Julkunen et al. (173) found that antinucleosome antibodies did not provide additional information in the diagnosis of SLE. Servais et al. (176) reported that antinucleosome antibodies were inferior to anti-dsDNA in the diagnosis of SLE. In contrast, other investigators have reported that antinucleosome antibodies had higher sensitivity than anti-dsDNA in the diagnosis of SLE (170 ,177). The discrepancies in these studies are in part a result of variations in patient selection and test methodology, including antigen preparation and study design.

Antinucleosome antibodies have been reported to be associated with lupus disease activity including nephritis in several cross-sectional studies of SLE patients of different ethnicities (170 ,174 ,178 ,179 ,180). However, other investigators have reported lack of correlation with disease activity (181). A 2-year follow up study of 101 SLE patients found no correlation between antinucleosome antibodies and lupus disease activity, renal disease, and disease damage (182).

Summary

Antinucleosome antibodies are prevalent in SLE and high serum antibody titers maybe a useful aid in the diagnosis of SLE especially in patients who test negative for anti-dsDNA and anti-Sm antibodies. Antinucleosome antibodies may be seen in drug-induced lupus erythematosus, MCTD, and systemic sclerosis. Prospective well-designed studies are needed to investigate the utility of antinucleosome antibodies in individual SLE patients for assessing disease activity and following the response to therapy.

Serologic Parameters and Renal Biopsy Findings in Lupus Nephritis

A number of studies (Table 48-4) have examined the relationship between renal biopsy findings and the serologic data obtained at biopsy (161 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190). Could the histologic type of lupus nephritis, histologic activity, and chronicity indices be predicted by anti-dsDNA, C3, and/or serologic parameters? The results of various studies are not necessarily comparable because of differences in morphologic classification, parameters measured, and patient selection, including consideration of the effects of previous or current drug therapy. All studies except two (187 ,190) were based on a single kidney biopsy that was performed within a few months after onset of the renal abnormality.

Hill et al. (188) found an excellent correlation between serum levels of anti-dsDNA and C3 with the overall amount and distribution of immune deposits in the renal biopsy as assessed by immunofluorescence. In contrast, a poor association between the degree of epithelial proliferation and

the histologic type of lupus nephritis was noted using the Baldwin classification system (191).

Table 48-4: Correlation of Serologic Abnormalities and Renal Histology

Source	No. of Patients	Results and Comments
Hill, 1978 (188)	59 with 77 biopsies	Excellent correlation between anti-dsDNA and C3 with overall amount and distribution of immune deposits; rheumatoid factor found in those with milder lesions; cryoglobulins correlated with more severe changes.
Hossiau, 1990 (189)	50	High anti-dsDNA and low C3 correlated with nephrotic syndrome with or without renal failure; class IV nephritis patients had higher anti-dsDNA titer than those with class III or V nephritis.
Nossent, 1991 (161)	35	High histologic activity index correlated with IgM antinuclear antibody and IgM anti-dsDNA. No correlation between histologic type and serologic abnormalities.
Clough, 1980 (183)	11	IgM anti-dsDNA was higher than IgG anti-dsDNA in class IV nephritis. IgG anti-dsDNA was higher than IgM anti-dsDNA in class III nephritis.
Hashimoto, 1983 (186)	20	Histologically active lesions, especially class IV nephritis, correlated with high titer and complement fixing IgG anti-dsDNA. Glomerular C3 deposits correlated with complement fixing IgG anti-dsDNA.
Feldman, 1982 (185)	34	Renal activity index but not chronicity index correlated with anti-dsDNA (Farr assay) and IgG anti-dsDNA by ELISA.
Esdaile, 1989 (184)	87	Low C3 was predictive of renal insufficiency, renal death and total SLE death; high anti-dsDNA associated with renal death and inversely related with nonrenal death.
Hecht, 1976 (187)	31 with repeat biopsy	Persistently normal C3 was associated with stability or improvement of renal lesion of repeat biopsy in some but not all patients. Anti-dsDNA showed better correlation with clinical histologic improvement.
Pillemer, 1988 (190)	55 with repeat biopsy	Normalization of C3 correlated better than decrease in anti-dsDNA titer with activity index during repeat biopsy.
Okamura, 1993 (192)	40	IgG but not IgM anti-dsDNA by ELISA correlated with renal histologic activity score and amount of electron dense glomerular deposits.

Houssiau et al. (189) reported a good correlation among the anti-dsDNA titer and the serum C3 (but not C4) level with functional severity of the renal disease and the World Health Organization (WHO) histologic classification of lupus nephritis. Patients with nephrotic syndrome or renal failure had a higher anti-dsDNA titer and a lower C3 level than those presenting with proteinuria alone and a normal serum creatinine level. Patients with class IV nephritis had a higher anti-dsDNA level than those with class III or V nephritis. In contrast, the serum C3 level did not correlate with the histologic type. Considerable overlap in values among the various clinical or histologic groups was found, however, so these associations are not applicable to the individual patient.

Nossent et al. (161) observed no correlation among the WHO histologic classification and various serologic parameters (anti-dsDNA, other types of ANA, C3, C4, C1q, immune complexes, and anticardiolipin antibodies) in 35 patients with lupus nephritis. Conversely, using the National Institutes of Health (NIH) renal histology index, the activity index correlated with serum titers of IgM ANA and IgM anti-dsDNA. Glomerular proliferation showed the best overall correlation with serologic parameters. Clough and Valenzuela (183) described a similar correlation between IgM anti-dsDNA and diffuse lupus nephritis.

Hashimoto et al. (186) reported a good correlation between histologically active lesions, especially in diffuse lupus nephritis, and high titers of IgG complement-fixing anti-dsDNA. Feldman et al. (185) obtained similar results, and they noted a good correlation between anti-dsDNA using the Farr binding assay and IgG anti-dsDNA using ELISA and renal activity, but not with the chronicity index. IgG, but not IgM, anti-dsDNA antibodies as measured by ELISA were found to correlate with the histological activity score and the amount of electron-dense deposits in 40 patients with untreated SLE who underwent kidney biopsy (192). The serum titer of anti-dsDNA antibodies was

significantly higher in patients with class IV nephritis as compared to those with class I, II, or III (192).

In contrast to these studies, Pillemer et al. (190) correlated serologic tests and histologic changes over time in 55 patients who had both initial and repeat renal biopsies. All patients received various immunosuppressive drugs for nephritis during the interval. At the time of the second biopsy, the serum C3 level had improved in 78% and the anti-dsDNA level decreased in 85% of patients. Patients with a normal C3 level at the time of the second biopsy had a significantly lower activity index than those with a low C3 level. The activity index was not significantly affected by a decrease in anti-dsDNA antibody titer. The duration of hypocomplementemia, however, was less consistent as a prognostic indicator. Esdaile et al. (184) also found a low serum C3 level to be a valuable predictor of renal insufficiency, renal death, and total SLE death in a study of the long-term outcome of 87 patients with lupus nephritis. In a similar study of 31 patients with SLE and serial kidney biopsies, Hech et al. (187) showed that normalization of the serum C3 level and a drop in the anti-dsDNA titer following drug therapy are associated with stabilization or improvement of the renal disease.

A prospective study of 17 patients concluded that serum C3 levels are more sensitive and specific than serum C4 values for monitoring disease activity in lupus nephritis (193).

Serologic Parameters in Renal Relapse

A prospective study of 46 newly diagnosed lupus nephritis over a period of 5 years revealed that 37% experienced at least one renal relapse. Serological findings including rising anti-dsDNA titer and hypocomplementemia were less pronounced during the relapse than at baseline and were not particularly helpful in predicting renal relapse (194).

“Silent lupus nephritis” refers to patients with histolopathologic diagnosis of lupus nephritis on kidney biopsy, but without clinical evidence of renal disease including normal urinalysis, creatinine, BUN, and creatinine clearance. Wada et al. (195) reported that 8 of 31 (28.8%) of patients with silent lupus nephritis developed overt renal disease over a 60-month follow-up. Elevated anti-dsDNA and hypocomplementemia predicted the development of clinical nephritis.

Summary

Kidney biopsy is useful in the management of patients with lupus nephritis. The histologic type of lupus nephritis and severity of renal damage as assessed by the activity and chronicity of the lesions are predictive of the outcome of lupus nephritis in most patients. None of the serologic parameters at the time of biopsy, either singly or in combination, can adequately and satisfactorily predict the histologic type or severity of the renal lesion in an individual patient.

Complement and Activation Products in Systemic Lupus Erythematosus

The in vivo activation of the complement system by complexes of anti-DNA and DNA antigen and other autoantibodies is central to the pathogenesis of the glomerular injury and, possibly, to other tissue damage in patients with SLE. Acute exacerbations of the disease often are associated with hypocomplementemia. Serial measurements of C3 and C4 are routinely ordered in clinical practice while testing for total hemolytic activity (i.e., CH_{50}) or C1q is sometimes used to assess lupus disease activity.

A recent prospective study of 53 SLE patients studied monthly noted that a decrease in the serum level of C3 and C4 was not consistently associated with global measures of disease activity. However, decreasing serum complement over time was strongly associated with lupus nephritis and hematologic abnormalities (196). Other investigators have reported that measurements of C3 and C4 are not reliable marker or predictor of lupus disease activity or to separate patients with mild disease from those with severe disease (197 ,198 ,199). Several reasons are cited why serum complement levels are imperfectly associated with lupus disease activity. There is a wide variation of normal complement protein concentration among individuals partly because of genetic factors. The serum protein concentrations are controlled by the rate of protein synthesis and catabolism that vary between individual subjects. Complement components including C3 and C4 are acute phase reactants and synthesis may increase in response to inflammation. Serum levels of complement proteins do not reflect what is happening in the tissues. Autoantibodies to complement components such as anti-C1q antibodies may activate complement such that the degree of complement activation is associated with these autoantibodies rather than lupus disease activity (200).

Investigators have postulated that small-vessel injury in SLE may occur without evidence of immune complex mediation by the release of split products of complement activation, such as anaphylatoxins. These activation products, such as C3a, C5a, and SC5b-9 can activate and attract inflammatory cells. This can lead to cell aggregation and vascular adherence, resulting in an occlusive vasculopathy and ischemia (28).

Correlation with Disease Activity

A number of studies have shown that measurement of the plasma concentration of activation products of complement, including iC3b neoantigen, C3a, C4a, C3d, C4d, and the terminal complex, C5b-9, can be useful in assessing disease activity and predicting exacerbations (201 ,202 ,203 ,204 ,205 ,206 ,207 ,208)

(Table 48-5). The consensus of these various studies is that measurement of the activation products is superior to the determination of serum C3 or C4 values. Many patients with clinically active disease and normal serum C3 or C4 levels have elevated activation products of complement. Nevertheless, except for a few (201 ,203 ,209), most of these studies emphasized differences between patient groups (e.g., active vs. inactive) rather than longitudinal determinations in individual patients.

Table 48-5: Activation of Complement as a Measure of Disease Activity in SLE

Source	Activation product	No. of Patients	Results and Comments
Negoro, 1989 (205)	iC3b neoantigen	40 untreated	Plasma levels elevated in 83% of patients; highly correlated with disease activity and renal activity index.
Hopkins, 1988 (201)	C3a and C5a	40	C3a level increased in all patients; occurred 1 to 2 months prior to flare; marked elevation in cerebritis; C5a levels less sensitive.
Wild, 1990 (207)	C4a and C3a	24	C4a levels higher in patients with severe disease than in those with mild disease; C4a correlated with anti-dsDNA and C1q assay for immune complexes; C4a superior to C3a.
Senaldi, 1988 (206)	C4d and C3d	48	C4d correlated better than C3d with disease activity; C3 and C4 did not correlate with disease activity.
Horigome, 1987 (202)	Terminal C attack	54	TCC correlated with circulating immune complexes, CH_{50}, C4, C3, complex (TCC) C5, and alternate pathway activity.
Garwryl, 1988 (203)	Terminal C complex	22	Elevated TCC correlated with 89% of all flares.(C5b-9)
Kerr, 1989 (204)	Factor B activation (Ba)	51	51% of patients with high Ba had severe multisystem disease; associated with cutaneous vasculitis; Ba correlated better than C4a and C3d with disease severity.
Buyun, 1992 (209)	BaBb, C4d, SC5b-9	86	C4d most sensitive in 86% with major flares
Porcel, 1995 (210)	C3, C4, C3a, C4a, iC3b	39	SC5b-9 most useful with 77% sensitivity and 80% specificity.
Mollnes, 1999 (211)	C4bc, Bb, C3a, C3bc, C5a, SC5b-9	21	Only SC5b-9 correlated with disease activity scores.
Nagy, 2000 (197)	C1rs-C1inh, C3b(Bb)P, SC5b-9	65	C3b(Bb)P was highly specific and sensitive indictor of disease activity.

Three prospective studies examined the value of complement activation products and conventional measurements of complement in monitoring disease activity. Buyon et al. (209) reported that an elevated serum level of C4d had the most sensitivity, being found in 86% of patients who subsequently developed a major exacerbation. The specificity was low, however, such that 69% of the patients who did not flare during the study period had abnormally elevated C4d. Porcel et al. (210) found the terminal complement complex to be the most useful in monitoring disease activity, with 77% sensitivity and 80% specificity. Nagy et al. (194) compared the serum levels of C8H50, C4.C3 to the plasma levels of C1rs-C1inh, C3b(Bb)P, and SC5b-9 in 65 SLE patients. In a smaller number of patients studied serially, C3b(Bb)P, a complex formed during the activation of the alternative pathway, showed the highest difference between active and inactive disease and the best correlation with SLEDAI. Long-term study of a larger group of patients are needed to confirm these findings.

In the SLE patients without evidence of nephritis, Mollnes et al. (211) found that the routine measurement of complement including activation products is of limited importance in predicting disease flares. They measured conventional complement tests and plasma level of C1rs-C1inh, C4bc, Bb, C3a, C3bc, C5a, and SC5b-9. There were 27 flares in 21 patients but none developed nephritis during the study period. Only the plasma level of SC5b-9 correlated with disease activity at the time of the flare.

Measurement of C3d in the urine has been shown to be a sensitive indicator of complement activation and has been reported to be helpful in assessing disease activity in lupus nephritis (212 ,213).

Conventional measurements of complement and split products may be useful in evaluating disease flares during pregnancy and in differentiating a lupus flare from preeclampsia. The serum levels of CH_{50}, C3, and C4 rise during pregnancy. In patients without SLE but with

preeclampsia, the plasma concentration of complement split products, including Ba (i.e., activation product of the alternative pathway), C3a, C4d, and SC5b-9, is increased; however, CH50 generally remains normal. In pregnant patients with SLE and disease exacerbation, there is a reciprocal rise in complement split products and a drop in serum C3, C4, and CH_{50}. A high ratio of CH_50 to Ba has been suggested to differentiate patients with preeclampsia from those with active SLE (214 ,215 ,216).

Summary

Serial measurements of serum C3 and C4 are useful in following SLE patients with nephritis and possibly those with hematologic abnormalities. Additional prospective studies are needed to compare the relative value (including cost-effectiveness) of the various complement activation products in assessing disease activity, major organ involvement such as nephritis, and in determining the possible effects of comorbid conditions, especially infections. A minor drawback of these assays is the need for special handling of the plasma specimen to prevent spurious activation of complement in vitro. The measurement of activation products of complement may be particularly useful in patients with isolated CNS involvement, who frequently do not exhibit hypocomplementemia (201).

Table 48-6: Summary of Serologic Abnormalities in Systemic Lupus Erythematosus (SLE)

1. Anti-double-stranded (ds)DNA and anti-Sm antibodies are serologic markers of idiopathic SLE. Their presence in patients suspected of the disease on clinical grounds confirms the diagnosis.
2. Mixed connective-tissue disease (MCTD) should be considered in a patient with overlapping features of SLE, polymyositis, and scleroderma in the presence of a high titer of anti-U1RNP and the absence of anti-dsDNA and other specific types of antinuclear antibody (ANA).
3. Pregnant SLE patients should be tested for anti-Ro/SSA antibodies, because their presence indicates a risk for neonatal lupus syndrome.
4. Anti-Ro/SSA antibodies are associated with SCLE, photosensitivity, ANA-negative SLE, and genetic deficiency of complement with LE-like clinical features.
5. Antihistone antibodies have limited diagnostic value for idiopathic SLE but are considered characteristic of drug-induced LE.
6. No single serologic test is predictive of disease exacerbation in SLE. The most useful parameters for assessing disease activity are anti-dsDNA and serum complement levels.
7. Measurements of the activation products of complement and soluble receptors are promising serologic markers predictive of disease severity and exacerbation.

LE, lupus erythematosus; SCLE, subacute cutaneous lupus erythematosus.

Cytokines and Soluble Receptors in SLE

A recent analytical review of biomarkers in SLE identified four markers of disease activity that have promising clinical value: soluble IL-2 receptors, thrombomodulin, soluble VCAM-1, soluble TNFR and α interferon (217).

Soluble IL-2 Receptors in SLE

Following activation, resting T lymphocytes express receptors for IL-2 (IL-2R) on the cell surface, and a high-affinity IL-2R enables T lymphocytes to proliferate in response to the cytokine. The receptor can be shed or released in vitro, or physiologically in vivo, and can be detected in supernatants of cell cultures, in blood, and in body fluids. Elevated serum concentrations of soluble IL-2R (sIL-2R) have been found in patients with conditions that are characterized by immune-system activation, including SLE, RA, chronic infections, and malignancies.

Association with Disease Activity

We have found a positive correlation between serum levels of sIL-2R and immunologic markers of disease activity in SLE, including reduced serum C3 and high cryoglobulin levels (29). Sequential studies in patients with active SLE have revealed a decrease in serum sIL-2R levels concomitant with a clinical response to steroid therapy. Our findings have been confirmed and extended by other investigators (218 ,219 ,220 ,221 ,222 ,223).

A prospective study of 71 unselected patients with SLE by ter Borg et al. (218) showed an elevation of sIL-2R in 18 of 21 patients who developed clinical exacerbations, which correlated with changes in anti-dsDNA antibodies and with C3 and C4 values. Of these exacerbations, 75% were preceded by a rise in sIL-2R levels, but changes in anti-dsDNA and C3 levels tended to precede the increase in sIL-2R. The serum concentrations of sIL-2R in patients with inactive SLE were higher than those of healthy individuals, suggesting that an ongoing T cell activation process was occurring in SLE, even during periods of clinical quiescence. The sIL-2R level increased further before disease exacerbation.

In lupus nephritis, Laut et al. (223) reported a correlation between sIL-2R and histologic activity and chronicity indices, along with presence of IgG and C3 in the kidney biopsy specimen. The serum level did not correlate with serum creatinine, suggesting that the high sIL-2R was not the result of decreased renal clearance. Significant elevation of sIL-2R occurred during lupus nephritis flare and appeared to more sensitive than anti-dsDNA and CH_{50} as a serologic marker.

Elevation of sIL-2R can occur in infections and should be taken into consideration in patients with lupus. Wong and Wong (224) found markedly elevated sIL-2R in patients with lupus and either active or inactive SLE and concurrent infection. Chronic infections, especially tuberculosis and

candida infection, were associated with higher levels of sIL-2R than with pyogenic and herpes zoster infections.

Gilad et al. (225) reported elevated levels of sIL-2R in the cerebrospinal fluid (CSF) of patients with stroke as the initial manifestation of SLE. The levels were significantly higher than those seen in the CSF of nonlupus patients with ischemic strokes.

Soluble Tumor Necrosis Factor Receptor

In a prospective cohort study, Aderka et al. (226) found the serum level of sTNF p55 and p75 correlated with lupus disease activity than did anti-dsDNA antibodies. In a cross-sectional study of 40 SLE patients, Davas et al. (227) reported that the serum level of sTNF correlated with SLEDAI and European community lupus activity measure (ECLAM) disease activity scores. The elevated serum level of sTNFR in patients with lupus nephritis decreased significantly 6 months after drug therapy. Other investigators have reported similar findings that support the value of measuring soluble TNF receptor in assessing disease activity (228 ,229 ,230).

Serum Thrombomodulin

Thrombomodulin is a glycoprotein expressed on the luminal surface of vascular endothelium and acts as a thrombin receptor. Following endothelial damage, soluble thrombomodulin is detected in the plasma and urine and is considered a maker of microvascular endothelial cell injury. Elevated serum level of thrombomodulin has been reported in active SLE especially those with nephritis (231 ,232). Ho et al. (233) found plasma concentration of soluble thrombomodulin correlated with the level of vascular cell adhesion molecule-1 and both were correlated with serum creatinine and SLEDAI score. Plasma thrombomodulin remained persistently elevated in patients with history of lupus nephritis independent of disease activity and serum creatinine suggesting endothelial cell activation in the kidneys (234). Compared to other serologic parameters, including anti-dsDNA, complement, C-reactive protein, and soluble vascular endothelial adhesion molecules, Boehme et al. (235) found that (235) soluble thrombomodulin correlated the best with lupus disease activity measured by SLAM scores.

Circulating Adhesion Molecules

Elevated plasma levels of soluble vascular adhesion molecule-1 (sVCAM-1) and other cell adhesion molecules in SLE and other diseases are considered markers of leucocyte and endothelial cell activation or damage. Ikeda et al. (236) found soluble VCAM-1 to be significantly elevated in class III and IV lupus nephritis and the plasma level correlated with high SLEDAI score and decreasing during remission. Other investigators have also noted a significant association of sVCAM-1 levels with active lupus nephritis (233 ,237).

Serum levels of sVCAM-1 were found to be increased in patients with primary and secondary antiphospholipid syndrome associated with SLE, especially in those patients with severe and recurrent thromboses (238).

SLE patients presenting with demyelinating syndrome and patients with multiple sclerosis have been found to have elevated levels of sVCAM-1 in paried samples of serum and CSF. Intrathecal synthesis of sL-selectin but not sVCAM-1 was seen in SLE (239 ,240). To understand the significance of these findings, further studies on SLE patients presenting with other neuropsychiatric syndromes should be undertaken.

Summary

The measurement of sIL-2R, thrombomodulin, sTNF-R, and/or sVCAM-1 appears to be useful for assessing disease activity especially in lupus nephritis and may be particularly helpful in those patients who test negative for anti-dsDNA.

Additional prospective longitudinal studies are essential to determine whether serial measurements can predict disease exacerbation, provide additional information to conventional laboratory parameters in monitoring response to therapy, and to examine the effects of infections and other comorbid conditions in SLE patients.

Application of Multiple Serologic Measurements in Systemic Lupus Erythematosus

It is clear that no single serologic test can adequately assess or predict the clinical course of SLE in individual patients. A few studies have examined application of a panel of serologic reactions to improve sensitivity and correlation with disease activity.

In an early study, Schur and Sandson (241) concluded that a combination of complement-fixing anti-dsDNA and CH_{50} correlated better with active disease, especially lupus nephritis, than either of the serologic tests alone. In a more recent study, Lloyd and Schur (21) found that serial measurement of a combination of CH_{50}, C3, C4, and circulating immune complexes by C1q-binding assay appears to be the most useful. Anti-dsDNA antibodies did not significantly increase the usefulness of this panel (see Chapter 13 , Complement and Systemic Lupus Erythematosus).

In a prospective study of 48 unselected patients with SLE, Abrass et al. (23) found that circulating immune complexes, as determined by a solid-phase C1q-binding assay, correlate with active disease manifestations, particularly nephritis or arthritis, but not with skin or other organ involvement. A change in disease activity, prompting the physician to make a change in management, was predicted by the results of solid C1q-binding test. Neither C3 nor anti-dsDNA correlated with disease activity, and neither gave additional information when combined with use of the solid-phase C1q-binding test.

Using a battery of laboratory tests, Morrow et al. (242) failed to identify a single test that reliably distinguished between severely active, moderately active, and inactive disease groups of patients with SLE. Determination of circulating immune complexes by polyethylene glycol precipitation, platelet count, and erythrocyte sedimentation rate distinguished the active from the inactive disease group. Patients with severely active disease and involvement of three or more systems were different from the less active group by solid-phase C1q-binding assay for immune complexes, anti-dsDNA, CH_{50}, and lymphocyte count, but patients with neuropsychiatric involvement and those with thrombocytopenia were the most difficult to sort out. Only 44 of patients could be classified accordingly into clinical grades when combinations of four out of five laboratory tests were used. Isenberg et al. (243) were unable to find a correlation between clinical disease activity and multiple serologic reactions to dsDNA, ssDNA, RNA, synthetic polynucleotides, and cardiolipin. In a retrospective study of complement and circulating immune complexes (as tested by five different assays) in 33 patients, Valentijn et al. (25) concluded that although disease activity correlates with serum levels of CH_{50}, C3, or C1q (by binding assay), the sensitivity and predictive value of the serologic parameters are low. In 20 of patients, one or more parameters constantly was abnormal regardless of disease activity. On the other hand, in a small subset of patients, a patient-specific activity parameter could be identified.

In a cross-sectional study of 100 patients, Clough et al. (244) found that a combination of sIL-2R, Westergren sedimentation rate, and anti-dsDNA antibody by ELISA correlated best with disease activity as measured by SLAM index. In contrast, the serum levels of C4, iC3b, and Bb correlated poorly with disease activity.

A 12-month longitudinal study of 53 patients with lupus showed an incidence of disease flare of 0.69 per patient-year of follow-up and a good correlation with serologic abnormalities. Active nephritis was associated with high anti-dsDNA antibodies as measured by Farr method, low C3, and low C4. High anti-dsDNA level also was associated with musculoskeletal and cardiopulmonary involvement. The odds ratio for lupus flares in asymptomatic patients with high anti-dsDNA was three; for those with low serum C3, it was two (245).

Conclusions and Recommendations

Many reports evaluating the application of serologic tests in the assessment and prediction of disease activity have been rife with shortcomings. No uniform index of clinical disease activity has been used, and certain groups of patients (e.g., those with nephritis) were either over- or under-represented in the test populations. Conclusions often were based on a single test sample, and length of the follow-up period was not adequate. Serologic tests were not standardized, so comparison of the various studies is not feasible. Despite these obvious faults, it is clear that no single serologic test available today is ideal and applicable to all patients with lupus. Considering the heterogeneity of the clinical disease, it is unlikely that a single such test will be found. Serologic abnormalities in a patient with active lupus nephritis are not necessarily the same as those in another patient with skin rash, fever, hematologic changes, and/or serositis.

Newer serologic tests, such as determination of complement split products, sIL-2R and other soluble factors, are undergoing further evaluation, and a combination of anti-dsDNA and serum complement now is generally used in clinical practice. The ELISA test for anti-dsDNA probably is the most widely available test in clinical practice. The clinician should become familiar with the advantages and limitations of the particular assay that is used in the laboratory to which specimens are sent. In patients who continually do not have anti-dsDNA antibodies (even after using different assay methods), measuring the serum titer of some other ANA, such as anti-Sm or anti-Ro/SSa, may be useful (83). The serum C3 concentration is measured more frequently than CH_{50}, although the latter probably is more a more sensitive parameter (24 ,242). In my experience, serial measurement of serum cryoglobulins is a useful parameter, while tests for circulating immune complexes, such as the C1q solid-phase binding assay have limited value.

It must be remembered that there are some patients in clinical remission who have persistently abnormal serologic findings (12 ,13). Careful monitoring of specific organ functions, such as renal function, remains an important aspect in the assessment of disease activity and response to therapy (see Table 48-6).

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Chapter 49

Differential Diagnosis and Disease Associations

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SLE's mimicry of other diseases was first reviewed in 1954 by Harvey (1), who listed 24 different diagnoses made on his patients during the early stages of their disease. Usually, the greatest difficulty is separation of SLE from closely related connective tissue disorders. Table 49-1 summarizes the clinical data in order to make these differences readily apparent. Presentations of diverse entities ranging from thyroiditis, gluten sensitivity, syphilis, and viral, as well as parasitic infections have all been misdiagnosed as lupus (2 ,3 ,4 ,5 ,6 ,7).

Is it Really Lupus?

In addition to obtaining a detailed history, attempting to correlate any past features that might have been manifestations of SLE with the current illness, the physician must perform a thorough physical examination (see Chapter 32) and make a careful laboratory survey. This should include determination of the presence of antinuclear antibody (ANA), rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), creatine phosphokinase (CPK), C3 complement, Westergren sedimentation rate, and also a complete blood count (with differential and platelet counts), blood-chemistry profile, Venereal Disease Research Laboratories (VDRL) test, partial thromboplastin time, urinalysis, chest radiography, and electrocardiography. If these tests are not diagnostic and SLE is strongly suspected, assays for anticardiolipin antibody, anti-Ro/SSA, anti-La/SSB, anti-DNA, anti-Sm, and antiribonucleoprotein (anti-RNP) should be done, as well as a serum-protein electrophoresis. The Mayo Clinic has advocated a cascade algorithm for screening positive ANAs (8). Along with a thorough clinical evaluation, a diagnosis can be derived 90% of the time.

Our group evaluated 44 patients with a positive ANA and no other abnormal commonly derived tests or autoantibodies (i.e., Sm, RNP, Ro, La, Scl-70, RF, C3, C4, CPK) who did not fulfill the ACR criteria for SLE and were referred for a rule-out-lupus consultation (9). At 6 months, 43% fulfilled criteria for SLE, 32% had fibromyalgia, and 9% had seronegative rheumatoid arthritis (RA). Obtaining a bone scan, lupus band test, serum-protein electrophoresis, and specific tests such as antineuronal antibodies, antibodies to histone-DNA complexes, or antiribosomal-P antibodies helped to make final determinations.

The differential diagnosis of connective-tissue disorders is complicated by the overlap of coexisting rheumatic syndromes. Two large clinics have reported that 25 and 33 of their patients, respectively, had features of two connective-tissue disorders (10 ,11). Co-existing SLE and scleroderma, as well as SLE and polymyositis, have been found in a number of patients. If a positive anti-RNP is present, the overlap syndrome may represent a distinct entity termed mixed connective-tissue disease (MCTD).

Complicating these issues is the recognition that autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, Sjögren syndrome, or Raynaud phenomenon can be isolated processes for years before evolving into an established connective-tissue disease. Thirty percent of Isenberg's 215 British lupus patients also had at least one other autoimmune disorder (12). As early as 1956, Talbott and Ferrandis (13) observed frequent transitional forms from one rheumatic disease to another. Some patients, who appear to have fulfilled all or most of the clinical criteria for a diagnosis of active RA, lose the exclusive features of this malady at some future time and manifest unmistakable SLE, polyarteritis, polymyositis, or scleroderma. Currently, it is not possible to determine whether the disorder was present from the beginning of symptoms and subsequently changed into another disorder.

Why is it necessary to differentiate among these conditions? Arriving at a specific diagnosis is necessary to understand the course and prognosis of the illness and treat it effectively. For example, if gold therapy had been instituted in a patient who was thought to have RA and urinary or hematologic abnormalities developed, it would be assumed that they were a reaction to the treatment. If ANA had been initially sought and found, however, gold therapy might not have been used, and the changes noted would have been recognized as being evidence for progression of the underlying disease.

Table 49-1: Differential Diagnosis of Connective Tissue Disorders

Parameter	SLE	RA	PSS	MCTD
Sex incidence	90% female	75% female	66% female	80% female
Age of majority	10–50 y	20–40 y	20–50 y	All ages
Family history	+ for LE or RA in 12% or more	Often +	0	Rarely +
Disease duration	Mo-y	Mo-y	Mo-y	Variable
First changes	Arthritis, rash	Arthritis	Skin	Arthritis, Raynaud's phenomenon
Cardiac involvement (clinical)	33%	65% at autopsy; clinically rare	+; perfusion and conduction abnormalities seen	Myocarditis in children (clinical)
Skin and mucous membranes	Alopecia, butterfly erythema, scaling erythematous papules, ulcers	Subcutaneous nodules	Tightness of skin of hands, face, neck; hyperpigmentation of involved skin	Rashes of SLE, PSS, and membranes dermatomyostitis
Ocular	Iritis, retinal vasculitis or infarcts; Sjögren's syndrome	Scleritis, Sjögren's syndrome	Sjögren's syndrome	Rare; Sjögren's sydrome
Adenopathy	Moderate	Minimal	0	Minimal
Pleurisy or lung disease	Most cases	Rare	Interstitial fibrosis common	30%
Pericarditis	30%	Clinically rare	Rare	25%
Generalized abdominal pain and tenderness	Often	0	Dysphagia common; bowel motility decreased	Dysphagia common
Hepatomegaly	Occasional	0	0	0
Splenomegaly	10%	Rare	0	0
Joints involved	All joints, esp. minor	All joints	Minor	Erosive arthritis in 30%
Arthritic deformity	Frequent, nonerosive	Often erosive	Often	20%
Myalgia	48%	Frequent	+20%	50%
Raynaud's phenomenon	26%	Occasional	Common	80%
CNS involvement	Personality changes, convulsions and localized deficits, fatigue	Rare	Rare	10%
Laboratory				
Urine abnormalities	46% at some time	0	Creatinuria	Lupus nephritis in 10–40%
WBC and differential	Leukopenia in 43%	Leukocytosis, acute phase	Normal	Leukopenia in 35%
Anemia	10% hemolytic; 56% < 11.0 g Hb	Normocytic	0	41%
Uremia	5–10%	0	Occasional	0
Hyperglobulinemia	Common	Frequent	40%	80%
LE cells	+in 75%	+9% only	+5%	14%
ANA	+95%	+25%	+50% speckled, CREST-centromere	+100% speckled
Muscle biopsy	Usually 0	Usually 0	Myositis uncommon	Often positive
Skin biopsy	Suggestive +	0	Diagnostic	+ lupus band test or PSS
Dermatomyostitis and Polymositis	**Polyarteritis Nodosa**	**Rheumatic Fever**	**Serum Sickness**	**Behçet's Syndrome**
66% female	40% female	Equal	Equal	Mostly female
10–50 y	All ages	2–19 y	All ages	10–50 y
0	0	0	0	0
Mo-y	Variable	Mo-y	Weeks	Mo-y
Myalgia, skin changes, or weakness	Asthma, polyneuritis, abdominal pain, or fever	Arthritis	Urticaria	Orogenital ulcers
Occasional	Occasional	Most in acute phase	0	0
Periorbital edema, dusky erythema, Gottron's nodes	Hives, necrotic ulcerations, cutaneous and subcutaneous nodules	Erythema marginata, subcutaneous nodule	Hives, angioneurotic edema	Recurrent aphthous stomatitis, cutaneous vasculitis
0	Rarely, retinal hemorrhages and exudates	0	0	Uveitis in 66%
Rare	Minimal	Minimal	Minimal	0
Rare	0	0	0	0
Rare	0	Often	0	0
0	Often	Occasionally	Occasionally	Inflammatory bowel disease, occasionally
0	20%	+ with failure	0	0
Occasional	0	Rare without subacute bacterial endocarditis	0	0
Rare	Major	Major	All	55%
0	0	0	0	Rare
Marked	Common	Frequent	Rare	0
Common	Rare	0	0	0
0	25%	Chorea	0	22%
Creatinuria	Hematuria and red cell casts	0	0	0
Normal; eosinophila occasionally	Leukocytosis, eosinophilia in 18%	Leukocytosis	Leukocytosis, eosinophilia	0
Uncommon	50%	Normocytic	?	0
0	Common	0	0	0
0	Occasionally	0	0	Occasional
0	0	0	0	0
+30%	+20%	0	0	0
Usually +	Suggestive if +	0	0	0
Suggestive	Suggestive	0	Suggestive	0
20% of dermatomyositis cases associated with malignancy; proximal muscles involved; EMG may be diagnostic, CPK level elevations common	Association of asthma, eosinophilia, hypertension and polyneuritis suggests diagnosis; Biopsy or *p*-ANCA + in only 50%	Preceding streptococcal infection Diastolic heart murmur almost pathognomonic; elevated antistreptolysin titer		HLA-B5 associations, antibody to human mucosal cells

CNS, Central nervous system; EMG, electromyelography; *p*-ANCA, antineutrophil cystoplasmic antibody; WBC, white blood cell.

The Misdiagnosis of Lupus

Many people who are told they have or might have SLE do not. Hochberg et al. noted that only one third of patients who were told they had lupus by a physician actually fulfilled the American College of Rheumatology (ACR) criteria for SLE (14). One hundred forty-nine patients were referred to the University of Alabama for management and/or consultation for suspected SLE; 37 (25%) probably had only fibromyalgia and 15% had an undifferentiated or incomplete autoimmune syndrome (15). Two hundred sixty-three patients referred to the University of Florida Autoimmune

Disease Clinic between 2001 and 2002 carried a presumptive diagnosis of SLE. The actual diagnosis could only be confirmed in 49% of them (16). Although many patients might have a cross-over syndrome or undifferentiated connective-tissue process, at least of half of those carrying a diagnosis of SLE that is unconfirmed do not have any inflammatory process. The misdiagnosis of lupus leads to unnecessary, toxic and expensive treatments, stigmatization of patients, pointless lifestyle and dietary restrictions, and upset family relationships and reproductive planning. Even more disturbing are the frequent feelings of anger toward the rheumatologist who tells the patient they do not have the disease. Although most of these patients have fibromyalgia (see Chapter 63), factitious disorders mimicking SLE also have to be considered (17).

Positive Antinuclear Antibody Testing: How Often Is It Lupus?

All too often, rheumatologists are referred patients who feel well or have vague symptoms and have a positive ANA to “rule out lupus.” A positive ANA often is found in patients with other disorders, even in seemingly healthy patients. A positive ANA had a 10% predictive value for SLE at a university medical center (18) and a 23% value in a large consultative rheumatology practice (19). A suburban Orange County, California rheumatology group studied 276 patients who were referred for a positive ANA without a diagnosis (20). After a comprehensive evaluation, 52 (18.8%) were diagnosed with SLE, 44 (15.9%) had an organ-specific autoimmune disease, 8.3% had an infectious disease, and 2.9% had neoplasia. No diagnosis was made in 13.4%. Of 1,010 ANA tests ordered during a 10-month period at the Beth Israel Hospital laboratory in Boston, 153 were positive. Seventeen patients turned out to have lupus, and other rheumatic diseases were identified in 22, for a predictive lupus value of 11% (21). On the other hand, 130 soldiers who donated sera while being inducted into the armed forces who ultimately developed lupus were studied (22). One hundred fifteen had at least one SLE autoantibody (78% had ANA), which presented a mean 3.3 years prior to diagnosis. Sixty-two ANA-positive Canadian patients were evaluated a mean 5.4 years later, only 3 had developed a connective tissue disease, and none were diagnosed with SLE (23).

Antinuclear Antibody-Negative Lupus

Positive ANA is only one of 11 criteria that are used to define SLE according to the ACR classification. As noted in Chapter 2 , 4 of the 11 criteria must be present to make a diagnosis, but the ANA is so central to current concepts of SLE that many rheumatologists find it inconceivable for SLE to be present without it.

Several reports have documented the delayed appearance of ANA in patients suspected of having SLE. In view of my own group's studies (24 ,25) documenting a mean of 3 to 4 years between onset of symptoms and time of diagnosis, this is not surprising. Cairns et al. (26) reported 11 patients with lupus nephritis in whom a negative ANA persisted for years before becoming positive. Bohan (27) and Enriquez et al. (28) presented several well-documented cases. Persillin and Takeuchi (29) found ANA in the urine and pleural fluid of a patient with diffuse proliferative nephritis and nephrotic syndrome for some time before serum ANA was present. Low antibody concentrations in the serum secondary to loss in body fluids can be present, as was noted by Ferreiro et al. (30).

Numerous reports in the 1960s and 1970s examined the ANA-negative lupus subgroup, but only animal substrates for ANA were considered to be reliable at the time (31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,43). Many patients actually had discoid lupus erythematosus (DLE) or subacute cutaneous lupus erythematosus (SCLE) and did not meet ACR (then ARA) criteria, but the remaining number of ANA-negative patients with lupus was larger than necessary. This became documented when human cell line ANA substrates were introduced. Provost's group evaluated 28 patients with SLE who had titers of 1:20 or less with a mouse liver substrate (31). Using a rat liver substrate, three (11%) had a positive ANA. With human spleen imprints, 16 (57%) were positive, 9 (32%) were positive on a KB cell-line substrate, and 8 (28%) were positive with an Hep-2 cell line. These results emphasize how a negative ANA can become positive merely by using another substrate, thereby converting ANA-negative lupus to ANA-positive lupus. Reichlin (44) has stated that with a KB or Hep-2 substrate, 98% of all patients with SLE are ANA-positive, because non-DNA–containing antigens such as Ro/SSA are better represented when these cell lines are studied. Unfortunately, human cell lines are less specific, although they are more sensitive. Larger numbers of healthy people have positive ANAs when human cell lines are used. Only 17 of 447 patients (3.8%) with idiopathic SLE who were tested between 1980 and 1989 on Hep-2 substrate were ANA-negative (45). They were evenly divided into three groups: (1) antiphospholipid syndrome, (2) renal biopsy documented lupus in patients who had received steroids and chemotherapy, and (3) skin biopsy positive patients who also fulfilled ARA criteria. Several reports also have documented patients with high-titer cardiolipin antibody, recurrent thromboses, and negative ANAs who fulfilled the ACR criteria for SLE (46 ,47). A 2004 literature review found 164 cases of ANA negative lupus published since 1976, or which 97% were described before 1987. Only 27 (16%) of the cases were tested on a suitable substrate (48).

Technical inaccuracy, prozone phenomenon, variations in microscope quality, ANA hidden within circulating immune complexes (CICs), in vivo binding of ANA by tissues, substrate specificity, low-cutoff dilutions, and use of monospecific antisera are other causes of negative ANAs in patients with SLE. Inadequate fixation of the substrate may lead to antigenic deficiency, allowing leaching of the pertinent antigens leading to negative ANA results. Occasionally, patients with positive LE-cell preparations

and negative ANAs have been observed (49 ,50 ,51). Wide variations in the reproducibility of ANA tests and difficulties in standardization also are problems that have not yet been overcome (52 ,53). Phenotyping B cells may help diagnose true SLE (54).

In summary, if a KB or Hep-2 cell-line substrate is used to detect ANA, 90% of the ANA-negative patients who meet ACR criteria can be shown to be ANA-positive. If these substrates are not available, specific tests for anti-Ro/SSA may be useful (55). Other ANA-negative patients with lupus may fulfill DLE, SCLE, or juvenile RA (JRA) definitions without fulfilling ARA criteria. ANA-negative patients with lupus usually fall into three categories: (1) antiphospholipid syndrome, (2) early disease, and (3) previously positive ANA made negative by steroids, cytotoxic drugs, or uremia (56). True ANA-negative lupus probably comprises less than two of all SLE cases. Many patients who claim to have ANA-negative lupus do not have SLE (57).

Undifferentiated Connective-Tissue Disease

Coined by LeRoy in 1980 (58), the term undifferentiated connective tissue disease (UCTD) has undergone many evolutions. Between 1982 and 1987, ten rheumatic disease centers enrolled 410 patients for a landmark study. All had symptoms for less than 1 year. Fifty-seven had RA, 57 SLE, 37 poly/dermatomyositis, 46 scleroderma, and 213 early “connective tissue disease.” The latter was defined as patients with isolated Raynaud phenomenon, unexplained polyarthritis, or isolated keratoconjunctivitis sicca, and at least three of the following: Raynaud, polyarthritis, sicca symptoms, myalgias, rash, pleurisy, pericarditis, central nervous system (CNS) symptoms, pulmonary symptoms, peripheral neuropathy, false-positive test for syphilis, and elevated sedimentation rate. These patients have been monitored for nearly 20 years and the following observations have been made (59 ,60 ,61 ,62 ,63):

- Twenty percent with unexplained polyarthritis developed RA; one of 67 developed SLE.
- Among 31 with isolated Raynauds, one developed SLE.
- Among 115 with UCTD, 33 were still UCTD, 12 developed SLE, four developed RA.
- UCTD had an 87% 10-year survival compared with 56% for scleroderma.
- Thirteen percent of the UCTD patients evolved SLE. They were more likely to be younger, African American, and have rashes autoantibodies.

In other words, at 10 years, approximately one third with UCTD had no disorder, one third remained UCTD, and one third evolved a defined rheumatic disorder. Six hundred sixty-five European UCTD patients were followed 5 years later (64). Two hundred thirty, or (34.5%) developed a defined connective-tissue disease, 12% remitted and 64.5% remained UCTD. From most to least frequent, the defined disorders were RA, Sjogren, SLE, MCTD, scleroderma, vasculitis, and inflammatory myositis.

Table 49-2: Practice Point: A Working Definition for UCTD

1. Mandatory: inflammatory arthritis in >1 joint or Raynaud or keratoconjunctivitis sicca
2. Mandatory: positive antinuclear antibody, rheumatoid factor or anti-CCP
3. Need three of the following: myalgias, autoimmune rash, serositis, persistent fever without infection, adenopathy, elevated sedimentation rate or CRP, antiphospholipid antibody

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Must not fulfill ACR definitions or criteria for any other rheumatic process.

Smaller-scale related efforts came to similar conclusions (65 ,66 ,67 ,68) and one provides an excellent review of earlier studies (69). The presence of malar rash, oral ulcers, elevated anti-DNA, or low C4 complement made the evolution to lupus more likely (70). The reader is referred to two excellent reviews of the subject (69 ,71).

There are probably several UCTD patients for every individual who fulfills the ACR criteria for SLE. UCTD patients are responsive to antimalarials, methotrexate, and corticosteroids, but rarely have organ-threatening disease requiring aggressive management. Table 49-2 lists this writer's working diagnosis for UCTD, which updates definitions derived in the 1980s for the above-cited studies.

Incomplete Lupus

This misleading term has appeared in the literature to denote patients who are thought to have SLE but do not fulfill four ACR criteria. These individuals range from those with biopsy documented nephritis to idiopathic thrombocytopenic purpura with a positive ANA to fibromyalgia. Twenty-eight Swedish patients with “incomplete SLE” were followed for a median of 5.3 years. Fifty-seven evolved criteria fulfilling SLE (72). Swaak et al. managed 122 European incomplete lupus patients. Twenty-seven met full criteria shortly after study entry, but only 3 more did over the next 3 years (73). Nevertheless, 43% were taking corticosteroids 3 years later. Too many patients who carry this label undergo unnecessary treatments and become stigmatized and medicalized; hence, the term should not be used and replaced with those who meet definitions for UCTD.

RA and SLE

Clinical Differentiation

RA and SLE share many clinical and serologic features, an overlapping that was recognized in the 1950s and 1960s by the publication of hundreds of papers on “RA with LE cells.” (See pages 464 to 476 in, *Lupus Erythematosus* 2nd Ed. for an extensive review of these clinical and serologic

features.) RA and SLE usually can be distinguished easily from each other, especially if the former is ANA-negative or erosive. When RA displays extra-articular involvement or is ANA-positive, however, it occasionally is difficult to differentiate from SLE. When a patient presents with a new inflammatory arthritis that has overlapping features of both diseases, it may take 6 to 12 months of clinical observation before a definitive diagnosis can be made.

Extra-Articular Differentiation

Extra-articular RA may include serositis, Sjögren syndrome, subcutaneous nodules, cutaneous vasculitis, anemia, and other features that are observed in SLE. Ropes (74) compared 142 patients with SLE to a cohort of patients with RA. The latter had a 6% incidence of LE cells, a 1% incidence of sun sensitivity (vs. 34% in SLE), and a 4% incidence of alopecia (vs. 46% in SLE). The incidence of thyroid antibodies is increased in both disorders (75). Felty syndrome consists of a positive ANA, splenomegaly, arthritis, leukopenia, and an increased incidence of cutaneous vasculitis. Several patients of my acquaintance with Felty syndrome were misdiagnosed as having lupus. Felty syndrome is characterized by antigranulocyte (as opposed to antilymphocyte) antibodies and elevated complement levels (76). Close examination, however, reveals that the overwhelming majority of those with Felty syndrome are middle-aged men, that anti-DNA is never present, and that most have circulating cryoglobulins (77 ,78 ,79). CNS involvement and renal disease are absent. Another differentiating feature between RA and SLE is the lack of kidney involvement in those with RA. In what might be the definitive study, Davis et al. (80) reviewed the records of 5,232 patients with RA who were followed at UCLA between 1955 and 1977. Of these, 28 (0.5%) had renal disease of all types, only 4 of whom (0.1%) had glomerulonephritis. Also, 3 of the 4 met the ACR criteria for SLE, and 1 had MCTD. Davis et al.'s literature review of glomerulonephritis in RA (80) demonstrated that most of the cases could be accounted for by gold- or penicillamine-induced nephropathy, interstitial nephritis, amyloid, or diabetes.

Laboratory and Serologic Differentiation

RF was present in 15% of 166 patients with SLE who were studied by Ginzler's group in detail (81), in 18% of Wolfe et al.'s 124 patients with lupus, and in 22.7% of 365 patients tested with idiopathic SLE who were followed by Wallace (unpubl. observations). Its presence is associated with milder disease. The availability of anti-CCP has further helped differentiate SLE from RA. Among 231 patients in Isenberg's group tested for anti-CCP, only 3 (<1%) were positive. Two of the 3 had erosive arthritis (82 ,83). Four percent of 250 and of 365 patients with RA in each of two reports had reduced complement levels (84 ,85).

Numerous investigators have looked for ANA in RA, and its incidence has ranged from 3% to 88%, with an average of 25% (86 ,87 ,88). A subset of RF-negative, ANA-positive RA has been described (89 ,90). Many of these patients have JRA; most have erosive disease and a good prognosis. Chapter 28 discusses RF further.

Co-existence of Systemic Lupus Erythematosus and Rheumatoid Arthritis

Do SLE and RA co-exist? It has long been known that patients may start with a diagnosis of RA or SLE that becomes SLE or RA over a period of years. Assuming that MCTD is not present, however, the true co-existence of these conditions is rare. Despite the frequent clinical overlap between RA and SLE features, the combination of advanced, deforming, erosive RA and a significant degree of biopsy-proven SLE is an extremely unusual finding.

Occasional case reports have appeared documenting a true co-existence (91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103). Of my own group's 464 patients with idiopathic SLE, one had classic seropositive, erosive, nodular RA with biopsy-documented proliferative SLE nephritis and nephrotic syndrome. The concurrence of SCLE in patients with RA who are Ro/SSA-positive is more common (104 ,105). Cohen and Webb (106) reported the development of SLE in 11 Australian patients with typical RA who were observed over a 17-year period, but the total number of patients with RA followed was not stated. Brand et al. (107) presented 11 co-existing cases; most had class II genetic determinations of both disorders. Panush et al. (108) have identified a true co-existence in 6 of 7,000 patients with RA who were evaluated over an 11-year period. It was concluded that rhupus did not occur more frequently (0.09%) than expected from the chance concurrence of SLE and RA (1.2%). Van Vollenhoven's group at Stanford found “rhupus” in 13 patients among 1,507 with RA and 893 with SLE (109). Seven appeared to have transformed from SLE to RA. Among 22 “rhupus” patients in Mexico City an increased prevalence of HLA-DR1 and DR2 alleles were found (110).

Juvenile Rheumatoid Arthritis and Systemic Lupus Erythematosus

JRA has been classified into systemic (i.e., Still disease), oligo-articular, and polyarthritis subsets. Several large-scale studies have observed ANA in approximately 60% of patients with JRA, particularly oligo-articular disease in young girls with uveitis (111 ,112 ,113 ,114). One study has determined that ANA in this subset is directed against an RNP that requires both RNA and protein moieties for antigenic integrity (115). Despite the high frequency of ANA, other antibody systems, such as anti-RNP, anti-Sm, anti-Ro/SSA, anti-La/SSB, anti-nDNA, and anti-PM1, rarely are found.

Approximately 2.5% of patients with classic deforming polyarthritis originally diagnosed as JRA later develop multisystem lupus (116 ,117 ,118). In one study, two of 85 patients with JRA evolved into SLE both had anti-DNA while still carrying a JRA diagnosis (119). Of 509 referrals to a pediatric rheumatology unit, 110 had a positive ANA. Only

10 had SLE (120). These distinctions often are clouded by the high incidence of ANA and the relatively low incidence of RF in JRA (121). ANA-negative childhood SLE also has been reported (122).

Relationship of Systemic Lupus Erythematosus to Other Autoimmune and Rheumatic Diseases

Scleroderma and Other Fibrosing Syndromes

Although ANA is present in most patients with scleroderma, other serologies associated with SLE are observed in a small minority with scleroderma. These include LE-cell preparations (123), antiphospholipid antibodies (124 ,125), and other nuclear antigens (126). Anticentromere antibodies usually are associated with the calcinosis cutis, Raynaud phenomenon, esophageal motility disorder, sclerodactyly, and telangiectasia (CREST) syndrome but can be found in up to 5% of patients with pure SLE (127). In contrast to SLE, familial occurrence of scleroderma is rare. Clinically, sclerodactyly, telangiectasias, calcinosis, and malignant hypertension with acute renal failure are almost unheard of in patients with SLE. It is important to differentiate among SLE, MCTD, and scleroderma, because the latter rarely is responsive to steroids or cytotoxic agents. Conversely, one would not attempt to treat SLE or MCTD with penicillamine.

Features that are relatively unique to both scleroderma and SLE are infrequently observed in patients who do not have MCTD. Dubois et al. (123) reviewed 14 cases of co-existent scleroderma and SLE in detail in 1971 and summarized the literature to date. Unfortunately, their work was hampered by the lack of accepted criteria for scleroderma, SLE, or MCTD. Since then, case reports have appeared of autoimmune hemolytic anemia (128 ,129), high levels of anti-nDNA (130), lupus nephritis (131 ,132), and discoid lupus (133 ,134 ,135 ,136) occurring in patients with scleroderma. Patients with anti-Scl antibodies probably have lupus rather than scleroderma if anti-dsDNA is present (137). Scleroderma may evolve into SLE and vice versa (138); morphea (139) and linear scleroderma can be seen with SLE (136 ,140 ,141). One case of neonatal LE with morphea (142) and four of eosinophilic fasciitis with SLE have been presented (143 ,144 ,145 ,146).

The extreme rarity of retroperitoneal fibrosis in SLE has been noted (143 ,147 ,148 ,149). Dialysis associated nephrogenic fibrosing dermopathy has been reported in SLE (150).

Polymyositis and Dermatomyositis

In contrast to SLE, patients with polydermatomyositis are less often women and rarely have an autoimmune family history. Also, different skin lesions are present (i.e., Gottron nodules and heliotrope rashes), a co-existing malignancy may occur, serositis is rare, and nephritis, liver inflammation, and hematologic abnormalities are absent. Lupus can present as a focal, acute myositis (151). Rarely, MCTD may evolve into a pure poly/dermatomyositis. A low- grade myositis with muscle enzyme levels two to three times normal may be seen in lupus that responds to low doses of corticosteroids. (See Chapter 33 for a detailed discussion of lupus myopathy and its comparison with other inflammatory myopathies.)

Systemic Vasculitis

Although polyarteritis nodosa is relatively rare, it can be mistaken for SLE. In contrast to patients with SLE, those with polyarteritis nodosa usually are men and include all age groups equally. Cutaneous vasculitis may be more prominent, as may eosinophilia, wheezing, and nerve and bowel symptoms. The ANA often is negative; two cases of coexistence of these entities has been reported (152 ,153). Hypersensitivity angiitis and serum sickness may mimic SLE at first but, ultimately, can be distinguished by a self-limited course, an absence of ANA, and rarity of severe visceral involvement. Ordering an antineutrophilic cytoplasmic antibody often can differentiate lupus from microscopic polyangiitis and Wegener granulomatosis (154).

Behçet syndrome can mimic SLE with its uveitis, oral and genital ulcers, CNS involvement, and frank synovitis, but in Behçet syndrome, the ANA is negative and certain ethnic predispositions (i.e., Japanese and Turkish) as well as HLA predispositions (i.e., the B5 haplotypes) can be observed. There is little doubt that some ANA-negative patients with lupus actually have Behçet syndrome. The lack of any diagnostic test for Behçet syndrome further complicates the picture, and one case of co-existent disease has been reported (155). One case of SLE in a child with Kawasaki disease has been reported (156).

Large-Vessel Vasculitis

SLE is a disease of the small arteries and medium-sized arterioles, but it can rarely affect larger-caliber vessels. Large-vessel vasculitis is not associated with autoantibody formation. Elderly people more commonly develop polymyalgia rheumatica and giant-cell arteritis, however, and SLE occasionally is included in the differential diagnosis as musculoskeletal symptoms are present and an age-related positive ANA may be found (157 ,158). A true concurrence of giant-cell arteritis and SLE has been reported twice (159 ,160). Takayasu pulseless arteritis is found in young women, who mostly are Japanese, but also in other Asian and Hispanic women. One Japanese literature review described 10 cases of Takayasu arteritis with co-existent SLE (161). Saxe and Altman (162) reviewed 18 cases in the literature and concluded that co-existence of these diseases was coincidental. The co-existence of large-vessel vasculitis with SLE is coincidental, possible, and rare (163 ,164 ,165).

Crystal-Induced Arthropathies

Although 29% of patients with SLE are hyperuricemic (usually secondary to nephritis, diuretics, or chemotherapy), clinical gout is rare (166). This could result from the predominance of menstruating females among those with active SLE. Until 2000, fewer than 20 cases have been described in the literature (167 ,168 ,169 ,170 ,171 ,172 ,173 ,174); most were males taking diuretics. Recently, two groups examined the clinical features of 15 and 10 patients, respectively, with gout and SLE. Over 90% had nephritis, many had been transplanted, were on diuretics and cyclosporine, and the lupus was almost always inactive (175 ,176). Wallace et al. (177) reviewed the negative association between gout and RA. Only 3 of their 464 patients with idiopathic SLE had clinical gout, including a 25-year-old woman with nephritis who had tophaceous deposits. One report reviewed three young women with SLE and tophaceous deposits; all were underexcretors of uric acid (178). It has been proposed that patients with SLE (who often have decreased synovial fluid complement levels) have a natural barrier to gout, because urate requires the presence of near-normal synovial fluid complement levels to induce inflammation (168).

The rarity of pseudogout in patients with SLE has been reviewed by Rodriquez et al. (179).

Fibromyalgia

See Chapter 63 .

Dermatitis Herpetiformis

Thomas and Su (180) found nine patients with concomitant dermatitis herpetiformis and SLE who were followed at the Mayo Clinic from 1950 to 1981 and reviewed the literature. Five other reports have appeared, the most important of which are those of Aronson et al. (181) and Davies et al. (182).

Sarcoidosis

SLE and sarcoidosis share many immunologic features (183). Both manifest hyperglobulinemia, decreased skin test and lymphocyte responsiveness, lymphopenia, impaired antibody-dependent cellular cytotoxicity, and increased levels of circulating immune complexes (CICs). Cryoglobulins and antilymphocyte antibodies may be present in both disorders, and up to 32% of patients with sarcoidosis may have a positive ANA. Differential diagnosis can be a problem (184 ,185), but despite these similarities, only eleven cases of co-existence have been reported in the English-language literature (183 ,184 ,186 ,187 ,188 ,189 ,190 ,191 ,192).

Amyloid

It would be expected that patients with SLE have an increased incidence of amyloid, as do those with RA or ankylosing spondylitis. Cathcart and Scheinberg (193) enumerated many reasons why SLE and amyloid should co-exist. For example, both have a common pathogenetic pathway, and polyclonal B-cell proliferation is seen in both. Benson and Cohen (194) found serum levels of amyloid protein A to be elevated in 25 cases of active SLE (although these were one half the levels seen in an RA group). This α-globulin is a precursor of the major protein constituent of secondary amyloid fibrils. Serum-amyloid P component also can be deposited in lupus tissues without evidence of clinical amyloid (195 ,196), and may be protective against lupus (197 ,198). Despite this, fewer than 20 cases of the co-existence of SLE and amyloidosis have been reported (199 ,200 ,201 ,202 ,203 ,204 ,205 ,206 ,207 ,208 ,209 ,210 ,211 ,212 ,213 ,214 ,215 ,216 ,217 ,218).

Seronegative Spondyloarthropathies and Psoriasis

Nashel et al. (219) estimated that 500 concurrent cases of ankylosing spondylitis (AS) and SLE should be present in the United States, but this figure does not take into account the differences in catchment groups (AS–white males; SLE–females, especially nonwhites). They presented the first true case of co-existence and reviewed three cases reported earlier. None of these met both AS and SLE criteria, but one since has appeared (220). Kappes et al. (221) noted the difficulty in differential diagnosis, because patients with SLE may have sacroiliitis by bone scan and be HLA-B27 positive. Only one case of SLE and reactive arthritis and one case of discoid lupus in AS has been reported (222 ,223).

Several reviews have drawn attention to the co-existence of psoriasis and SLE (224 ,225 ,226 ,227 ,228). A 1980 report presented 23 cases of co-existence at the Mayo Clinic (10 met ACR criteria for SLE, and 13 had DLE) between 1950 and 1975 and reviewed 15 reports of 33 cases (11 of which antedated 1960) (226). Of these, 63% were female, SLE and psoriasis each appeared first one half of the time, and 80% had discoid lesions that usually were distinct from psoriatic patches (appearing and disappearing independently), but 7 of 27 biopsied lesions had pathologic features of both disorders. DLE can be misdiagnosed as psoriasis (229), may flare with ultraviolet B or psoralen ultraviolet A (PUVA) therapy (224 ,225), and SCLE can be induced during PUVA treatments in patients who are Ro/SSA positive (227 ,228). Despite the not uncommon concurrence of LE and psoriasis, only one case of psoriatic arthritis and SLE has been reported, and no HLA studies were cited in any of these reports (230).

Association of Systemic Lupus Erythematosus with Other Disorders

Several disorders have increased or decreased associations with SLE, and others can mimic its presentation and must be considered in the differential diagnosis. The relationship among Raynaud phenomenon, hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome, biliary

cirrhosis multiple sclerosis, myasthenia gravis, thyroiditis, inflammatory- bowel disease, syphilis, Klinefelter syndrome, sickle cell anemia, autoimmune hemolytic anemia, Sjögren syndrome, thrombocytopenic purpura, pemphigus, chronic active hepatitis, and SLE are discussed in other chapters (see the index for specific listings). Additional associations and differential diagnostic considerations are reviewed here. The reader is referred to an excellent discussion by Lorber et al. (230) regarding the rationale for such associations.

Porphyria

Both porphyria and SLE are characterized by fever, rash, sun sensitivity, leukopenia, anemia, arthralgias, and CNS abnormalities. Although almost 50 concurrent cases have been reported, many of these patients have not fulfilled the established criteria for SLE, and almost all of their symptoms could be explained by porphyria alone (231 ,232 ,233 ,234 ,235). Two comprehensive evaluations of 55 and 158 patients with porphyria cutanea tarda patients (233 ,234) found that none met the ACR criteria for SLE, although 12 were ANA positive. One review of 38 patients with porphyria (189) found that 8 of 15 with acute intermittent porphyria were ANA positive; one had SLE. Filotou reviewed 9 cases of acute intermittent porphyria in the literature; 8 had pre-existing SLE (236).Gibson and McEvoy found that 15 of 676 patients with porphyria had concurrent SLE. Nine had discoid lupus, 5 had SLE, and 1 had SCLE. Porphyria was precipitated by hydroxychloroquine therapy in 2 (237). Our group has had 2 concurrent cases among 2,000 lupus patients in 20 years. The ability of chloroquine to induce cutaneous porphyria further complicates the differential diagnosis.

Angioimmunoblastic Lymphadenopathy with Dysproteinemia and Autoimmune Proliferative Syndrome

Angio-immunoblastic lymphadenopathy with dysproteinemia (AILD) is a hyperimmune state that presents with rash, polyclonal gammopathy, Coombs-positive hemolytic anemia, hepatosplenomegaly, anergy, and decreased T-cell suppressor levels. It is fatal within months without treatment. AILD can resemble SLE (238 ,239 ,240 ,241 ,242) in that sicca syndrome, symmetric peripheral polyarthritis, and positive serologies can be observed (243 ,244). In their literature review, Rosenstein et al. (242) discussed several patients who followed the pattern of having an established autoimmune disease terminate with AILD, and they speculated that it represents a malignant transformation of immune-mediated disorders. Patients with autoimmune proliferative syndrome (e.g., ALS), characterized by a defect of the Fas-mediated apoptosis pathway, are usually children. Most have antinuclear and antiphospholipid antibodies (245 ,246).

Carcinoma

Chapter 69 discusses the occurrence of malignancies in those with SLE. The initial presentation of a patient with fevers, weight loss, adenopathy, and joint pains requires consideration of autoimmune and malignant disorders. Renal cell carcinomas can present with necrotizing vasculitis, Raynaud phenomenon, cryoglobulinemia, positive ANA, false-positive syphilis serologies, and elevated levels of CICs (247 ,248 ,249). Resection of the tumor usually reverses these findings. Mycosis fungoides can mimic chronic cutaneous lupus (250). A case of a woman with breast carcinoma and postradiation pneumonitis and serositis with a positive ANA and LE-cell preparation that disappeared after corticosteroid therapy also has been reported. Other malignancies are associated with ANAs (251). ANAs were detected in 27.7% of 274 Spanish patients with malignancies, but in only 6.4% of healthy subjects (252). Paraneoplastic rheumatic symptoms were seen more often in those who were ANA positive. For example, 31% of 204 patients with hepatocellular carcinoma had a positive ANA (253). Patients with immunoblastic sarcoma, lymphoma (254 ,255 ,256), Burkitt lymphoma (257), hairy-cell leukemia (258), ovarian carcinoma (259), adrenal adenoma (260), myelodysplastic syndromes (261 ,262), and Meigs syndrome (263) were thought to have SLE on initial presentation.

Tumor-associated antigen CA 19–9, which is a fairly specific marker for gastrointestinal adenocarcinomas, was positive in 6 of 19 patients with SLE in one report (264), and CA 125 in active SLE in another study (265).

Infectious Diseases

Chapter 45 discusses the propensity of patients with SLE to develop infections, and specific infectious associations with the disease. Problems relating to differential diagnosis are presented here. Additionally, a variety of infections (e.g., toxoplasmosis, schistosomiasis, leishmaniasis) can present as SLE with autoantibodies (266 ,267 ,268 ,269).

Leprosy

Leprosy rarely occurs in association with SLE (270 ,271 ,272), but the presence of deforming arthritis, alopecia, rash, and neuropathy in both conditions can make the differential diagnosis confusing (273 ,274 ,275 ,276 ,277). A positive ANA or rheumatoid factor is found in 3% to 36% of leprosy cohorts, but other antibody systems are absent (273 ,274 ,275 ,276 ,277). Mackworth-Young et al. (278) have found a common idiotypic determinant that is shared by patients with SLE and lepromatous leprosy.

Tuberculosis

Tuberculosis and SLE have overlapping chest and CNS features, as well as symptoms of fever, malaise, and weight loss (279). Feng and Tan (280) found concurrent tuberculosis in

16 of 311 patients with SLE (5%) who were seen in Singapore between 1963 and 1979, Tan et al. in 11% of 526 Hong Kong and 3.6% of 556 Turkish patients (281 ,282 ,283). The coexistence of tuberculosis correlated with steroid dosing and renal involvement and was frequently extrapulmonary (283). Isoniazid prophylaxis is safe and effective (284 ,285).

Viral Infections

Viral infections may display overlapping features with those of lupus on initial presentation, including intense fatigue. The chronicity of certain viral infections, such as the Epstein-Barr virus (EBV), herpesvirus cytomegalovirus, and viral hepatitis in young women, as well as the tendency of patients with SLE to develop infections, makes this a complex issue (286 ,287). Infections with these viruses can induce a low-titer ANA, anticardiolipin antibody, RF, anti-DNA and cryoglobulin among others (288 ,289). Similarly, SLE may be associated with IgM antiviral antibodies (290 ,291 ,292).

Increased antibody titers to EBV capsid antigen, early antigen, and nuclear antigen and by PCR compared with those of controls have been noted in patients with SLE (293 ,294 ,295 ,296 ,297), and false-positive Monospot test results have been reported (298). Harley's group has suggested that EBV can induce lupus (299) and that nearly all lupus patients have seroconverted. Nearly all adults with SLE (195 of 196) in one study were exposed to EBV (300). $CD8^+$ T cells may defectively regulate viral loads in SLE (301). Fevers, fatigue, adenopathy, and leucopenia can represent EBV and/or SLE, especially in adolescents (302).

Surveys have shown that 5% to 69% with SLE are reactive to cytomegalovirus antibodies representing viral induction or activation of SLE, simultaneous disease, or an immune suppressive mediated viral illness (300 ,303 ,304 ,305). The high prevalence of varicella zoster in lupus is probably associated with reduced CD4 T cell responses to the virus (306). A study of 44 patients with parvovirus B19 infection demonstrated an association with a transient, subclinical autoimmune state, complete with expression of anti-nDNA and antilymphocyte antibodies in most patients (307). This can be confused with SLE or may co-exist or flare it (308 ,309 ,310 ,311 ,312 ,313 ,314 ,315 ,316 ,317 ,318 ,319 ,320 ,321 ,322).

Winfield's group reported that the IgM in sera from children with acute infectious mononucleosis and hepatitis A is reactive with different antibody epitopes than from those with SLE (323). The measles virus genome along with elevated antibody titers has been found in lupus nephritis patients (324). See Chapter 45 for a more complete discussion of infections in lupus.

Human Immunodeficiency Virus and AIDS

The presentation of human immunodeficiency virus (HIV) infection can mimic that of autoimmune phenomena (325 ,326). Fevers, lymphadenopathy, rash, renal dysfunction, neurologic and hematologic disorders, sicca syndrome, and polyarthralgias can be observed. HIV positivity is associated with the presence of the lupus circulating anticoagulant (although thrombosis does not occur), hemolytic anemia, ANA, RF, CICs, immune thrombocytopenia, polyclonal hyperglobulinemia, and leukopenia. Anti-Ro/SSA and anti-La/SSB are not seen (327 ,328 ,329 ,330 ,331).

Barthels and Wallace discussed two cases, reviewed the literature, and presented an algorithm for following SLE patients with false-positive AIDS testing (332). Approximately 40 cases of concurrent AIDS and SLE have been presented (326 ,333 ,334 ,335 ,336 ,337 ,338). Of the 30 cases that were reported prior to 2002 and reviewed by Palacios et al. and Daikh et al., only 18 fulfilled the ACR criteria for SLE (339 ,340). Interestingly, about half are males, have nephritis, are children (especially with congenital acquired immune deficiency syndrome [AIDS]), and are African American. In a fascinating report, Fox and Isenberg followed a lupus patient who was infected with HIV while under their care (341). Aggressive antiretroviral therapy can re-activate lupus (342 ,343), whereas cyclophosphamide can reactivate HIV (344). Stored sera showed the precise time of HIV seroconversion, which resulted in clinical improvement and the disappearance of autoantibodies. Kaye (345) hypothesized that SLE somehow may be protective of AIDS. Assuming that 500,000 Americans have SLE and that 150,000 have AIDS, at least 400 concurrent cases would be expected. This negative correlation becomes more impressive when one considers that if 10% of patients with SLE had autoimmune hemolytic anemia or other complications that required transfusions (e.g., uremia, surgery) between 1978 and 1983, when the U.S. blood supply was unsafe, up to 50,000 should have been at risk of becoming infected with HIV (345), but not a single report has stated that any converted to HIV seropositivity (346).

Approximately 10% to 20% of patients with SLE will have intederminant reactivity patterns against various glycoproteins that are associated with HIV-1, human T-cell lymphotropic virus (HTLV)-1, and HTLV-2 (347 ,348 ,349 ,350 ,351 ,352 ,353 ,354). Occasional reports of concurrent disease have appeared (355 ,356 ,357 ,358 ,359); lupus activity may be suppressed (360).

Interestingly, many patients with HIV infections have antibodies to RNP. It has been suggested that immunization with anti-U1 snRNP potentially can block HIV infectivity (361 ,362). High IL-16 levels associated with SLE also might be protective (363).

Miscellaneous Disorders

Skin lesions of chronic granulomatous disease can mimic those of DLE (364 ,365 ,366 ,367 ,368 ,369) and co-exist with SLE (370 ,371). Thallium poisoning can result in ANA formation and mimic SLE (372 ,373). Down syndrome is associated with an inflammatory arthropathy that sometimes resembles SLE (374 ,375 ,376 ,377) and can co-exist with it. Two cases of lysinuric protein intolerance (378 ,379), Moyamoya disease (380 ,381), and prolidase (382) with SLE have been reported. Cases of Hunter syndrome (383), Osler-Weber-Rendu (384),

amyotrophic lateral sclerosis (385), Fabry disease (386), Werner syndrome (387), Noonan syndrome, Wilson disease, Hermanky-Pudlak, osteopoikilosis, stiff person syndrome, hemophilia A, Rosai-Dorfman disease, autoimmune neuromyotonia, and multicentric reticulohistiocytosis (388 ,389 ,390 ,391 ,392 ,393 ,394 ,395 ,396 ,397 ,398) with SLE have appeared.

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Chapter 50
Mixed Connective-Tissue Disease and Overlap Syndromes

Robert W. Hoffman

Mixed Connective Tissue Disease

Historical Perspective

The first full-length publication describing what the authors called “mixed connective-tissue disease” (MCTD) was reported by Sharp et al. in 1972 (1). The patients described by Sharp et al. were proposed to be distinct based upon the presence of high levels of antibodies against an extractable nuclear antigen (ENA) that was RNase- and trypsin-sensitive. Subsequently, it was shown that ENA contained both the RNase- and trypsin-sensitive ribonucleoprotein (RNP) antigen, and the RNase- and trypsin-resistant Smith (Sm) antigen (2 ,3). We now know that the RNP antigen consists of a complex containing a series of small nuclear ribonucleoproteins (snRNP) including three polypeptides (70kD, A, and C) that associate noncovalently with U1RNA as part of the spliceosome complex (4 ,5 ,6). The spliceosome is found in the nucleus of eukaryotic cells and has the physiologic function of assisting in the excision of introns and the processing of premessenger RNA to mature messenger RNA (5). The RNP antigen is also known by a variety of other names including nuclear RNP (nRNP), U1-snRNP and U1RNP (6).

Clinically, the patients initially reported by Sharp et al. from Stanford were described as having overlapping features of SLE, scleroderma, and polymyositis (1). The patients were felt to be distinctive based on the absence of serious renal or central nervous system (CNS) involvement, and their favorable clinical response to treatment with corticosteroids (1). The initial studies on MCTD that were begun at Stanford continued at the University of Missouri-Columbia by Sharp et al. beginning in 1969. Sharp's collaborative studies with other centers resulted in a seminal paper in 1976 describing MCTD patients identified from five academic medical centers, including the University of Missouri-Columbia, Stanford University, the Mayo Clinic, the University of Cincinnati, and Northwestern University (7). Numerous studies on MCTD by Sharp et al. followed in the ensuing decades from the University of Missouri-Columbia, including prospective longitudinal studies on a large cohort of patients, some of whom had been followed for as long as 30 years (7 ,8 ,9 ,10 ,11 ,12). Dr. Sharp has recently published a historical review describing the collective body of this work (13). It should also be acknowledged that a number of investigators from the United States, Japan, Mexico, and Europe have also made important contributions to defining and further characterizing MCTD. These include Drs. Donato Alarcon-Segovia, Robert Bennett, Evelyn Hess, Eva Hedfors, Halsted Holman, Mitsuo Homma, Reiji Kasukawa, Ingvar Pettersson, Morris Reichlin, Bernard Singsen, Eng Tan and their colleagues, to mention only a few (1 ,4 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23). The work of these individuals and others has substantially advanced our understanding of the clinical, immunologic, and genetic features of MCTD since its original description, now over three decades ago.

Definition

Four widely recognized criteria for classification of patients as MCTD have been published (22 ,23 ,24 ,25 ,26 ,27 ,28 ,29). Some authors currently favor the criteria proposed by Alarcon-Segovia and Villarreal, and later validated by Alarcon-Segovia and Cardiel, based on its simplicity and perceived general applicability (22 ,23). Table 50-1 shows this proposed classification algorithm. The other published classification criteria are substantially more cumbersome to apply outside of a clinical research setting. Unfortunately, there has been no international consensus conference that has addressed the topic of disease classification criteria in MCTD since the international conference on MCTD held in Japan in 1986 (18).

It is important to acknowledge that controversy does exist in the literature over nomenclature of MCTD (10 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38). Although most critics accept that a recognizable group of patients as those called “MCTD” exist, there is dispute over the nomenclature and whether MCTD should be considered a distinct disease rather than a syndrome on

the continuum of another rheumatic disease, such as SLE or scleroderma (30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38).

Table 50-1: Diagnostic Criteria for Mixed Connective Tissue Disease*

Serologic criteria
Anti-RNP antibody must be present at a moderate-high level in serum
AND
Clinical criteria
There must be at least three out of the five of the following clinical findings
Edema of the hand
Synovitis
Myositis
Raynaud
Acrosclerosis
AND this must include either synovitis or myositis

*Alarcon-Segovia and Villarreal
**Anti-RNP was defined in this study using hemagglutination. A titer equal to or greater than 1:1,600 was required in their study to be considered “moderate or high.”

Following the initial description of MCTD by Sharp et al., the concept of MCTD clearly has evolved based upon the work of Dr. Sharp and many other investigators (10). In the last two decades, considerable advances have been made in two areas that assist investigators in the classification of rheumatic disease patients; these are characterization of autoantibodies and genetic markers of the rheumatic diseases (39). Studies using these have been of paramount importance in advancing the classification of all systemic rheumatic diseases, including MCTD (39). In MCTD, for example, it has been shown that the frequency of HLA-DR4 is increased in patients compared to health controls in population- based studies done on several continents, including North American, South America, and Europe (10 ,40 ,41 ,42 ,43 ,44 ,45). It has been proposed that as newer classification criteria of MCTD are developed, these should take advantage of advances in serologic and genetic markers that might be particularly useful in the classification of MCTD. These might include serologic testing for antibodies reactive with the U1–70kD polypeptide of the RNP antigen and molecular genotyping for the presence of HLA haplotypes associated with MCTD (e.g., HLA-DR4 bearing haplotypes) (10 ,39 ,40 ,41 ,44 ,45).

Finally, recent studies on disease pathogenesis serve to refine and more clearly delineate our understanding of MCTD. For example, studies of autoantibodies in disease pathogenesis have advanced our understanding of MCTD and recent studies in an animal model support a direct role for immunity against the U1–70kD polypeptide of the RNP antigen in disease pathogenesis. Studies in murine models have demonstrated that a single immunization with autologous U1–70kD polypeptide of the RNP antigen plus U1RNA can induce anti-RNP immunity and autoimmune lung disease characteristic of MCTD (46 ,47). Disease pathogenesis and animal models are discussed in greater detail in the section that follows (see Pathogenesis).

Clinical Features

General Features

In the absence of a universally accepted classification criteria and with our understanding of MCTD having evolved over that past three decades, review of the literature on MCTD poses some challenges. This is particular true for rare complications of the disease described as case reports or small series of patients in the literature. Despite these challenges, there are now numerous well-characterized clinical series reporting on substantial numbers of patients from across the world that define the clinical features of MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Table 50-2 shows a summary of the most common clinical features of MCTD.

The primary clinical features of MCTD are Raynaud phenomenon, swollen fingers or hands, arthralgia, with or without associated arthritis, esophageal reflux or dysmotility, acrosclerosis (i.e., sclerodactyly), mild myositis, and pulmonary involvement of a variety of forms (see Table 50-2) (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Additional clinical features that have been commonly reported include: malar rash, alopecia, anemia, leucopenia, lymphadenopathy, and trigeminal neuralgia. The characteristic serologic findings are a high-titer FANA with a speckled pattern and the presence of antibodies to RNP at moderate to high levels in the serum; some authors require the absence of antibodies to Sm to classify patients as MCTD (10).

Table 50-2: Clinical Features of Mixed Connective Tissue Disease*

Clinical Findings	At Diagnosis (%)	Cumulative Feature (%)
Raynaud phenomenon	89	96
Arthralgia/arthritis	85	96
Swollen hands	60	66
Esophageal dysmotility	47	66
Pulmonary disease	43	66
Sclerodactyly	34	49
Pleuritis/pericarditis	34	43
Skin rash	30	53
Myositis	28	51
Renal disease	2	11
Neurologic disease	0	17

*Burdt et al. (9)

Prevalence

Currently, there is a paucity of epidemiologic data on MCTD. A nationwide multicenter collaborative survey on MCTD from Japan reported a prevalence of 2.7% (51). It has been reported by Sharp et al. that at a tertiary referral center known for expertise in MCTD, the disease was seen less frequently than SLE or rheumatoid arthritis (RA), but more commonly that polymyositis, dermatomyositis, or scleroderma (10).

Sex Distribution

MCTD is more common in females than it is in males. It appears to have a sex distribution similar to that seen in SLE (10). In a Japanese nationwide survey, MCTD was found to have a female to male ratio of 16:1 (51), whereas the longitudinal prospective clinical series of Burdt et al. reported an 11:1 ratio of women to men among patient from a tertiary referral center in the Midwestern United States (9). Lundberg and Hedfors reported a 4:1 ratio among patients selected for anti-RNP antibodies rather than MCTD per se who were studied at Huddinge University hospital in Stockholm, Sweden (15).

Skin

Raynaud phenomenon is one of the most common manifestations of MTCD in all clinical series. It has been reported to be present at diagnosis in 90% to 95% of patients (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). In some patients it may diminish in severity or resolve over time (9). There are a small number of patients who will have associated digital infarcts. Pathologic and radiographic studies have reported the presence of an obliterative vasculopathy in these patients (55). Digital infarcts appear to correlate with the presence of severe obliterative vasculopathy.

As in other organs, the vascular endothelium appears to be a major target of the pathologic process in MCTD (55).

Swollen fingers or swelling of the hand is very common in MCTD, particularly at the onset of disease (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Total hand edema can occur but is less common. Acrosclerosis (also known as sclerodactyly) occurs with or without proximal scleroderma and is typically a later manifestation of the disease. Nailfold vascular changes identified by unaided direct visual inspection or by one of several methods of capillary microscopy occur in MCTD. These are characterized by vascular dilatation and vessel loss or “drop out” (56). Skin rashes are present in 50% to 60% of patients (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). A malar rash similar to that typical of SLE has been reported to be common. Discoid lesions are also occasionally present. The scleroderma-like features of squared telangiectasis over the hands, and face, periungual telangiectasis and sclerodactyly with or without calcinosis cutis also occurs in some patients with MCTD. In contrast, truncal scleroderma is rare or absent in most series (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,57 ,58).

Gottron papules or a heliotrope rash, typical of dermatomyositis, are also seen in MCTD. Erythema nodosum, hyper- or hypopigmentation of the skin is uncommon but has been reported (10 ,59 ,60). Nodules appear to be uncommon despite the fact that arthritis and rheumatoid factor are common features of the disease.

The sicca complex has been found to be present in approximately one fourth to as high as one half of all patients with MCTD (11 ,61); these patients also frequently have anti-SS-A/Ro antibodies (61). Oral ulcers are reported to be present in patients with MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Genital ulcers and more severe lesions resulting in nasal septal perforation have been described (62).

Joints

Arthralgias, along with Raynaud phenomenon, are present in almost all patients with MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,63 ,64 ,65). Arthritis is also very common in MCTD. Arthritis ultimately develops in 50% to 60% of patients (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,55 ,63 ,64 ,65 ,66 ,67). Rheumatoid factor is also common in MCTD, occurring in 50% to 75% of patients. In fact, despite the fact that MCTD was initially observed among patients with overlapping features of SLE, scleroderma, and polymyositis, it is now recognized that MCTD patients have many features in common with RA. This includes increase in the frequency of the HLA-DR4 susceptibility gene and immune responses against serum immune globulin (i.e., rheumatoid factor) and immunity against heterogeneous nuclear ribonucleoprotein (hnRNP) in the form of antibodies and T cells (68 ,69 ,70). MCTD patients may fulfill the American College of Rheumatology (ACR) classification criteria for RA (10). In contrast to RA, however, most MCTD patients have no bony erosions or only small marginal erosions with well-demarcated edges (63 ,64 ,65 ,66 ,67). Occasionally, patients will develop RA-like deformities, including boutonniere deformities and swan neck deformities. More severe, erosive arthropathy has been reported to be associated with HLA-DR4 (44). A severe destructive arthritis including arthritis multilane has been reported in MCTD (65). A Jaccoud's-like arthropathy, similar to that seen in SLE with or without erosions, has also been reported (63 ,64 ,65 ,66 ,67).

Muscles

Myalgias are common in MCTD, being reported in 25% to 50% of patients. Myositis has been reported to be present in 20% to 70% of patients (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). The majority of MCTD patient, however, are not weak (9 ,59). Mild myositis with normal or modest elevation of muscle enzymes and normal electromyogram are the findings that are most common in MCTD; patients may be completely asymptomatic (9 ,10). In contrast, however, myositis can be severe in some patients and can be indistinguishable from dermatomyositis (10). Patients such as this may meet Bohan

and Peter's criteria for classification of myositis (71). Lundberg et al. have published findings of a longitudinal study comparing myositis patients with or without RNP antibodies (60). They found that patients with anti-RNP antibodies and myositis appeared to respond quickly to treatment with corticosteroids and that their myositis rarely relapsed after initial treatment (60). The pathologic muscle finding reported in MCTD include lymphocytic infiltrates, which may be either perivascular (within the endomysium vessel wall or perimysium) focal fiber necrosis, and occasionally perifascicular atrophy (59). Patients with MCTD may develop fibromyalgia and in a some patients this can be a clinically dominant aspect of the disease (72).

Pulmonary System

Pulmonary involvement can be a serious complication of MCTD and is the most common disease-related cause of death in MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Symptoms can include cough, dyspnea on exertion or at rest, and pleuritic chest pain. The physical examination may reveal basilar rales or cardiac finding compatible with pulmonary hypertension. Often, however, the physical examination of the lungs is normal. More sensitive testing may be required to detect early pulmonary disease, such as pulmonary function testing measuring lung diffusion capacity for carbon monoxide (DLco) (8 ,9).

Decreased DLco has been shown to be a sensitive measurement for detecting pulmonary involvement in MCTD and appears to be effective when used for periodic screening of patients as an approach to identify those with early pulmonary disease (8). Some patients may have an abnormal chest radiograph or abnormal pulmonary function testing result. The most common radiographic findings are small, irregular opacities of the basilar or less commonly the middle lung fields, although changes can include interstitial abnormalities, pleural effusions, infiltrative lesions, or pleural thickening. High resolution computerized tomography of the chest may reveal finding of fibrosis or alveolitis that may not be detectable using plain film radiography of the chest. Pathologic changes that may be found on biopsy or at autopsy include: interstitial fibrosing pneumonitis, obliterative vasculopathy of pulmonary vessels with intimal proliferation and medial hypertrophy of the pulmonary arteries and arterioles with minimal or no interstitial fibrosis, plexiform lesions, and vasculitis (8 ,9 ,10 ,18).

Pulmonary hypertension is the most common disease-related cause of death in MCTD (9). In the longitudinal study of Burdt et al. 13% (6/47) of the patients died of pulmonary hypertension (9). Additionally, there is evidence in MCTD that treatment of pulmonary hypertension can result in prolonged survival in some patients; therefore, early identification and proper treatment of pulmonary hypertension is very important (see Treatment section later in the chapter) (8 ,9 ,10 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80).

Rare pulmonary manifestations that have been reported in MCTD include pulmonary hemorrhage and diaphragm dysfunction (81 ,82 ,83).

Gastrointestinal System

Esophageal motility disorders with symptomatic esophageal reflux, including heart burn or regurgitation of food, is common in MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,84 ,85 ,86 ,87 ,88 ,89 ,90). Less commonly, patients may experience difficulty in swallowing or pain. Uncommon features of gastrointestinal involvement in MCTD that have been reported include: pseudodiverticula along the antimesenteric boarder (similar to that described in scleroderma), mesenteric vasculitis, pancreatitis, bacterial overgrowth syndrome, malabsorption, protein-loosing enteropathy, pseudo-obstruction, serositis, colonic perforation and gastrointestinal bleeding (84 ,85 ,86 ,87 ,88 ,89 ,90). There are reports in the literature of chronic active hepatitis, biliary cirrhosis and Budd-Chiari syndrome in patients with MCTD.

Cardiac System

When sought using methods such as electrocardiography or echocardiography, evidence of cardiac abnormalities are reported to be common in MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,91 ,92 ,93 ,94 ,95). Approximately 20% of patients will have abnormalities when examined by using either electrocardiography or echocardiography. As discussed above, pulmonary involvement is common in MCTD and may result in cardiopulmonary disease, such as pulmonary hypertension. Pulmonary hypertension can result in associated cardiac changes, such as right ventricular hypertrophy, right atrial enlargement, intraventricular and atrioventricular electrical conduction abnormalities. Pericarditis has been reported to occur in 10% to 30% of MCTD patients (91 ,92). Myocardial involvement may be found with severe myopathy or when pulmonary hypertension is present. Additional cardiac abnormalities that have been described have included: septal hypertrophy, various left ventricular abnormalities, mitral valve prolapse, intimal hyperplasia of the coronary arteries, and endocardial abnormalities. Although atherosclerotic heart disease has now been recognized as a significant complication of other rheumatic diseases, including SLE and RA, similar findings have not yet been reported in longitudinal studies on MCTD (9).

Nervous System

The presence of neuropsychiatry manifestations of MCTD were first emphasized by Bennett and colleagues (96 ,97 ,98). They reported that over half of the 20 patients that they studied with MCTD had neuropsychiatry findings, including aseptic meningitis, psychosis, seizures, peripheral neuropathy, trigeminal neuropathy, and cerebella ataxia (96 ,97 ,98).

Trigeminal neuralgia can rarely be the presenting feature of MCTD (99 ,100). Trigeminal neuralgia can manifest as neuralgic pain or as partial or complete anesthesia over the distribution of one or more branches of the trigeminal nerve (100). Vascular headaches have been frequently described in MCTD (101). Aseptic meningitis has been reported to be associated with the use of nonsteroidal anti-inflammatory agents particularly ibuprofen in MCTD. A peripheral, predominantly sensor polyneuropathy occurs in MCTD. Although MCTD was initially described to be notable for the absence of serious neurologic involvement, we now recognize that serious neurologic disease can occur in some MCTD patients (10 ,50 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103).

Cerebellar dysfunction, psychosis and seizure have been reported to occur in MCTD. Additionally, severe neurologic problems that have been rarely reported (most often in the form of case reports) in MCTD include: cauda equina, transverse myelitis, stroke, and cerebral hemorrhage. The precise relationship of these to MCTD has not been clearly established (102 ,103).

Renal Disease

Renal involvement is present in approximately 25% of patients with MCTD (104). In those patients with renal involvement, focal proliferative glomerulonephritis is more common than diffuse proliferative glomerulonephritis (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,104 ,105 ,106 , 107 ,108 ,109). Diffuse proliferative glomerulonephritis has been reported to occur but is uncommon in MCTD. Patients with diffuse renal involvement often initially have or subsequently develop anti-Sm and/or anti-double stranded DNA (anti-dsDNA) antibodies (10 ,110 ,111). It appears that the development of anti-Sm antibodies (particularly against the Sm-D peptide) occur when there is ongoing immune spreading and more severe disease (111).

In MCTD, membranous glomerulonephritis with or without nephrotic syndrome may rarely occur. Intimal proliferation within arteries and ischemic changes have been seen in MCTD. Scleroderma-like renal crisis has also been reported to occur in MCTD (10). Patients with MCTD and concomitant Sjögren syndrome may develop interstitial nephritis and can have associated finding such as renal tubular acidosis (11).

Hematologic Disorders

Hematologic abnormalities are common in MCTD (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54 ,112 ,113 ,114 , 115). Mild lymphadenopathy occurs in approximately 25% to 50% of patients. Lymphadenopathy is often an initial feature of disease and tends to decrease over time; although it may re-appear with a flare of disease. The development of massive lymphadenopathy and pseudolymphoma has been observed.

Anemia, lymphopenia, and leukopenia are all common in MCTD, occurring 50% to 75% of patients. Antilymphocyte antibodies have been found to be common and have been reported to correlate with disease activity (10 ,112). Anemia of chronic disease is one of the most common finding in several series of MCTD. Coombs positive immune-mediated hemolytic anemia has been reported to be rare feature of MCTD (113).

Thrombocytopenia occurs in MCTD but appears less commonly that leucopenia or anemia (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Finally, there are case reports describing idiopathic thrombocytopenic purpura (ITP), red cell aplasia, and thrombotic thrombocytopenic purpura (TTP) in MCTD (114). Coagulation abnormalities appear to be rare but have been described.

Miscellaneous Systemic Features

Malaise and low-grade fever may occur in MCTD. The disease has been reported to present as fever of unknown origin (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Rarely, patients may have high-grade fever without an identifiable infectious agent as the cause of the fever. In these patients it has been inferred that MCTD was the cause of their fever, however, it is important to recognize that a careful search for infection should always be completed before fever is attributed to the underlying disease (116). Orofacial and ocular vascular involvement have been described in MCTD (117 ,118).

Sicca symptoms are common in MCTD and Sjögren syndrome is present in 25% to 50% of patients (11 ,61). Patients frequently will have antibodies to SSA/Ro and these may develop after the onset of MCTD. Anti-SSB/La is found in a small number of MCTD patients occurring in less than 5% (11). Photosensitivity and malar rash appear to be increased among MCTD patients who are positive for SSA/Ro antibody (11). Autoimmune thyroiditis and persistent hoarseness have also been described in MCTD.

Children

MCTD in a child was described within 1 year following the initial report of MCTD in adults (119). Early studies in children helped establish that the disease could occur in any age group and the examination of pathologic material from children who had died as a result of the disease provided some of the earliest insights into the vascular proliferative lesions that are fundamental to the immunopathologic process underlying MCTD (120 ,121). We have gained a more complete understanding of MCTD in children over the past three decades, as clinical series containing larger numbers of children with increasing length of follow-up have been published (120). Taken together, these studies demonstrate that MCTD can present at any age and that MCTD in children appears to have clinical manifestations of disease and disease outcome similar to that seen in adults (9 ,110 ,120).

The initial report of MCTD in a child was publishing in 1973 by Sanders et al. (119). Subsequently, Singsen et al.

published a seminal paper on MCTD describing the disease in 14 children (21). These children were characterized clinically as having arthritis, Raynaud phenomenon, sclerodermatous skin findings, fever, abnormal esophageal motility, and evidence of myositis (21). Serologically these children had high levels of antinuclear antibodies exhibiting a speckled patter and high levels of antibodies against ENA/RNP as measured by hemagglutination with their specificity against RNP confirmed using immunodiffusion (21).

These early clinical and serologic studies established that the newly recognized disease also occurred in children (21 ,49 ,119). In contrast to most of the outcome studies reported recently, these initial studies from Singsen et al. at the Los Angeles Children's Hospital found that renal involvement and thrombocytopenia appeared to be common, and that the prognosis was unfavorable in a significant number of the small group of children they had studied (21 ,49). Recent studies have challenged these initial observations that MCTD is different in children, although some studies from Japan have also reported that MCTD in that country may have a worse prognosis when the disease has its onset in childhood (9 ,110 ,120). Subsequent longitudinal studies from Europe and the United States, however, have found childhood MCTD to have the same core clinical manifestation as seen in adults, including Raynaud phenomenon, swollen hands, arthralgia, arthritis, mild myositis, telangiectasias, and sclerodactyly (9 ,110 ,120). These studies have also reported that MCTD in children has a relatively favorable outcome in approximate 70% of patients, with 5% to 20% having complete remission of their disease following treatment (9 ,110 ,120).

As in adults, pulmonary involvement appeared to be an important feature of the disease in children and the development of pulmonary hypertension has been reported in two series from the United States and in one series of patients from Japan (29). Thrombocytopenia, as initially reported by Singsen et al., has not been found to be clinically significant in other series (21 ,110 ,120). Infection complicating the disease has been reported as a major cause of death in MTCD (9 ,10 ,116). Coexisting sicca syndrome has been reported by some to be common in children with MCTD and there are rare patients reported with neurologic involvement in MCTD, which can be severe (61 ,120).

Pregnancy

A small number of published studies have examined the effects of MCTD on pregnancy (122 ,123 ,124). Lundberg and Hedfors have retrospectively examined pregnancy outcome in 40 pregnancies among 20 patients with high-titer RNP antibodies from Stockholm, Sweden (122). They found fetal and maternal outcome was excellent with no evidence of flares of disease during pregnancy or in the postpartum period (122). These finding were in contrast to somewhat less favorable outcome for pregnancy in MCTD reported by Kaufman and Kitridou from Los Angeles, where they found parity was decreased and fetal wastage was increased similarly among patients with either MCTD or SLE (123 ,124). Overall, the outcome of pregnancy in MCTD with careful clinical monitoring at centers with experience in managing MCTD appears highly favorable (122, Hoffman RW- personal observation).

Serologic and Immunology Studies

Antinuclear and Anti-RNP Antibodies

Patients with MCTD have antinuclear antibodies against a series of nuclear antigens. The presence of antibodies against U1RNP is required for the diagnosis by its original definition and subsequently proposed classification criteria (1 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,126 ,127 ,128 ,129). The typically MCTD patient will have high titers of fluorescent ANA exhibiting a speckled pattern of immunofluorescence with the commonly used HEp-2 cell line, as well as with other human or mouse tissue substrates (6). Patient will have antibodies against ENA and RNP, which typically will be present at high levels in untreated patients at the onset of disease. The development of anti-RNP antibodies occurs in close temporal relationship to the onset of clinical disease, with symptoms developing within 1 year after the appearance of this antibody in most patients (9 ,10 ,111 ,130). Although in active disease there is no close correlation between the level of anti-RNP and disease activity or severity, the level of anti-RNP antibodies may diminish or even disappear over time in some patients with treatment, particularly in those treated with cytotoxic drugs such as cyclophosphamide (9).

To fully understand the complex nature of autoantibodies found in MCTD and SLE, it is useful to review the structure of the spliceosome and its major components. The spliceosome contains a series of snRNP associated with a series of uridylic acid rich (U) RNAs. In the normal cell these are involved in the complex process of excising introns during the processing of premessenger RNA to the final mature messenger RNA molecules (5).

The primary antigenic components of the spliceosome include uridylic acid rich (U) RNAs (U1, U2, U4/6, and U5), which are associated with snRNP peptides. The snRNP complex contains both the Sm and RNP antigens (2 ,3). The RNP antigen consists of three polypeptides (70kD, A, and C) noncovalently associated with U1RNA; this is illustrated in Fig. 50-1 . The Sm antigen consists of 8 polypeptides (Sm-D1–3, B1–2, E, F, and G) that are noncovalently associated with U1, U2, U4/U6, and U5 RNA (4 ,131). The primary reactivity found in MCTD is with the U1–70kD polypeptide (previously called the 68kD polypeptide) (6 ,10 ,111), although patients sera may react with the U1-A or less commonly with the U1-C polypeptide of RNP (10 ,111). An individual patient's sera may react with some or all of the U1-associated polypeptides. At the onset of disease it has been reported that the most common reactivity is with the 70kD polypeptide or with the A polypeptide and that over time there is immune

spreading to other components of the complex (111). In longstanding disease, especially in patients who have received cytotoxic drug therapies such as cyclophosphamide, there may be so-called epitope contractions where antibody reactivity with one or more of these snRNP polypeptides deceases or disappears (9). There are a number of studies that have found that serologic reactivity with the U1–70kD polypeptide is closely associated with clinical finding of MCTD and is superior to measuring antibodies to RNP alone (10 ,40).

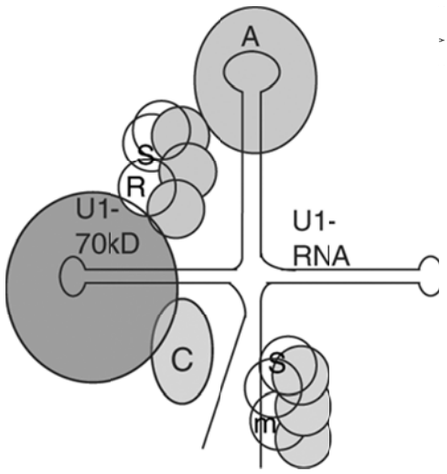

Figure 50-1. This illustration shows the 70kD polypeptide bound to U1-RNA. Also shown are the other U1-associated polypeptides, A & C which form the complex. The Sm polypeptides B, D, E, F, & G, which bind to U1, U2, U3, and U4/6 RNA, are also illustrated here. (Modified from Greidinger and Hoffman (148)).

In contrast to the reactivity with RNP typically found in MCTD, the snRNP antibody reactivity characteristically seen in patients with SLE is directed against the Sm antigen and its associated polypeptides. Approximately one third of patients classified as SLE will develop anti-Sm antibodies. The most prevalent antibodies found in sera that are reactive with the Sm antigen are directed against the Sm-B and Sm-D polypeptides, although reactivity with the other Sm polypeptides (Sm-E, F, and G) has been described (130). It appears that immune spreading to develop reactivity with the Sm-D polypeptides occurs later in the anti-snRNP immune response. The presence of anti-Sm-D antibodies is associated with the presence of renal disease and a worse clinical outcome (110).

There is a subset of patients with reactivity to the U1-specific polypeptides (including 70kD) as well as with the U1- to U6-associated Sm-B and Sm-D polypeptides at the time of initial presentation. Also, some patients that initially only have reactivity with the U1-specific polypeptides may have immune spreading and develop antibodies against the Sm-B and/or Sm-D polypeptides while under observation. As discussed above, such patients may develop more typical SLE-like clinical manifestations, have significant renal involvement and a worse prognosis (10 ,110).

There is a small group of patients that exhibit reactivity with both Sm and RNP at the time of diagnosis. Using some classification criteria the presence of antibodies against Sm disqualifies these patients as being classified as MCTD (10). One classification criteria proposed by Sharp et al. would eliminate any patient with significant levels of antibodies against Sm or dsDNA from being classified as MCTD (10). It appears that patients with anti-Sm antibodies are at increased risk for the development of renal involvement and may have a different major histocompatability genotype (HLA-DR2) than patients with isolated reactivity to RNP (HLA-DR4) (40 ,41 ,110).

Thus, there appear to be both genetic and clinical differences between patient that have immune reactivity limited to U1RNP (particularly U1–70kD) compare to those that exhibit immune spreading and develop immune reactivity against both U1RNP and Sm polypeptides.

Immune self-reactivity with U1RNA, rheumatoid factor, SSA/Ro, and viruses Antibodies against U1RNA are common in MCTD (12). Antibodies levels against U1RNA have been described as correlating with disease activity in at least one study, although others have not been able to confirm such a strong correlation between these (9 ,12 ,131). Interestingly, the RNA binding domain on the 70kD polypeptide has been identified as the dominant T-cell epitope recognized by MCTD patients; suggesting a link between B- and T-cell responses against U1RNA (132 ,133 ,134 ,135 ,136 ,137 ,138 ,139). It has been shown recently that U1RNA can activate murine and human cells through triggering innate immune receptors, including Toll-like receptor 3 (TLR3) and possibly others (140). These emerging studies support the broader concept that autoimmune diseases may have defects in both the acquired and innate arms of the immune system. Perhaps both are important in triggering and/or sustaining systemic autoimmune disease (140).

Rheumatoid factor is common in MCTD, occurring in 50% to 75% of patients (1 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,40 ,41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53 ,54). Antibodies to SSA/Ro are also common, occurring in one third of the 55 MCTD followed in a recent longitudinal study. In that study, anti-SSA/Ro was found in 33% of patients and SSB/La in 4% (11). Antibodies against phospholipids have been detected in MCTD, occurring in 15% of patients in one study (141 ,142). Unlike SLE, however, the presence of antiphospholipid antibodies was not found to be associated with an increased risk of clotting in MCTD (141). Many of these antiphospholipid antibodies in MCTD may be directed against $ß_2$ microglobulin (142). Although not associated with clotting, their presence has been reported to be associated with an increased risk for the development of pulmonary hypertension (9).

Antibodies against viruses or their products have been examined in MCTD. A cross-reactive epitope on 70kD and certain retroviral antigens has been reported, although there is no definitive evidence for retroviral triggering of MCTD (143). There has also been association between past infection or immunization against cytomegalovirus and the development of anti-RNP antibodies in some, but not all studies, where this has been examined (144 ,145 ,146).

T cells reactive with U1–70kD and U1-A snRNP polypeptides have been identified from the peripheral blood of patients with MCTD (133 ,134 ,135 ,136 ,137 ,138 ,139). T-cell clones reactive with U1–70kD and heterogeneous nuclear ribonucleoprotein

from the peripheral blood of MCTD patients have been extensively characterized. It has been shown ex vivo that such T cells can provide help to autoantibody producing B cells. Several features of the autoantibodies identified in MCTD are characteristic of a T-cell–dependent B-cell response suggesting a central role for T cells in disease (139). This is discussed further in the section on Pathogenesis below.

Immunopathology

Autopsy studies on children reported by Singsen et al. provided some of the earliest information on the histopathology of MCTD and supported the concept that MCTD was a distinct entity (49). They found that there were widespread proliferative vascular lesions, involving small, medium, and large vessels. They reported involvement of the coronary, renal, and pulmonary arteries, as well as the aorta with endothelial proliferation and an obliterative vasculopathy (49). They found that plasmacyte containing inflammatory infiltration of tissue was common and that vasculitis was also present. Subsequent studies have confirmed and extending these early findings (18 ,49 ,59 ,98 ,147).

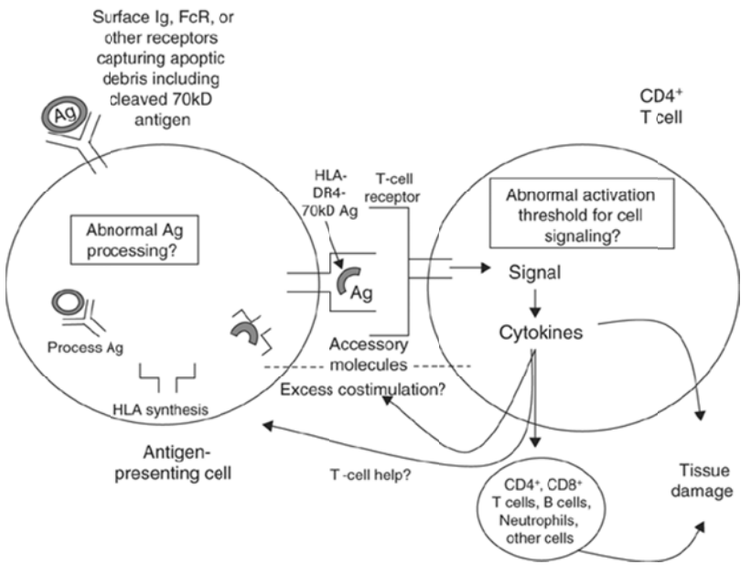

Figure 50-2. This illustrates one hypothetical model of the pathogenesis of MCTD. In this model, apoptotic material, including the 70kD polypeptide, are take up by an antigen-presenting cells (APC), processed and presented to an autoreactive T cells in the context of the major histocompatibility complex antigen HLA-DR4. CD4-positive T cells respond to antigen by producing cytokines that assist in the expansion of themselves in an autocrine fashion, as well as in the differentiation and proliferation of autoantibody producing cells. Tissue injury may be mediated directly by these cells or by soluble factors released from them. The autoreactive T cells may to have abnormal threshold for TCR triggering that renders them prone to autoimmunity. (Modified from Hoffman (139)).

Pathogenesis

An overarching model of disease pathogenesis in MCTD requires incorporation of a large amount of information on genetics, environmental influences, self-antigen modification, immune effector cells, and immunoregulation (139 ,148). Figure 50-2 shows a proposed model of disease pathogenesis in MCTD. Substantial knowledge has been gained from clinical observational studies and animal models have also helped to shape our current understanding of the pathogenesis of MCTD. Genetic factors and environmental factors contribute to susceptibility to MCTD. Immune effector mechanisms and abnormal immune regulation appear to be important features of the disease. Immune cells (T cells, B cells, antigen-presenting cells) and their products (cytokines, chemokines, antibodies) may all be important in pathogenesis. New experimental animal models are potentially instructive in identifying the molecular mechanisms of disease pathogenesis.

Genetic Factors

A useful model of genetic contributions to susceptibility to autoimmunity is the threshold liability model first proposed by Wright and as elegantly illustrated in studies dissecting the genetics of murine lupus in the New Zealand Mixed 2460 lupus-prone mice by Wakeland et al. (149 ,150 ,151). In this model, combinations of disease-susceptibility and disease-resistance genes interact with environmental factors; when a certain threshold is reached disease occurs. Environmental factors may lower the threshold for disease. In cases where the genetic burden is large enough (e.g., certain complete complement deficiencies such as C1q deficiency) an environmental trigger may not be necessary for the disease to develop (152). In MCTD major histocompatibility genes have been identified as having positive or negative associations with disease susceptibility; specifically, HLA-DR4 has been shown to be associated with anti-RNP antibody responses and with MCTD per se in a number of studies among several different populations, including patients from the United States, Mexico and Europe (10 ,39 ,40 ,41 ,42 ,43 ,44 ,45). HLA-DR2, DRw53, and DQw3 have also been reported to have positive association with MCTD (10). In contrast, the HLA class II phenotype/genotype most closely associated with scleroderma, HLA-DR5, and its subtypes, has been shown to have a negative association with MCTD (10). Regarding select immunoglobulin gene repertoire or regulation, immunoglobulin allotypes have been found to be associated with anti-RNP responses/MCTD in some but not all studies (10).

As for other multigenetic human diseases, the genetic contributions to the development of MCTD appear complex. Although the data are limited, reports of familial MCTD are rare (153). In longitudinal studies of families of affected individuals, the presence of anti-RNP antibodies was virtually never found among unaffected family members (9). These findings are also consistent with recent studies among military personnel where the development of anti-RNP antibodies was closely associated temporally with the development of clinical disease (129).

Environmental Factors

Several environmental factors that modify disease susceptibility or trigger disease have been proposed; most convincing among these is the influence of female sex hormones as suggested by the marked female-to-male ratio of the disease and other findings. There are also studies suggesting that in some patients Epstein Barr virus, retroviruses, or a newly identified virus may play a role in triggering disease (155 ,156). Cytomegalovirus has also been suggested as being able to elicit anti-RNP antibody response in the absence of disease (144 ,145 ,146). Vinyl chloride exposure has been associated with the development of MCTD-like syndrome (156).

B Cells in Pathogenesis

The presence of antibodies against RNP is required for the diagnosis of MCTD based upon the initial description by Sharp et al. and as defined by the currently proposed classification criteria (Table 50-1). The fact that anti-RNP antibodies are present in high levels in patients sera, have undergone isotype switch to the IgG class, and that anti-RNP producing B cells exhibit nucleotide substitutions typical of an antigen-driven immune response has all been presented as evidence that anti-RNP immunity in MCTD is an antigen-driven immune process. Anti-RNP antibodies may be reactive with the U1–70kD, A, or C polypeptides of the U1-snRNP complex (9). Patients may also have antibodies against the Sm-B peptide, which can be complexed to U1 or another member of the Sm-associated U1–6 RNA. IgG antibodies directed against U1–70kD appear to be those most closely linked to disease pathogenesis (9). The presence of antibodies against multiple individual components of the U1RNA/RNP snRNP macromolecular complex and the fact that immune spreading to different components of the complex occurs is supportive of the hypothesis that MCTD is an antigen-driven immune process (111 ,139).

Although anti-RNP antibodies are required for the diagnosis of MCTD and their development is closely linked chronologically to the onset of clinical symptoms, they have not been proven to directly mediate tissue injury. Studies in the recently developed animal model of MCTD may, however, allow for this to be examined definitively (see below) (46 ,47 ,148).

T Cells and APC in Pathogenesis

T cells are believed to play a central role in the pathogenesis of MCTD (132 ,133 ,134 ,135 ,136 ,137 ,138 ,139). As discussed above, autoantibody producing B cells produce high levels of IgG antibodies and exhibit features of T-cell–derived cytokines driven affinity maturation. Epitope mapping of anti-RNP antibodies reveal that epitope spreading typical of T-cell–dependent B-cell responses is identifiable (139). T cells may also produce cytokines or other soluble factors that recruit other cells to sites of inflammation or that directly mediate tissue injury.

Antigen-specific T cell may drive both T- and B-cell responses as illustrated in the model shown (Fig. 50-2). Autoantigen reactive human T cells have been identified and characterized from the peripheral blood of MCTD patients, including T cells reactive with U1–70kD, U1-A, and hnRNP A2 (136). These T cells have been linked to the presence of autoantibody production ex vivo and have been shown to be able to provide help for autoantibody production in vitro.

There is substantial evidence suggesting that in autoimmunity T cell hyperactivity and presentation of apoptotically modified self antigen presentation may be important in pathogenesis (Fig. 50-2). Rosen et al. were the first to show that snRNP antigens (including U1–70kD) undergo

site-specific cleavage during apoptosis and are found clustered in vesicles on the cell surface where they may be accessed by immune molecules (157 ,158). It has been proposed that structural modifications of the antigens, such as could occur during apoptosis, may render them more immunogenic and be important in breaking immune tolerance (157 ,158 ,159).

Tissue Injury and Innate Immune Signaling in Pathogenesis

A central feature to immunopathogenesis in MCTD may be local factors influencing tissue injury. We know based upon pathologic studies that one of the primary targets of tissue injury is the vascular endothelium. Clinically this is demonstrated by the almost uniform presence of Raynaud phenomenon and the potentially lethal development of pulmonary hypertension that remains as the primary disease-related caused of death (9). Although antiendothelial antibodies have been described, there is very little information on how tissue specific local responses may participate in the development of pathologic lesions. This important area awaits further investigation.

Very recently the potential importance of innate immune signaling through the TLR or other innate immune signaling pathways been recognized in autoimmunity (141 ,160 ,161). The finding that the dominant T-cell epitope on U1–70kD resides entirely within the RNA-binding domain of the RNP molecule, the fact that U1RNA antibodies are tightly linked to MCTD and finally the fact that U1RNA can activate human and murine cells through TLR3 and TLR7 all suggests that the innate immune signaling is of substantially potential importance in pathogenesis in MCTD (132 ,140). Although additional work is clearly needed, the schema shown in Fig. 50-2 illustrates how this information may be unified in a single model of disease.

Experimental Animal Model of MCTD

A variety of experimental murine models of autoimmunity and normal mice that have had single genes inactivated (so called “knock-out mice”) have substantially advanced our understanding of autoimmunity (162). Although murine models of SLE have been very informative, most of these have focused on the development of anti-DNA antibodies and renal disease and do not replicate MCTD. Recently, a model of MCTD has been described that has reactivity against RNP and develops pulmonary lesions characteristic of MCTD; rather than the renal lesions that are more typical of SLE. In this model, mice that are transgenic for the HLA-DR4 susceptibility gene that were immunized with U1–70kD polypeptide plus U1RNA or complete Freund adjuvant (containing a complex mixture of antigens including RNA). In that we know that MCTD and the overlap syndromes are characterized by immune responses against ribonucleoprotein antigens and typically lack renal involvement, this model may prove to be distinctively informative for advancing our understanding of disease pathogenesis.

Course and Prognosis

There can be an evolution of MCTD from mild to more severe disease developing over time (9). Patients typically exhibit Raynaud phenomenon, arthralgia, and swollen hand with or without polyarthritis at the outset of their disease. This often leads to a diagnosis of RA, another connective tissue disease or undifferentiated connective tissue disease (see Undifferentiated Connective Tissue Disease and Overlap Syndromes) at initial presentation. Prospective studies have shown that pulmonary or esophageal dysfunction may be detectable prior to the onset of clinical symptoms when sensitive diagnostics were used, such as pulmonary diffusion capacity for carbon monoxide and esophageal manometry) (8 ,9 ,87 ,88).

Long-term outcome studies by Burdt et al. found that with treatment certain features of the disease, including arthritis, swollen hands, serositis, myositis, erythematosus skin rash, Raynaud phenomenon, and esophageal hypomotility diminished (9). In contrast, sclerodactyly, diffuse sclerosis, pulmonary dysfunction, nervous system involvement, and pulmonary hypertension were less responsive to treatment and became the dominant residual features of the disease over time (9). In those unusual patients who develop serious renal involvement their prognosis was less favorable (9). The patients who develop pulmonary hypertension had the worst prognosis and this occurred in 23% of the 47 patients followed over a period of up to 30 years (8 ,11). In patients treated with corticosteroids or corticosteroid and cyclophosphamide, some had prolonged remission of their disease and some were able to discontinue all medications. Despite prolonged remission there were patients who later had recurrence of their disease, including those who developed membranoproliferative glomerulonephritis (9 ,10). Overall, however, the majority of patients were able to lead functionally normal lives.

Treatment

There are no large controlled clinical trials in MTCD and, therefore, management must be designed using data from controlled trials of other diseases and observational studies that typically include small numbers of patients (163 ,164 ,165 ,166 ,167 ,168 ,169 ,170). There are no drugs that have been approved in the United States by the Food and Drug Administration (FDA) specifically for the treatment of MCTD.

Arthralgia and mild synovitis can be treated with nonsteroidal anti-inflammatory agents and hydroxychloroquine (169 ,170). In patients where these measures are ineffective, disease-modifying antirheumatics can be used, similar to the approach used in the treatment of RA. There are some particular issues that should be considered, however.

Because MCTD can have associated lung disease, methotrexate therapy must be monitored closely, because, in theory, this could exacerbate MCTD lung disease. As in SLE, there are concerns that antitumor necrosis therapy could potentially exacerbate disease or affect the development of anti-dsDNA antibodies and induce renal disease or CNS disease in MCTD.

Raynaud phenomenon is treated with protective measures that maintain total body warmth and prevent peripheral cooling. The use of gloves should be encouraged and may assist patients (169 ,170). Calcium channel blockers are effective at reducing the severity and frequency of episodes of Raynaud phenomenon. Other approaches have been tried, such as regional sympathectomy, although it remains unclear how effective these may be. In patients with severe Raynaud phenomenon and complications such as digital infarctions, measures used in scleroderma, such as prostaglandin therapy, may be considered. Physical and occupational therapy may be helpful to maintain mobility and facilitate function.

Esophageal reflux symptoms can be effectively controlled in most patients with proton pump inhibitors. Many patients require chronic therapy to control symptoms. Evaluation for Barrett esophagitis should be done, although the timing of such evaluation has not been clearly defined. Dilatation of the esophagus may be of benefit for patients with strictures. There are case reports of severe, refractory esophageal involvement responding to aggressive immunotherapy with corticosteroids and with cyclophosphamide (168). Diarrhea in MCTD may be related to bacterial overgrowth syndrome and can result in malabsorption.

Clinically significant myopathy in MCTD can be treated in most patients with corticosteroids. The addition of methotrexate should be considered in more severe or refractory cases (169 ,170). In individuals with elevation of the serum creatinine, but without clinical weakness, low-dose corticosteroids or no treatment may be adequate with continued monitoring. Aggressive treatment, resulting in complete normalization of the serum creatinine, may not be readily achievable in patient with myositis nor required in patients without symptoms. Fibromyalgia may be the cause of muscle pain in MCTD patients. As in other rheumatic diseases, fibromyalgia may develop later during the course of the disease (72).

Pulmonary function should be monitored in MCTD as pulmonary disease is common and is the most frequent disease associate cause of death. Annual pulmonary function testing with DLco, plain film radiograph of the chest and two-dimensional echocardiogram have been empirically recommended. High-resolution computerized tomography of the chest may be indicated when abnormalities are found on the plain film radiographs or pulmonary function testing. Right heart catheterization may be required to evaluate pulmonary hypertension when significant abnormalities are detected by echocardiography or by pulmonary imaging studies. Intensive immunosuppressive therapy, including high-dose corticosteroids and intravenous cyclophosphamide, may benefit interstitial lung disease and pulmonary hypertension in MCTD (9 ,74 ,79 ,80 ,163 ,164). Although controlled clinical trial data is lacking, it appears rational to treat pulmonary hypertension in MCTD using similar agents to those used to treat primary pulmonary hypertension including the selective use of oxygen, anticoagulants, vasodilators, and prostacyclin (80). Although in MCTD the presence of antiphospholipid antibodies appears to be less clearly linked to clotting than in SLE, their presence has been associate with the development of pulmonary hypertension (9).

Thrombocytopenia may responds to corticosteroids, however, in patients who fail to respond, intravenous immune globulin and splenectomy may be beneficial. Patients may also respond to cytotoxic therapy with cyclophosphamide or other agents (169 ,170). Sicca symptoms are common and can be treated with supportive measures of ocular lubrications, preventative dental care, lubrication for dry skin, and vaginal lubrication for dyspareunia. A trial of pilocarpine therapy may be indicated in patients with more severe symptoms and those who fail to obtain adequate relief with supportive measures alone (11 ,168). Specific treatment is typically not required for mild anemia or leucopenia.

Erectile dysfunction and altered sexual response are now recognized to be common problems, particularly in chronically ill populations. The use of sildenafil and similar compounds may benefit such patients after they have had careful evaluation. There is also some evidence from studies of systemic sclerosis that nitric oxide inhibitors may benefit Raynaud phenomenon.

Pregnancy

Patients should be counseled regarding contraception to prevent unplanned pregnancy, especially when taking medications that could be harmful to the fetus. High-risk obstetrical evaluation and care should be provided to the mother prior to and during the planned pregnancy. Attention should be given to the identification of any additional risk factors that often occur in such patients, such as the presence of anti-SSA/Ro antibodies that have been associated with congenital cardiac disease, including heart block and antiphospholipid antibodies that have been associated with increased risk for fetal loss. Most drugs used to treat MCTD are not approved for use during pregnancy.

Undifferentiated Connective TIssue Disease and Overlap Syndromes

Undifferentiated connective tissue disease is said to be present when a patient lacks adequate clinical or diagnostic features to fit a recognizable clinical syndrome. Conceivably this may range from the presence of a single clinical or laboratory finding, such as a positive antinuclear antibody test or the presence of arthralgia, to a more

complete syndrome with presence of a number of clinical and/or serologic features. The term was first used by LeRoy et al. in 1980 to describe the early phase of connective tissue diseases when the findings were nonspecific and often indistinct (171). Subsequent authors have similarly used the term UCTD, although the criteria for inclusion in later studies have not been uniform (172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181 ,182). Additionally, studies that have been reported have not always included laboratory testing for complete serologic characterization of antinuclear antibody present in the patients sera. It should be emphasized that the term “UCTD” as applied by LeRoy et al. was not intended to describe “overlap” syndrome patients who have two or more distinctly recognizable rheumatic diseases (171). Until there is more complete understanding of the pathogenesis of the rheumatic diseases and better markers that accurately predict organ damage and disease outcome, there will continue to be controversy in the area of disease classification (10).

Table 50-3: Autoantibodies Associated with Overlap Syndromes

Autoantibody Specificity	Clinical Features
tRNA synthetase	Myositis with arthritis and pulmonary involvement
PM/Scl	Overlapping features polymyositis and limited scleroderma
Ku	Polymyositis and systemic sclerosis
RNA polymerase II	SLE overlap

Another approach to classification of rheumatic disease is the use of autoantibodies as disease markers. It has been proposed that manifestations of disease in a single patient reflects the constellation of autoantibodies specifically present and that these may evolve over time (9 ,11 ,39 ,129). If in fact the presence of specific autoantibodies is linked to select aspects of disease, the classification of disease primarily based upon the autoantibodies present may be useful (39). Studies on immune spreading of autoantibodies and on epitope contraction potentially provide one explanation on how evolving and even fluctuating clinical manifestations may be linked to autoantibody production (9 ,11 ,39 ,110 ,111 ,129). Table 50-3 summarized a number of uncommon antibodies that have been associated with systemic rheumatic diseases with protean and often overlapping clinical manifestations.

Undifferentiated Connective Tissue Disease

There may be evolution of disease in a patient initially classified as UCTD into a recognizable rheumatic disease over time or the patient may remain without adequate features for classification as a well-recognized rheumatic disease (171 ,172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180 ,181). Alarcon et al. have published a series of studies examining the evolution of UCTD and classification of the rheumatic diseases (172 ,173 ,174 ,175 ,176 ,177). Their work would indicate that the 90% to 100% of patients who present initially with a well-recognized diagnosis will retain that diagnosis over time and that a moderate percentage of patients with UCTD will evolve into a recognizable disease, although many will remain classified as UCTD. Bombaridieri et al. reported that the patients with UCTD they studied did not progress into distinctly recognizable rheumatic diseases, but remained classified as UCTD when observed over time (177 ,178 ,179). Thus, it would appear that UCTD is a common rheumatic syndrome that may remain stable over time and appears to have a favorable outcome (172 ,173 ,174 ,175 ,176 ,177 ,178 ,179 ,180).

UCTD is reviewed in more detail in Chapter 49 .

Overlap Syndromes

There are a number of so-called overlap syndromes that have been described (180 ,181 ,182 ,183 ,184 ,185 ,186 ,187 ,188 ,189 ,190 ,191 ,192 ,193 ,194 ,195 ,196). Many of these are identifiable by the presence of a specific antibody. Table 50-3 shows some examples of these syndromes and their associated antibodies.

Synthetase Syndromes

Synthetase syndromes are characterized immunologically by the presence of antibodies reactive with aminoacyl transfer-RNA synthetases. The first to be reported were characterized by the presence of antibodies against the Jo-1 antigen; we now know that these autoantibodies are directed against histidyl-tRNA synthetase (188 ,189 ,190). Clinically, the patient possessing anti-Jo-1 antibodies have inflammatory muscle disease plus the presence of additional widespread connective tissue disease signs and symptoms. Patients may have Raynaud phenomenon, arthralgia, arthritis, sicca symptoms, telangiectasias, dermatomyositis-like rashes, dysphagia, and pulmonary fibrosis (188 ,189 ,190).

PM/Scl

Patients with PM/Scl antibodies are characterized by having features of both polymyositis and scleroderma (191 ,192). Additionally, the illness may include Raynaud phenomenon, tendon inflammation, and concomitant pulmonary involvement. Although sclerodactyly or mild proximal scleroderma may be present, there is the absence of widespread sclerodermatous skin involvement. However, severe renal involvement with scleroderma-like kidney disease has been described to occur in these patients.

Ku

The patients first describe has having antibodies against the Ku antigen were found to have overlapping features of

polymyositis and systemic sclerosis (192). Patients with antibodies against Ku may also have a wide range of clinical manifestations including features of SLE, MCTD, Sjögren syndrome, and systemic sclerosis (193 ,194).

RNA Polymerase II

Patients with a number of clinical syndromes can have antibodies against one or more of three RNA polymerases, including RNA polymerase I, II, and III (195 ,196). A moderate number of patients with diffuse scleroderma or less commonly patients with limited scleroderma may have antibodies against RNA polymerase I and RNA polymerase III (195). Patients classified as SLE-overlap syndrome have been reported to have antibodies against RNA polymerase II in the absence of antibodies against RNA polymerase I and III (196). These patients have been reported to frequently have antibodies reactive with other specificities including Ku, RNP, and topoisomerase I, in addition to antibodies to RNA polymerase II (196). This finding is consistent with the concept of epitope spreading against multiple antigenic determinants that may develop over time in some patients, as discussed above.

Coexisting Rheumatic Diseases

A patient may simultaneously develop two coexisting rheumatic diseases where their coexistence is not causally related but rather is based upon a chance association. This is especially more likely for those diseases that are common, such as the coexistence of RA and fibromyalgia, or RA and Sjögren syndrome (181 ,182 ,183 ,184 ,185 ,186 ,187). Small clinical series of such patients have been described and the literature is replete with case reports of patients said to have overlapping features of different autoimmune diseases. The precise relationship of these to each other ultimately waits a more complete understanding of the genetics and pathogenesis of autoimmunity and of the connective tissue diseases (10).

Mixed Connective-Tissue Disease and Overlap Syndromes: Summary Points

- MCTD was described by Sharp et al. among a group of patients with clinically overlapping features of SLE, scleroderma, and polymyositis who had high levels of antibodies reactive with a novel antigen which is now known to be RNP.
- Primary clinical features of MCTD are Raynaud phenomenon, swollen fingers or hands, arthralgia, with or without associated arthritis, esophageal reflux or dysmotility, acrosclerosis (i.e., sclerodactyly), mild myositis, and pulmonary involvement of a variety of forms.
- Serologic hallmark of MCTD is the presence of high levels of antibodies against the RNP antigen.
- RNP antigen also known as nRNP, U1-snRNP or U1RNP.
- Several criteria for classification of MCTD have been published and include clinical and serologic criteria.
- Laboratory abnormalities common in MCTD are high titer antinuclear antibodies, rheumatoid factor, leukopenia, and anemia.
- U1–70kD polypeptide of RNP plays a key role in MCTD.
- Genetic studies have shown that there is an association between HLA-DR4 and MCTD in several populations.
- MCTD can be mild to severe with most patients able to lead functionally normal lives.
- Pulmonary hypertension is the main disease associate cause of death and has been found to be present in over one fifth of patients.
- Absence of controlled clinical trials in MCTD requires that rational treatment is based upon evidence-based approaches derived from other rheumatic diseases.
- Overlap syndromes are often incorrectly used as synonymous with MCTD.

There are a number of distinctive overlap syndromes that have been reported, such as those associated with antibodies against Jo-1, PM/Scl, Ku, and RNA polymerase II.

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Chapter 51

The Mother in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) affects predominantly young women with normal fertility, who frequently get pregnant. In the past, pregnancy in lupus was often fraught with serious disease flares, fetal, and even maternal death. The course and outcomes of pregnancy in this disease has changed substantially in the last 55 years and pregnancy in lupus patients has now become commonplace.

A lot of insight has been recently gained on the immunobiology of both normal and lupus pregnancy. Moreover, much has been discovered on determining factors and biologic surrogate markers of a successful versus a failed pregnancy. The role of sex and pregnancy hormones on the maternal immune response and on the tolerance of a normal semiallogeneic fetus has been elucidated. A brief introduction will be given on the immunobiology of normal pregnancy and the role of sex hormones with direct reference to abnormalities seen in patients with SLE.

Immunobiology of Normal Pregnancy

Pregnancy is a physiologic condition characterized by a persistent local tolerance of the maternal immune system to the paternal human leukocyte antigens (HLA) expressed by the fetus. A normal semiallogeneic embryo is organized to evade hostile maternal cellular and humoral immune responses (1). This local immune tolerance is not associated with a reduced maternal immune response to exogenous stimuli, or with inability of the mother to produce antibodies against HLA (2). Several alterations occur in the normal immune system during pregnancy, both systemically and locally, that prevent fetal rejection.

The placenta represents the site of immune maternal-fetal interface. It has a maternal side, the decidua, which is the hypertrophic pregnant endometrium, and a fetal side, the trophoblast. The trophoblast has an inner layer of mononuclear cytotrophoblast and an outer layer of multinucleated syncytiotrophoblast, derived from fusion of cytotrophoblasts.

How is the maternal immune system alerted of the semiallogeneic trophoblastic cells invading the uterine wall and how does it respond?

The normal human endometrium hosts a significant number of leukocytes, the percentage and phenotype of which change during the menstrual cycle and pregnancy (3). Leukocytes account for 10% of stromal cells during the proliferative phase, 20% during the secretory phase, and 30% of endometrial stromal cells in early pregnancy decidua. Cells of the innate immune response, such as natural killer (NK) cells, represent about 70% of these leukocytes and are the largest leukocyte subset present in the decidua (3 ,4). Macrophages and dendritic cells (DC) are also diffusely distributed within the decidua, usually strictly adhering to extravillous trophoblast and close to the spiral arteries and endometrial glands (5). Cells of the adaptive immune response such as T and B cells, although a numeric minority, are critically important for normal pregnancy outcomes, as will be discussed later.

Endometrial leukocytes are derived from peripheral blood leukocytes that are locally chemotaxed and subsequently home in the decidua (6). During insemination, transforming growth factor beta-1 (TGF-ß1) found in seminal fluid stimulates production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and results in recruitment of inflammatory cell infiltrates in the uterus (7). Uterine NK cells (uNK) derive from peripheral blood NK cells (8). Based on intensity of surface expression of CD56, an isoform of neural cell adhesion molecule (NCAM), peripheral blood NK cells are divided in two populations: CD56dim, representing 90% of the NK population and CD56bright, which is the remaining 10% (7). CD56dim cells express high levels of CD16 a low affinity receptor for IgG complexes (FcRIII) associated with their cytotoxic potential (9). CD56bright cells do not express CD16 and are the main source of NK cell-derived regulatory cytokines. $CD56^{bright}CD16^{-}$ cells represent the pool from which uNK cells derive. The uNK cell phenotype changes during the normal menstrual cycle and early pregnancy (10). During pregnancy, there is decreased expression of many activation markers, and almost all uNK have been found to express one or more inhibitory receptors. The most pertinent are CD94/NKG2A, KIR2DL4, and ILT-2 (LIR-1) (11). In addition, uNK selectively express CD9, galectin-1, and glycodelin, which are associated with immunomodulatory functions (12).

Although uNK represent the major immune cell type during early pregnancy, they do not persist throughout gestation.

Antigen-presenting cells (APCs), such as macrophages and DC are a stable population present throughout the tissue from implantation to parturition. Macrophages represent about 20% of the decidual leukocytes (13). Although activated in vivo, as indicated by expression of HLA class II, CD11c, and CD86, these macrophages exhibit a phenotype of alternative activation, associated with immune suppressive activity (14). This phenotype is characterized by surface expression of B7-H1, ILT3, DC-SIGN, MS-1, and factor 13. In addition, decidual macrophages spontaneously secrete anti-inflammatory cytokines, such as IL-10 and TGF-ß1, and are a major source of prostaglandin E2 (PGE2), responsible for the suppressive function of decidual cell mixtures demonstrated in early in vitro studies (15).

The DC present in the human decidua derive strictly from myeloid DC and exhibit a predominantly immature phenotype (CD83-) (16). When immature DC present antigens to T cells, this leads to antigen-specific T cell tolerance, whereas antigen presentation by mature DC causes activation of naïve or memory helper T cells, cytotoxic T cells, and B cells (17). Immature DC in the decidua could capture fetal antigens from the invading trophoblast and present peptides to local maternal T cells, thus inducing tolerance to those antigens (16 ,18). Moreover, macrophage-derived PGE2 greatly enhances the ability of the DC to produce IL-10 and decreases its ability to secrete IL-12, thus leading to the development of a tolerogenic DC (19).

Because the human conceptus represents a semiallograft to the maternal host, immunologic tolerance to the fetal allograft is essential for its maintenance. In humans, $CD4^+$CD25bright regulatory T cells (Treg) are known to play an important role in the development and maintenance of tolerance in peripheral tissues (20). These cells express high levels of CD152 (CTLA-4) and suppress effector T cell proliferation and action via direct cell contact. It was recently shown that absence of $CD4^+CD25^+$ Treg led to failure of gestation, because of immunologic rejection of the fetus in a mouse model, suggesting that $CD4^+CD25^+$ Treg cells mediate maternal tolerance to the fetus (21). The presence and action of $CD4^+$CD25bright Tregs was recently characterized in human pregnancy (22). $CD4^+$CD25bright Tregs represent about 6.5% of the total $CD4^+$ cells in the peripheral blood of nonpregnant women. In early pregnancy, there is a significant numeric expansion of these cells in the peripheral blood (8.5%, $p<0.05$). In human deciduas, 5% to 6% of decidual cells are $CD4^+$ cells. Early pregnancy deciduas from patients undergoing therapeutic abortion show tremendous enrichment of $CD4^+$CD25bright Tregs (21.8% of $CD4^+$) compared to those in patients with spontaneous abortion (7.1% of $CD4^+$, $p<0.0001$), indicating a potential role of these cells in maintenance of human pregnancy (22).

The extravillous trophoblast (EVT), which represents the fetal side of the maternal-fetal immune interface, has 50% genetic contribution from the father, and is thus an allogeneic normal tissue in the mother. However, unlike other situations in which allogeneic cells come into contact, as in transplantation, there are fundamental differences. The trophoblastic cell is not a normal somatic cell, but a normal extraembryonic cell. The highly polymorphic HLA class I (HLA-A and HLA-B) and class II molecules displayed by somatic cells, initiating recognition of the allograft and its subsequent rejection are not displayed by trophoblastic cells (23 ,24). Instead, EVT expresses an unusual combination of three HLA class I molecules: a less polymorphic HLA-C and two others, HLA-E and HLA-G, which are known to be dominant ligands for inhibitory receptors on maternal NK cells, macrophages, and DC (24). HLA-E is virtually monomorphic and would be perceived as “self” by maternal uNK cells expressing the inhibitory receptor CD94/NKG2A, thus preventing cytotoxicity (25). The quasimonomorphic HLA-G is recognized by the inhibitory uNK cell receptors KIR2DL4 and ILT2 (LIR-1), as well as by inhibitory receptors ILT-2 and ILT-4 (LIR-2) on macrophages and DC, and by ILT-2 on T cells (26). HLA-C allotypes are recognized by diverse inhibitory KIR2D receptors present on uNK cells (27). Given the stable presence of maternal macrophages and DC in the decidua throughout pregnancy, their exposure to trophoblastic antigens such as HLA-G, and their immunosuppressive phenotypes, HLA-G is a prime candidate potentially responsible for driving APC behavior beneficial to the maintenance of human pregnancy (16 ,28).

Another mechanism for protection of pregnancy against rejection is the abundant expression of Fas ligand (FasL) by maternal syncytiotrophoblast (29). Activated lymphocytes expressing Fas would be driven to apoptosis upon FasL engagement on the surface of such trophoblastic cells.

Complement activation and complement-mediated cytolysis could readily occur at the maternal-fetal interface jeopardizing the integrity of pregnancy (30). Complement regulators have been identified on the surface of all trophoblastic cells protecting them from complement-mediated cytolysis. Membrane regulators such as decay acceleration factor (DAF, CD55), acting as a C3-convertase inhibitor, and membrane cofactor protein (MCP, CD46), acting as a cofactor for C3b-inactivator, are expressed on the trophoblast in association with CD59 (31). This is an important complement regulator that inhibits the assembly of the cytolytic membrane attack complex on the surface of the trophoblast (32).

Another mechanism of fetal protection is the expression of indoleamine 2,3-dioxygenase (IDO) on fetal-derived syncytiotrophoblasts at the maternal-fetal interface, an enzyme that catabolizes tryptophan (33). Inhibition of IDO in mice resulted in fetal rejection by maternal lymphocytes (34).

The uterine decidua and the fetoplacental unit produce large numbers of cytokines, which contribute to a shift of the immune response from T helper-1 (Th1) to Th2: successful pregnancy has been considered akin to a Th2 type of response (35), where cytokines IL-10, IL-4, IL-5, IL-6, and IL-13 predominate. Recurrent spontaneous abortion of unknown cause (pregnancy rejection) is mediated by a Th1 response (36), where interferon-γ (IFN-γ), tumor necrosis factor-alpha and -beta (TNF-α, TNF-ß), IL-2, and IL-12 predominate.

The hormonal milieu dramatically changes during pregnancy. Pregnancy-associated hormones modulate the number,

surface phenotype, and the biological function of immune competent cells in maternal blood and decidua, ascertaining protection and growth of a normal fetus.

Progesterone, the hormone of pregnancy has long been considered the “nature's immunosuppressant” (37). In pregnancy, progesterone causes a decrease in the NK cell numbers, activation and cytotoxicity via several ways: 1. direct action on NK cells (38), 2. through promoting Th2 differentiation in $CD4^+$ T cells, and 3. via progesterone-induced blocking factor (PIBF) on γδ-T cells. Progesterone also facilitates NK cell homing to the endometrium via induction of expression of homing receptors and addressins on peripheral NK cells and endometrium respectively, and possibly through upregulation of vascular endothelial growth factor (VEGF) and macrophage inflammatory protein-1b (MIP-1b) expression by endometrium (39). Endometrial stromal cells under the influence of progesterone produce IL-15, prolactin (PRL), and likely other unidentified factors, which regulate uNK cell proliferation, differentiation, and production of cytokines and other molecules that support placental and trophoblast development and promote local immunomodulation (40).

In the presence of progesterone, peripheral lymphocytes from healthy pregnant women produce PIBF, a 34-KD mediator protein (41). PIBF has been shown to exert immunomodulatory functions both in vitro and in vivo. It predominantly inhibits NK cell function (42). Neutralization of endogenous PIBF activity in pregnant mice by anti-PIBF antibody results in a 70% reduction in the number of viable fetuses, and this is associated with an increased splenic NK cell activity (42). Ninety percent of pregnancy loss mediated by anti-PIBF administration is corrected by the treatment of the pregnant animals with anti-NK antibodies. The second main mechanism of action of PIBF is the induction of a Th2 dominant cytokine response (43 ,44). During normal pregnancy, the concentration of PIBF in biological samples continuously increases until the thirty-seventh gestational week and is followed by a sharp decrease over the forty-first week of gestation (45).

In pathologic pregnancies, urinary PIBF fails to increase. Thus determination of PIBF concentration in the urine has been proposed as a useful surrogate test for threatened premature pregnancy termination (45).

Work on the immunoregulatory properties of steroid sex hormones shows the following: 17β-estradiol (E2) significantly reduces neutrophil chemotaxis through a receptor-dependent mechanism (46); in mice, estradiol reduces NK cell cytotoxicity in a dose-dependent manner, and augments polyclonal B cell activation, probably through suppression of NK cells (47). In vitro experiments with adult male mononuclear cells under various stimuli (e.g., phytohemagglutinin (PHA), anti-CD3, IL-2) showed that estradiol and pituitary gonadotropins had a variety of effects on T cell subsets, depending on the stimulus used (48): E2 significantly decreased $CD4^+$ cells and increased $CD8^+$ cells; prolactin had no subset-specific effects, but enhanced proliferation of peripheral mononuclear blood cells (PMBCs), as did follicle-stimulating hormone (FSH) and luteinizing hormone (LH); FSH decreased $CD4^+$ cells and enhanced $CD8^+$ cells, especially $CD8^+CD28^+$; LH increased $CD4^+$ cells. Human chorionic gonadotropin and somatotropin appear to suppress in vitro T cell functions. Peripheral T-lymphocytes from pregnant women express human chorionic gonadotropin/LH receptor messenger RNA (mRNA) transcript and the receptor protein, suggesting that immunomodulatory effects are receptor-mediated (49). Some of the actions of progesterone were detailed earlier. Cortisol is present at a fourfold concentration in the third trimester, and the free cortisol index is elevated (50). This fact might have been responsible for some of the clinical amelioration seen in lupus pregnancy.

It would appear, then, that various immunomodulatory events occur during pregnancy, especially at the maternal-fetal interface, which regulate the maternal immune response and result in tolerance, survival, and growth of the embryo.

Summary

- The fetoplacental unit is immunologically privileged by expressing (a) unique HLA antigens; (b) Fas ligand, which protects it from Fas-expressing lymphocytes; and (c) complement regulatory proteins, which protect it from complement lysis action.
- Successful pregnancy shows a shift to a Th2 response, with IL-10 playing a prominent role. Recurrent spontaneous abortion of unknown etiology is associated with a shift to Th1 response.
- Progesterone at the maternal-fetal interface, through PIBF, inhibits the function of uterine NK cells, and promotes a Th2 dominant cytokine response.
- Expression of IDO on fetal-derived syncytiotrophoblasts at the maternal-fetal interface catabolizes tryptophan and inhibits fetal rejection by maternal lymphocytes.
- Greatly increased levels of hormones such as progesterone, estrogen, cortisol, chorionic gonadotropin, and somatotropin modulate cellular immunity at the maternal-fetal interface.

Sex Hormones and Systemic Lupus Erythematosus

SLE is a chronic autoimmune disease known for its female proclivity and peak incidence during reproductive years (9:1 ratio of females to males). Early reports of SLE flares corresponding to the menstrual cycle (51), focused an era of investigations on the potential contributions of estrogens, androgens, and prolactin to the development of this disease (52). Sex hormonal factors play an important role in regulating disease onset, severity, and progression (53).

Observations suggesting hormonal disease modulation include reports of SLE flares precipitated by estrogen (54), oral contraceptives containing estrogen (55), and ovulation induction regimens (56). Conversely, ovarian failure has been associated with decreased risk of lupus flares (57). An association of lupus with Klinefelter syndrome, and its amelioration following testosterone administration, also imply that sex hormones modulate the prevalence and

expression of SLE (58). Clinical investigations of sex hormones in lupus have yielded confounding results because of small patient numbers, ethnic differences, long periods of reporting, and insufficient power to test respective hypotheses. A recent meta-analysis of all available studies enrolling nonpregnant female and male patients with SLE, and matched controls, with sex hormone measurements in the serum yielded enlightening results (53):

- Estradiol (17β-estradiol, the most potent and predominant estrogen in serum) was significantly higher in adult SLE patients compared to controls when all studies (20 studies) and female-only studies (8 studies) were considered. No significant differences in serum estradiol levels could be demonstrated between male-only lupus patients and healthy controls (12 studies).
- Serum testosterone levels were significantly suppressed in SLE patients compared to controls when all studies (19 studies) and female-only studies (8 studies) were considered. This was not observed in male-only studies (11 studies). Possible explanations include increased activity of aromatic hydroxylase or increased production of LH driving testosterone aromatization in women.
- Serum dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) levels were significantly lower in adults with SLE compared to controls when all studies were considered (14 studies). The paucity of studies precluded sex subset classification for DHEAS, or of common estimators for DHEA.
- Serum PRL concentrations were significantly higher in SLE adults when all studies were considered (14 studies). PRL levels were also significantly higher when female-only studies (10 studies) or male-only studies (4 studies) were considered separately. Summation of studies suggested that 21% of all SLE patients were hyperprolactinemic, compared to 3% of healthy matched controls.

To our knowledge, no genotypic variations in the enzymes of gonadal steroid synthesis have been identified in SLE patients. However, abnormal metabolism of estrogen and testosterone has been reported: women and men with lupus, and their first-degree relatives, have a higher rate of C16[α] hydroxylation of estradiol to estrone and estriol, which retain estrogenic activity (59). In addition, there is decreased C2 hydroxylation of estrogen, and increased C17 oxidation of testosterone, resulting in inactive metabolites (60).

Because a hallmark of SLE is B cell hyperactivity with production of multiple pathogenic and/or disease specific autoantibodies, it was hypothesized that female sex hormones modulate autoreactive B cells (61). Treatment with estrogen induces a lupus-like syndrome in BALB/c mice transgenic for the H-chain of a nephritogenic anti-DNA antibody (62). In untreated mice, high affinity anti-DNA B cells are rendered tolerant and SLE does not develop. Upon estrogen administration, autoreactive B cell tolerance is broken. Estrogen upregulates antiapoptotic Bcl-2 and inhibitory BCR signaling molecules CD22 and SHP-1. The resulting impaired apoptosis of autoreactive B cells allows for their expansion, high titers of serum anti-DNA, and nephritis with extensive glomerular IgG deposition.

In humans, estrogen increases IgG and anti-DNA production by SLE peripheral blood mononuclear cells (PBMCs) (63). In addition, it induces CD40L expression on B cells that in turn upregulates anti-apoptotic molecules and prevents BCR-mediated apoptosis (64).

Treatment of lupus-prone (NZBX NZW)F1 mice with PRL leads to elevated serum IgG and circulating immune complexes, early proteinuria, and premature death at 28 weeks from immune complex-mediated nephritis (61). PRL also breaks self-tolerance of autoreactive B cells in transgenic BALB/c mice leading to a lupus-like disease (65).

Prolactin levels correlated with clinical and serologic disease activity in humans (66). During lupus pregnancy, PRL was significantly higher than in control pregnant women, with highest levels late in pregnancy (67). Male SLE patients also had elevated serum PRL (68). Two double-blind, placebo-controlled studies of the prolactin antagonist bromocriptine in SLE patients showed decreased PRL levels, Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score, and flare rates per patient/month (69). Of interest is that 33 women with increased PRL and no diagnosis of a rheumatic disease had increased prevalence of autoantibodies, including anti-double-stranded DNA (dsDNA), anti-Sm, and anti-Ro/SSA, compared to PRL-normal controls (70). Chapter 16 discusses sex hormones in SLE.

Definition of Terms

The following terms are used in this and Chapter 52 (71 ,72 ,73 ,74):

- Fertility rate: Average number of pregnancies per pregnant woman.
- Parity rate: Average number of viable infants per pregnant woman.
- Adjusted fertility or parity rate: Corrected for years at risk for pregnancy (10-year intervals).
- Spontaneous abortion: Spontaneous termination of pregnancy prior to 20 weeks' gestation.
- Elective or induced (“therapeutic”) abortion: Voluntarily induced termination of pregnancy.
- Stillbirth or intrauterine fetal death (IUFD or fetal death): The death of the fetus after the 20th week of gestation. The rate is approximately 5 per 1,000 in the general population.
- Neonatal death is defined as death occurring in the first 28 days after birth. The background rate is 5 per 1,000.
- Perinatal death is the sum of stillbirth and neonatal death. Perinatal death is approximately 10/1,000 in the general population.
- Pregnancy loss or fetal loss is the sum of spontaneous abortions and perinatal deaths.
- Recurrent spontaneous abortion; recurrent fetal loss: Three or more (75), or two or more (76), consecutive spontaneous abortions or intrauterine fetal deaths, respectively.
- Recurrent spontaneous aborter: A woman with recurrent spontaneous abortions, or recurrent fetal loss.

- Intrauterine growth restriction (IUGR): Formerly intrauterine growth retardation; the newborn weight is below the 10th percentile for gestational age. Synonyms: small newborn for gestational age, intrauterine malnutrition.
- Preterm premature rupture of membranes (PPROM) is the spontaneous rupture of membranes (amniorrhexis) before the onset of labor and before 37 weeks' gestation
- Premature rupture of membranes (PROM) refers to the spontaneous rupture of membranes before labor after 37 weeks' gestation.
- Premature or preterm birth: Spontaneous termination of pregnancy with a live birth between 21 and 37 weeks of gestation.
- Full-term or term birth: Spontaneous termination of pregnancy with a live birth between 38 and 40 weeks of gestation.
- Pregnancy-induced hypertension (PIH): Presence of blood pressure ≥140/90 mm Hg on at least two occasions, 6 or more hours apart, during the second half of pregnancy in a previously normotensive woman (77 ,78 ,79).
- Preeclampsia: PIH with proteinuria of >0.3 g/L in the absence of urinary tract infection (77), or abrupt onset of hypertension and proteinuria, usually after 24 weeks of gestation (78 ,79).
- Severe preeclampsia: Characterized by one or more of the following: blood pressure of at least 160 mm Hg systolic, or 110 mm Hg diastolic on two readings 6 hours apart; proteinuria >5 g/24 hours, oliguria (<400 mL/24 hours), cerebral or visual disturbances, pulmonary edema, or cyanosis (80).
- HELLP syndrome: is a form of severe preeclampsia characterized by Hemolysis, Elevated Liver enzymes, Low Platelets (80).
- Eclampsia: Severe preeclampsia with malignant hypertension, seizures, and renal failure.

Table 51-1: Course of Systemic Lupus Erythematosus (SLE) in Pregnancy

Time Period	No. of Pregnancies	Flares		Remission (%)[†]	Maternal Death(%)	SLE Onset(%)
		All (%)	Postpartum (%)			
1950–1959	139	60	—	35	17.0	—
1960–1969	272	52	—	22	8.0	—
1970–1979	211	50	—	9	0.5	—
1980–1989	618	37	17	2	1.7	12.5
1990–1999	1,056 (2,275)*	43.6	11.8	0	0.17[‡]	0.19
2000–2005	2,000**	23.3	10.1	0	0.65[a]	4.5[c]
Studies in the 1980s						
Tozman (107)	24	25	13	8	0	0
Houser (96)	18	50	22	0	0	0
Zulman (112)	24	54	22	0	8	8
Hayslett & Lynn (95)	65	39	13	5	4	—
Fine (90)	52	23	20	0	2	<10
Varner (108)	38	35	8	0	3	21
Gimovsky (93)	77	23[d]	2[d]	0	0	0
Imbasciati (97)	26	46	27	0	11	37
Lockshin (113)[e,f]	33	27	—	0	0	—
Mintz (75)[e,f]	102	60	20	0	0	—
Meehan (101)	22	45	42	0	0	0
Bobrie (89)	76	34	15	0	0	18.4
Lockshin (114)[e]	80	21	0	0	0	8
Studies in the 1990s[b]						
Nossent & Swaak (129)	39	74	63	0	0	0
Wong (125)[e,f]	29	58	0	0	0	0
Oviasu (130)	53	2 (13)[g]	0	0	0	0
Petri (120)[e,f]	74	36	—	0	0	0
Tincani (123)[e]	25	44	0	0	0	0
Rubbert (131)	21	95	—	0	0	0
Derksen (116)[e]	35	17	0	0	0	0
Le-Thi-Huong (117)[e]	103	45	9	0	2	0
Lima et al. (119)[e]	108	57	35	0	0	0
Ruiz-Irastorza (122)[f]	78	65	—	0	0	0
Tomer (124)[f]	54	14.5	Excluded	0	0	0
Le Huong (118)[eh1]	62	27	6	0	0	0
Johns (126)	44	61	—	0	0	0
Rahman (121)[†]	141	63	—	0	0	0
Carmona (115)[e,h2]	60	25	9.4	0	0	3.3
Sittiwangkul (132)[h3]	48	36	—	0	0	0
Kobayashi (128)[h4]	82	20.7	4.9	0	0	0
Studies in 2000–2005[b]						
Georgiou (140)[f]	59	13.5 (76)[g]	19[g]	0	2.1	0
De Bandt (139)[e]	59	20.3	10.2	0	0	0
Brucato (133)[e,f]	147	17.7	0	0	0	0
Cortes-Hernandez (138)[e]	103	33.0[i]	19.4	0	0	1.9
Tan (147)	27	7.4	7.4	0	0	0
Ruiz-Irastorza (157)	38	26.3	0	0	0	0
Chandran (136)	52	5.8	3.8	0	3.2	5.8
SLE Inactive	31	0	0	0	0	0
SLE Active	21	14.3	9.5	0	1.9	14.3
Molad (143)[e]	29	20.7	20.7	0	0	0
Clowse(137)[e]	267	21[k]	NS	0	1.1	13.5
Chakravarty (135)	63	67.7[j]	0	0	1.6	6.3

*The 2,275 pregnancies include studies without information on maternal flares (127,162,172,173,174), and pregnancies considered under nephritis (171,175,176,177).
**The 2,000 pregnancies include 718 pregnancies without concrete data on maternal flares (142,149,309), and 437 pregnancies considered under nephritis (134,141,144,145,146,148,179).
[†]Spontaneous remission.
[‡]Two deaths in over 1,200 pregnancies (2 deaths in 765 pregnant women with flare data)
[a]Thirteen deaths in 2,000 pregnancies (13 deaths in 1,690 women)
[b]Most patients fulfilled American Rheumatism Association criteria for lupus before pregnancy
[c]SLE onset in 36 of 806 pregnancies with such data.
[d]Flares requiring hospitalization.
[e]Prospective studies.
[f]Controlled studies.
[g]See text.
[h]SLE inactive at conception: 1, in 100%; 2, in 90%; 3, in 94%; 4, in 95% of pregnancies.
[i]In 10.7% of pregnancies, the flares were severe.
[j]In 29%, the flares were severe.
[k]Only moderate and severe flares reported

Pregnancy and Lupus Activity—Controversy or Consensus?

The relationship of pregnancy and SLE activity has been controversial and contradictory, especially in the earliest studies. The subject is fascinating, and there has been a virtual explosion in the number of studies addressing lupus pregnancy, both by rheumatologists and by obstetricians.

Reports from the 1950s recommended against pregnancy, and implied that it was not advisable and that termination should be offered (81 ,82). The numerous studies that followed, especially in the last 20 to 25 years, indicate that pregnancy in lupus patients often has a successful outcome, if well timed and managed. Although there is general agreement about successful pregnancy outcomes, there are still variations in study results, arising from differences in study design, variability of SLE severity, the lack of uniformity in the definition of flares, and cultural and socioeconomic factors.

- Study design: The majority of studies from the 1950s through the 1980s were uncontrolled and retrospective (72 ,81 ,82 ,83 ,84 ,85 ,86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96 ,97 ,98 ,99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109), with the exception of three controlled studies (110 ,111 ,112). Since 1984, prospective and/or controlled studies have appeared, three in the 1980s (75 ,113 ,114), and 11 (115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,127) of the 19 published in the 1990s (115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129 ,130 ,131 ,132). Of the 17 studies published in 2000–2005 (133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149), 4 are controlled (134 ,140 ,148 ,149), 4 are prospective (133 ,137 ,138 ,143), and 9 are retrospective (135 ,136 ,139 ,141 ,142 ,144 ,145 ,146 ,147) (Table 51-1). All studies except 1 (149) utilized the American College of Rheumatology (ACR, formerly ARA) classification criteria for SLE (150).
- The great variability of SLE severity can skew study outcomes. For example, it has been well established that SLE

nephritis is more severe and has a worse outcome in African-American and African-Caribbean patients (151 ,152).

- The definition of flare has not been uniform, even in the last 5 years: it ranges from the use of clinical indices of signs and symptoms, the patient's opinion, to complicated analysis of laboratory data, the physician's global assessment, or the physician's decision to increase prednisone dosage. The availability of lupus activity indices allows for a standardized approach (153 ,154). Several indices (SLEDAI, Lupus Activity Index [LAI], Systemic Lupus Activity Measure [SLAM], and European Consensus Lupus Activity Measure [ECLAM]) have been adapted for use in pregnancy, as Systemic Lupus Erythematosus Pregnancy Disease Activity Index (SLEPDAI), Lupus Activity Index in Pregnancy (LAI-P), and modified Systemic Lupus Activity Measure (m-SLAM) (155 ,156 ,157). Modifications in the activity measures have been developed in recognition of differential diagnostic dilemmas between active SLE versus preeclampsia, which share certain symptoms and findings (headache, hypertension, proteinuria, seizures, red blood cells [RBCs] in urine), and versus pregnancy-associated problems (fatigue, edema, alopecia). Definitions of flare include a change in the physician's global assessment equal to, or greater than 1, an increase of 3 in SLEDAI, or of 0.26 in LAI (158). LAI has been successfully compared to the “gold standard,” the physician's global assessment (157). For further details on activity indices, please see Chapter 47 .
- SLE patient populations vary greatly in terms of cultural diversity and educational-socioeconomic background, which influence their perception of disease severity, their compliance with prescribed medications, and the site of their health care, ranging from private offices for patients of affluent background to university, tertiary hospitals for economically disadvantaged patients (159).
- Greater diagnostic uniformity in patient selection has been achieved by the use of the SLE classification criteria (preliminary since 1971 (161), revised in 1982 (150), and updated in 1997 (160). The availability of sophisticated and standardized laboratory tests (antinuclear antibody [ANA], complement C3 and C4, and antiphospholipid antibodies [APL]) has allowed confirmation of diagnosis and subsetting of patients, further contributing to uniformity of patient selection and clinical follow-up. In addition, 55 years of experience with the treatment of SLE patients in the poststeroid era have resulted in increased patient survival, a large patient population with controlled SLE, and, indeed, a modification of the natural history of the disease (162 ,163 ,164).

SLE Activity during Pregnancy

Pregnancy in SLE patients is common, the prognosis has greatly improved with proper management, and patients function well within society and their families (105). Their fertility is normal, at 2 to 2.4 pregnancies per patient, not only in quiescent periods of the disease, but also during active episodes when approximately 10% of total reported pregnancies occur (72 ,91 ,93 ,110 ,129 ,165).

In the presteroid era, exacerbations and remissions occurred almost equally during pregnancy, but severe postpartum flares were seen in virtually all patients (91). The course of pregnancy tended to be smoother in patients who conceived during a period of inactive lupus (83), and the value of steroid therapy in decreasing flares during pregnancy and postpartum was recognized in 1962 (111). A sizable proportion of patients developed preeclampsia (1.8% to 20%) (84 ,86 ,99). The debate whether hypertension alone or with proteinuria during pregnancy represents pregnancy-induced hypertension, preeclampsia, or active lupus nephritis has been longstanding (112 ,113 ,165), but differentiation is possible (166 ,167). The more acute the lupus, and the earlier the report, the worse was the outcome, with maternal death occurring in 10% to 25% of patients (81 ,86) (Table 51-1). In 1970, McGee and Makowski advocated steroids for 6 weeks after delivery (100).

In the mid-1960s to mid-1970s, three major studies, from New York, Mexico City, and Los Angeles, respectively, were published: Estes and Larson reported 79 pregnancies in 36 patients (83), Fraga et al. reported 225 pregnancies in 53 patients (110), and Dubois reported 217 pregnancies in 112 patients (84). The appropriate use of corticosteroids seemed to decrease pregnancy and postpartum flares to half of those in the 1950s (from 50%–75% to 24%–27%), and maternal mortality was very rare. The study by Fraga et al. from Mexico City was followed up by a prospective study by Mintz et al. (75).

Table 51-1 summarizes lupus pregnancy data over the last 55 years categorized by decade, and in greater detail for the 1980s, 1990s, and the years 2000 through 2005. The most striking change is a dramatic decrease in maternal mortality from 17% in the 1950s to 0.26% in the 1990s and a slight rise to 0.65% in the 2000s. Lupus flares during pregnancy range from 17% to 60%; however, in later reports the majority are mild and mostly musculoskeletal and cutaneous (75 ,116 ,129), with the exception of patients in whom disease began during pregnancy or postpartum. A good example are the studies of Cortes-Hernandez (138) and Chakravarty (135), where all flares were 33% and 67.7% respectively, but severe flares were seen in 10.7% of the former, and in 29% of the latter study. One third to one half of flares were seen in the postpartum period, and the rest are equally divided among trimesters. Throughout the last 55 years it is abundantly clear that patients whose SLE is inactive at conception have a lesser likelihood of disease flares, whereas active SLE at conception has been a very important factor for pregnancy flares during pregnancy and postpartum (95 ,98 ,135 ,136 ,137 ,138 ,143 ,144 ,145 ,168).

SLE onset during pregnancy is described in 12.5% of the pregnancies in the 1980s, in 0.19% during the 1990s (115), and in 4.5% of pregnancies in the 2000s (135 ,136 ,137 ,138) and is also associated with severe lupus activity during pregnancy. In addition, active SLE during pregnancy affects fetal outcome with greater perinatal loss, small infants (IUGR), more preterm births, and less live births (see Chapter 52).

Nine studies, spanning from 1962 to 2002, have examined the question of whether lupus flares are more frequent in pregnancy (75 ,111 ,112 ,113 ,122 ,125 ,138 ,168 ,169). Three studies used the patients as their own controls (111 ,112 ,138) and six used nonpregnant controls (75 ,113 ,122 ,125 ,169) (Table 51-2).

Table 51-2: Flares of SLE in Pregnant and Nonpregnant Patients

Study	Per 100 Weeks at Risk	Per Patient-Month	
Garsenstein et al. (111)			
32 weeks before pregnancy	0.91	0.04	
0–20 weeks of pregnancy	3.04	0.13	
21–40 weeks of pregnancy	1.62	0.07	
0–8 weeks postpartum	6.31	0.27	
9–40 weeks postpartum	0.84	0.04	
Study	**Flares/Pregnancies (%)**		
Zulman et al. (112)			
6 months before pregnancy	4		
1st trimester	13		
2nd trimester	14		
3rd trimester	55		
6 months postpartum	23		
Study		**Per Patient-Month**	
Wong et al. (125)			
Pregnant:	13 in 155 patient-months	0.08	$p<0.02$
Nonpregnant:	218 in 5202 patient-months	0.04	
Petri et al. (169)			
Pregnant:	1.6337/patient-year	0.14	$p<0.0001$
After delivery:	0.6392/patient-year	0.05	
Nonpregnant:	0.6518/patient-year	0.05	
Mintz et al. (75)			
Pregnant:	55 in 909 patient-months	0.06	p = NS
Nonpregnant:	19 in 468 patient-months	0.04	
Ruiz-Irastorza et al. (122)			
Pregnant:		0.08	$p<0.001$
Nonpregnant:		0.04	
Pregnant vs.:		0.09	p = 0.0015
themselves after puerperium		0.05	
Lockshin et al. (113)			
Similar in 28 pregnant and 21 nonpregnant patients			
Greater thrombocytopenia in pregnant patients			
Urowitz et al. (168)			
Flares similar in 79 pregnant and in nonpregnant control patients			
Flares were twice as frequent if SLE was active at conception (82%) versus inactive at conception (41%)			
Cortes-Hernandez et al. (138)			
Before pregnancy in 60 patients	0.4 flares per person-year		
During pregnancy, in 60 patients with 103 pregnancies	1.2 flares per person-year		$p<0.0001$

Garsenstein et al. noted a clear-cut increase in flares during the first half of pregnancy (3.04/100 weeks at risk versus 0.91/100 weeks for the 32 weeks before pregnancy), a small increase during the second half of pregnancy (1.62/100 weeks), a sevenfold increase during the 2 months postpartum

(6.31/100 weeks), and a return to baseline in the 9 to 40 weeks postpartum (111).

Zulman et al. reported the percentage of flares per number of pregnancies during the 6 months preceding pregnancy (4%), each trimester (13%, 14%, and 55%, respectively), and during 6 months postpartum (23%) (112). The flare rate during the third trimester and postpartum appears excessive.

Lockshin et al. reported similar flares in 28 pregnant and 21 nonpregnant patients, with the exception of greater thrombocytopenia in the pregnant patients (113).

Mintz et al. reported 55 flares in 909 months in their at-risk pregnant patients, not significantly different from the 19 flares in 468 months of their nonpregnant patients (p = NS). It is of note that all pregnant patients in this study, even without active lupus, were given 10 mg of prednisone daily (75).

Wong et al., however, found the rate of flares in their pregnant patients to be twice that of their nonpregnant patients ($p<0.02$) (125).

In a prospective study of 40 pregnancies in 37 women, Petri et al. defined flare as a change of over 1.0 in the physician's global assessment (scale of 0 to 3) since the preceding visit or during the previous 3 months (169). They noted 27 flares in 24 of 40 pregnancies, or 60%. The intrapregnancy flare rate of 1.6337 ± 0.30087 per person-year was significantly greater than the flare rate after delivery (0.6392, $p<0.001$), and the rate in nonpregnant patients (0.6518, $p<0.0001$). The majority of the flares were moderate (59%), or mild (30%), and only 11% were severe.

In a prospective, controlled study from the University of Toronto of 79 lupus pregnancies, flares were similar in pregnant and nonpregnant women, but were twice as frequent in women with active SLE at conception (82%) than in women with inactive SLE at conception (41%); inactive SLE at conception was deemed protective against flares in pregnancy (168).

A prospective, controlled study of 78 pregnancies in 68 SLE patients by Ruiz-Irastorza et al. found that 65% of pregnant patients flared, versus 42% of the 50 nonpregnant SLE controls (p = 0.015) (122). A flare was defined as an increase of ≥0.26 in the LAI. Flare rates per patient/month were 0.082 in the pregnant and 0.039 in the nonpregnant patients (p = 0.0015). A subset of 43 patients had higher flare rates during pregnancy versus the postpartum period, when SLE was controlled (0.093 vs. 0.049). Serious flares, affecting the kidneys and central nervous system (CNS), were equally distributed in the two groups.

A prospective study of 103 pregnancies in 60 patients by Cortes-Hernandez et al. showed that the prepregnancy flare rate of 0.4 per person-year, increased to 1.2 per person-year during pregnancy (138). Flare was defined as any clinical disease activity event that required a change in therapy, and patients were assessed with SLEDAI.

Summary

- There has been lack of uniformity in the definition of flare, but there are attempts at consensus.
- Six studies have found increased flares during pregnancy and postpartum, and three studies found a similar risk of flare regardless of pregnancy status.
- Flare rates are remarkably constant in the nonpregnant patients: when the data in the Garsenstein et al. (111), Mintz et al. (75), Petri et al. (169), Wong et al. (125), Ruiz-Irastorza et al. (122), and Cortes-Hernandez et al. studies (138) are expressed as flares per patient-month, these rates are 0.03 to 0.05 (0.039565, 0.040598, 0.054316, 0.041907, 0.039, and 0.03333 respectively), about one flare for every 2 years (Table 51-2). This suggests, however, that over four decades, the rate of flare in SLE has not changed materially.
- Most studies show that the proportion of severe flares has lessened.
- The differences in flares during pregnancy in these nine studies may be explained by differences in patient populations, flare definition, and evolving therapeutic strategies since the introduction of corticosteroids.

Course of Lupus in Pregnancy—Studies in the 1980s (Table 51-1)

Studies with a minimum of 10 lupus pregnancies are reviewed here, and Chapter 52 reviews fetal effects. Of 13 studies in the 1980s, 10 are retrospective (89 ,90 ,93 ,94 ,95 ,96 ,97 ,101 ,107 ,108 ,112) and three are prospective with control groups (75 ,113 ,114). Maternal flares ranged from 21% to 60%, with a mean of 33%. The definition of flares was not uniform: Gimovsky et al. (93) defined as flare that which required hospitalization, whereas Mintz et al. (75), in their prospective study, accounted for even mild mucocutaneous and articular flares. Postpartum flares occurred in 2% to 42% of pregnancies, with a mean of 17%. It is of interest that only two series claimed that there was a spontaneous remission of SLE in pregnancy (95 ,107), and there was no statement of remission in subsequent time periods. Maternal deaths were few, primarily noted in a study of patients with SLE nephritis (97), and ranged from 0% to 11%, with a mean of 1.7%, well below that of the 1950s and 1960s. Onset of lupus during pregnancy or postpartum was noted in 0% to 37%, with a mean of 12.5%.

Prospective Studies

Three prospective studies on lupus pregnancy appeared in the 1980s (75 ,113 ,114). In 1984, Lockshin et al. compared 33 pregnancies to nonpregnant patients (113). A flare was defined as new signs from a previously inactive organ system, or an increase in corticosteroid dosage, or the treating physician's statement that a flare was present. Five of 8 instances of thrombocytopenia were attributed to SLE (24%), 4 patients developed new proteinuria that subsided after delivery, and corticosteroid dosage was increased in 7 patients. There were no significant differences in flares between pregnant and nonpregnant groups, laboratory values, dosage, and number of patients treated with prednisone. These patients and controls seemed to have mild SLE, as 40% did not require steroids during the observation period.

In a 9-year prospective, controlled study from Mexico City, Mintz et al. reported 102 consecutive pregnancies in 75 patients (75), who were followed closely by a team of rheumatologists, obstetricians, and neonatologists. There were no elective abortions, and all patients received a minimum of 10 mg of prednisone daily from diagnosis of pregnancy to the end of postpartum. Of the 102 pregnancies, 10 started with active maternal disease, and in the remaining 92, a 60% flare rate was noted; 54% of flares occurred in the first trimester and 20% in the postpartum or post–spontaneous abortion periods. A matched group of women with SLE served as controls in this study and in a study of progestogens as contraceptives during the same 9 years (170). The pregnant patients had 55 flares in 909 patient-months and the controls had 19 in 468 patient-months, which is not statistically different (0.06 vs. 0.04 per patient/month). Most flares were mucocutaneous and articular, with mild fever and serositis, controlled by increasing prednisone to 15 to 45 mg/day. Severe flares included nephritis in 9 patients, CNS manifestations in 8, profound cytopenias in 1 and multisystem flare with vasculitis in 1. Most severe flares were treated with 60 mg/day, and two with 200 mg and 300 mg of prednisone per day, respectively. No maternal deaths occurred in this study of seriously ill patients.

In 1989, Lockshin (114) reported on 80 pregnancies, including 16 of the 33 in the 1984 study, without a control group. One third of the patients were on steroids at the time of pregnancy, which defines them as having mild disease, and, by global criteria, 26% had clinical evidence of active SLE during pregnancy.

Lupus Pregnancy Studies in the 1990s

The continued interest in SLE pregnancy produced 26 studies with 21 to 634 pregnancies each in the 1990s (115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129 ,130 ,131 ,132 ,162 ,171 ,172 ,173 ,174 ,175 ,176 ,177), for a total of 2,275 pregnancies in more than 1,200 women. In several studies (127 ,162 ,172 ,173 ,174), there is no information on maternal flares, and five studies (171 ,172 ,175 ,176 ,177) are considered under nephritis. Seventeen studies report on maternal flares and are included in Table 51-1 (115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,128 ,129 ,130 ,131 ,132). Eight studies were prospective (115 ,116 ,117 ,118 ,121 ,122 ,123 ,125), two were controlled (122 ,124), three were both prospective and controlled (120 ,122 ,125), and nine were retrospective (126 ,127 ,128 ,129 ,130 ,131 ,132 ,162 ,172) (Table 51-1). In 4 studies, the majority of pregnancies were conceived during inactive lupus at the prompting of the investigators: 100% (118), 95% (128), 94% (132), and 90% (115). Flares were seen in 43.6% of pregnancies, with a range of 2% to 95%. In the 2 months postpartum, flares ranged from 0% to 63%, with four studies reporting no postpartum flares (116 ,123 ,125 ,130). All of these patients were well cared for, and the great majority was in remission at pregnancy onset. In the 4 studies with SLE inactivity at conception, flares were 27.3% on average (20.7% to 33%), and postpartum flares were 4.9% to 9.4% (115 ,118 ,128 ,132). In the remaining 13 studies, flares occurred in 49.4% on average. Two patients were reported with SLE onset in pregnancy, 3.3% of 60 pregnancies (115), or 0.19% of 1,056 pregnancies in the 1990s.

There were only two maternal deaths in these 17 series of 765 women, for the very low maternal death prevalence of 0.26%. However, if we account for all publications of lupus pregnancies reported in the 1990s, including those without information on maternal flares, the prevalence of maternal death is the lowest ever, 0.17%, or 2 deaths in over 1,200 women.

Prospective and Controlled Studies

Wong et al. reported from Hong Kong on 29 pregnancies in 22 patients (125). Flares in 58% of patients included nephritis (*n* = 6), arthritis (*n* = 5), and vasculitis (*n* = 2). At the 30th week of pregnancy, steroid dose was increased to 10, 20, or 30 mg if the patients were taking less than 10 mg, 10 mg, or more than 10 mg daily, respectively. If a higher dose was required, it was maintained until 4 weeks after delivery. No postpartum flares or maternal deaths occurred. The flare rate during pregnancy was significantly higher than that of the nonpregnant patients (Table 51-2).

Petri et al. defined flare by the use of a visual analogue scale, graded from 0 to 3 (physician's global assessment) (120): of the 27 flares seen in 74 pregnancies (36%), 30% were mild, 59% were moderate, and 11% were severe, necessitating a prednisone increase of 43 ± 25 mg per day.

Tincani et al. (123) assessed SLE activity by the SLAM score(153) in 25 pregnancies and found 4 renal flares out of 11, with skin, joint, thrombocytopenia, and neuropsychiatric events accounting for the remainder. Most flares were treated with prednisone and azathioprine.

Derksen et al. used the SLEDAI to assess lupus activity every month, from 6 months before pregnancy to 6 months postpartum (116). Flares occurred in 6 of 35 pregnancies (17%), necessitating prednisone treatment in three patients with polymyositis, thrombocytopenia, and a combination of severe synovitis, vasculitis, and proteinuria. Increasing or new proteinuria without urine sediment abnormalities in an additional 5 patients was considered to be PIH and not treated.

In a multicenter study from France, Huong et al. observed flares in 34 of 75 patients with inactive lupus at conception (45%), with 9% of flares in postpartum and 2 deaths from opportunistic infection in mothers treated with steroids for nephrotic syndrome (117).

Lima et al. reported 108 pregnancies in 90 SLE patients, with 74 flares in 62 pregnancies (57%) (119). Most flares occurred in the second trimester (38%) and postpartum (35%). One in 5 flares (20%) was severe, 24% moderate, and 56% mild. Ten patients (9%) had active lupus at pregnancy onset and 2 had elective abortion. Active (*n* = 12) or prior nephritis (*n* = 2) in 14 pregnancies was present in 5 of 6 preeclampsia episodes. The patients were 79% white, and in 65%, SLE manifestations were cutaneous and articular, with nephritis, hematologic, and neuropsychiatric lupus in only 16%, 10%, and 9%, respectively. The patients were on prednisolone, azathioprine, and hydroxychloroquine, and flares were treated with dose modifications of these drugs.

The controlled study by Ruiz-Irastorza (122) was mentioned above (see SLE Activity During Pregnancy) and is

from the same lupus research unit in London as the study by Lima et al. (119). We are unaware of any patient duplication in these studies.

Tomer et al. (124) reported from Israel on 54 pregnancies of 46 SLE patients and 70 non-SLE pregnant controls, with only 14.5% flares during pregnancy, and the postpartum period was excluded from follow-up. The authors attributed the low flare rate to prophylactic prednisone that patients received; however, similar regimens did not prevent flares in the Mintz (75) and Tincani studies (123).

Huong et al. (117) reported 62 planned pregnancies in 38 women, who conceived after SLE inactivity for a year on ≤20 mg prednisone per day (118). Prednisone was maintained at ≥10 mg/day upon diagnosis of pregnancy and flares occurred in 27%, with 6% in postpartum. Flares were moderate, except for a patient with class IV nephritis. Preterm birth and cesarean section were common.

Rahman et al. from the University of Toronto studied 141 pregnancies in 73 lupus patients and defined active lupus as SLEDAI ≥1 (121). Flares were present in 63% of pregnancies, and renal flares were predictive of fetal loss. Patients were most commonly treated with steroids, antimalarials, and azathioprine.

Carmona et al. from the University of Barcelona reported 60 pregnancies in 46 SLE patients, with inactive SLE at conception in 54 of 60 (90%) (115). There were mostly moderate flares in 25% during pregnancy (0.044/patient/month), and 9.4% in postpartum. Hypertension and smaller gestational age at delivery were significantly associated with lupus nephritis in 10 pregnancies (50% vs. 11.6% and 35.9 vs. 37.3 weeks). This is the only study in the 1990s that reports SLE diagnosed in 2 patients during pregnancy, or 3.3%. The authors used prednisone prophylaxis in the last month of pregnancy and first month of postpartum from 1985 to 1994 and stopped in 1995.

Retrospective Studies

Six retrospective studies in the 1990s reported on 868 pregnancies in 470 women (126 ,128 ,129 ,131 ,132 ,162). The largest was by Pistiner et al. on 634 pregnancies in 307 women (162). Flares in the six studies were mostly mild and ranged from 20.7% to 95% (mean, 57.3%, with only two studies reporting postpartum flares (128 ,129). There was no SLE onset during pregnancy or maternal deaths in these series. Patients from a large private practice (Dr. Wallace) had well-controlled lupus (162), with less nephritis (28%) and neuropsychiatric disease (11%), whereas the indigent SLE population at Los Angeles County- University of Southern California Medical Center in the same metropolitan area had more than 50% prevalence of nephritis (159).

Studies in 2000 to 2005

Of the 20 studies published in 2000 to 2005 (133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,157 ,178 ,179), four are controlled (134 ,140 ,148 ,149), 1 is controlled and prospective (133), 4 are prospective (137 ,138 ,143 ,157), and 11 are retrospective (135 ,136 ,139 ,141 ,142 ,144 ,145 ,146 ,147 ,178 ,179) (Table 51-1). All studies except 1 (149) utilized the ACR (formerly ARA) classification criteria for SLE (150). The 6 studies that address SLE nephritis and pregnancy and a study on pregnancy outcome of SLE women with kidney transplants (179) will be discussed in the nephritis section, and Chapter 52 discusses two studies that deal primarily with fetal outcome (149 ,178).

Georgiou et al. from the University of Ioannina, Greece reported 59 pregnancies in 47 SLE patients, with 59 nonpregnant SLE controls and 59 healthy pregnant women (140). Flares are described in 8 of 59 pregnancies (13.6%), and 6 of 8 had nephritis. The authors noted, however, clinical features of SLE during the first trimester in 57%, the second and third trimester in 13% each, and after delivery in 19%. The majority of patients were treated with ≤10 mg of prednisone/day, azathioprine, and hydroxychloroquine, and flares were treated with ≤60 mg of prednisone/day. There was one maternal death in a patient with severe nephritis.

De Bandt et al. from Paris reported 59 pregnancies in 31 women, with 55 (93%) of pregnancies during “inactive” SLE (SLEDAI ≤4) and on ≤10 mg of prednisone/day (139). These patients would not be considered inactive by the Toronto group (121). There were 12 flares (20.3%), 6 severe during pregnancy (4/6 in active patients), and 6 mild in postpartum.

Brucato et al. from the University of Milan reported on 147 pregnancies in 111 lupus patients, in a study designed to assess anti-Ro effects on pregnancy outcome (133). There were 26 major flares in 147 pregnancies (17.7%), and no postpartum flares or maternal deaths. In 100 pregnancies of anti-Ro positive women there were 2 infants with congenital heart block.

Cortes-Hernandez et al. from Barcelona examined predictors of fetal and maternal outcome in 60 SLE patients with 103 pregnancies (138). Of the flares in 33% of the pregnancies, 10.7% were severe, with the majority, 19.4%, in the postpartum period. Most flares were musculoskeletal and cutaneous, 4 were renal, and 1, thrombocytopenia. Onset of SLE in pregnancy was seen in 2 of 60 patients (3.3%), or 2 of 103 pregnancies (1.9%). SLE was active at conception in 6.8% of pregnancies (12% of patients). Cesarean section was required in 20% of deliveries. Preeclampsia occurred in 2, PIH in 5 (4.9%), gestational proteinuria in 12 (11.7%), and thrombotic events in 4 patients with APL antibodies, in spite of heparin treatment. Increased SLE activity in pregnancy was noted, with 1.2 flares per person-year during and 0.4 before pregnancy. Predictors of flare were more than 3 flares before gestation with a SLEDAI of ≥5, a higher SLEDAI during SLE evolution, and prior chloroquine treatment.

Tan et al. from Singapore reported 27 pregnancies in 18 Asian women (147). SLE was in remission at pregnancy onset in 26 of 27 (96.3%), renal impairment was present in 7 pregnancies/6 women, 2 of whom were on chronic dialysis. Flares were seen in 7.4% of pregnancies, all in postpartum. Preeclampsia (18.5%) occurred in 5 of 6 pregnancies with pre-existing hypertension.

Ruiz-Irastorza et al. found 26.3% flares in 38 women during pregnancy, in their evaluation of the LAI (157).

Chandran et al. in a retrospective study from India, examined 52 pregnancies in 31 women, 31 with inactive, and 21 with active SLE, including 12 patients with nephritis and 6 with neuropsychiatric (CNS) SLE (136). Flares were defined as any SLE activity that needed a change in therapy, or as a SLEDAI increase of >5, in contrast to definitions of >1 or >3 (121). There were no flares, maternal deaths, or SLE onset in the inactive SLE group, whereas the active group had 14.3% flares, 9.5% in the postpartum, and 3 patients with SLE onset in pregnancy (14.3%), 1 of whom died of severe flare with pulmonary hemorrhage (1.9% of 52 pregnancies).

Molad et al. reported 20 patients with 29 pregnancies from Israel and defined mild SLE activity as SLEDAI of 2, active SLE as SLEDAI ≥4 at the onset of pregnancy, and postpartum SLE flare as onset of new signs of SLE in a previously normal organ with or without abnormal laboratory studies, an increase of serum anti-dsDNA or a decrease in serum C3 and C4 (143). High pregestational activity by SLEDAI, and laboratory data compatible with active SLE were predictive of postpartum flares (20.7%).

Clowse et al. from Johns Hopkins University studied the impact of increased SLE activity in 267 pregnancies in 203 women over a 16-year period, and focused on moderate and severe flares (137). SLE activity was quantitated by a variation of the physician's global assessment, the Physician's Estimate of Activity (PEA), graded from 0 to 3. Lupus activity was considered high with scores of ≥2, and low ≤2. High activity was seen in 57 of 267 pregnancies (21%), was distributed evenly throughout the three trimesters, and was more common before 1995, in African-American women, and in women with SLE nephritis. High activity during the 6 months prior to conception was followed by high activity during pregnancy in 58% of 12 patients, and in only 8% of 84 women with low activity before pregnancy, the difference being highly significant. This study corroborates very well the results of prior studies (98 ,138 ,168), and the recommendation to plan conception after SLE remission of 6 months. In addition, fetal loss was 42% in high activity pregnancies, and only 11% in low activity pregnancies. There were 36 patients diagnosed with SLE during pregnancy (13.5%), and 3 maternal deaths (1.1%). The majority of patients were treated with prednisone (62% with low, 95% with high activity), one third with hydroxychloroquine, and 25% of high activity patients received azathioprine. The impact on fetal outcomes was substantial: pregnancy loss was threefold in high-activity pregnancies, full-term births were reduced (26% vs. 61% in pregnancies with no or mild activity), and live births were less (77% vs. 88%).

Chakravarty et al. from Stanford University reported 63 pregnancies in 48 women with SLE, 35% of whom had prior renal disease and 10% had previous CNS SLE (135). Active SLE at conception was defined as the use of ≥10 mg of prednisone per day, the use of any immunosuppressive drug, or a SLEDAI ≥2. Flares were defined as in the SELENA study (Safety of Estrogen in Lupus Erythematosus National Assessment) (180) and distinguished into mild/moderate and severe. There were 67.7% flares, 29% of them severe, all noted during pregnancy. Preeclampsia developed in 12/63 pregnancies (19%), and was severe in 7, with most of the patients having prepregnancy nephritis. The 4 of 48 women (8.3%) who developed SLE during 63 pregnancies (6.3%) had severe flares, with preeclampsia in 2 of 4. Two patients had HELLP syndrome, and 1 of them died later (1.6%). Thrombocytopenia was associated with a relative risk of 3.2 for preeclampsia, but the finding was not significant when the first pregnancy only was considered.

Pregnancy with pulmonary hypertension during SLE or antiphospholipid syndrome (APS) is an uncommon event with high maternal mortality, up to 56% (181 ,182). Most deaths occur within 3 days of delivery from acute cor pulmonale, because of high maternal blood volume and high pulmonary vascular resistance. Hopefully, the prognosis will improve with the introduction of pulmonary vasodilators (epoprostenol, bosentan, sildenafil).

CNS lupus during pregnancy can be accompanied by severe fetal prematurity and IUGR and poor maternal outcome (183).

Summary

Conclusions about SLE activity during pregnancy can be summarized as follows:

- Fertility in women with lupus is normal, except for amenorrhea and infertility during periods of severe disease activity.
- Despite the variability of definition and assessment of disease activity and disease severity, flare assessment is increasingly done with standardized methods, the majority of patients have inactive or well-controlled disease at the onset of pregnancy, and therapy is prompt and decisive.
- The average probability of flare during SLE pregnancy and postpartum has decreased substantially over the last 55 years, from 60% in the presteroid era, to 23.3% in the beginning of the 21st century. In contrast with studies of the 1950s, 1960s, and 1970s, most flares after 1980 are minor, with arthritis and cutaneous manifestations. Severe flares occur in approximately 10% to 29% of pregnancies and, for this reason, SLE pregnancy should still be handled as a high-risk pregnancy.
- Risk factors for flares include SLE activity during the 6 months preceding conception, and pre-existing renal disease (see also below). Conversely, conception during quiescence or remission is associated with a lesser risk for flare (8% to 15%). Mild lupus without vital organ involvement rarely exacerbates during pregnancy.
- Most studies avoid corticosteroid treatment for prevention of flares, and instead watch the patient very carefully and raise steroid dose at the slightest indication of flare.
- Most pregnant women in the 2000s were treated with steroids, hydroxychloroquine, and azathioprine.
- There is a greater prevalence of preeclampsia and PIH in lupus patients, especially with prior nephritis and antiphospholipid antibodies (see below).
- Onset of SLE during pregnancy or the postpartum period was seen in approximately 20% of patients (13% to 50%) in

older reports (95 ,97 ,110 ,184), and in 0% to 13.5% in recent reports (135 ,136 ,137 ,138): it can be a severe, catastrophic illness, with dire maternal and fetal outcome. A high index of suspicion for SLE should prevail when, during pregnancy or the early postpartum period, a young woman has unexplained rashes, arthritis, alopecia, proteinuria with active urine sediment, psychosis, chorea, pleuropericarditis, or vasculitis, alone or in combination. Delay in diagnosis and management can be lethal, whereas prompt, aggressive treatment is lifesaving.

Course of Lupus Nephritis during Pregnancy

Reports on lupus pregnancy from the 1950s and 1960s mention nephritis in the context of severe flare, increasing proteinuria, azotemia, or acute anuric renal failure, hypertension, preeclampsia, onset of nephritis during pregnancy, and even maternal death (83 ,91 ,103 ,111). Active nephritis at conception, often not detected early nor treated aggressively, can put the mother and fetus at grave risk.

On the other hand, in the late 1960s, 1970s, and 1980s, several papers reported an uneventful pregnancy course and better prognosis when renal function is normal and lupus nephritis has been inactive for 3 to 6 months before conception (75 ,83 ,89 ,90 ,95 ,97 ,98 ,107). This finding has been proven true through the 1990s and the 2000s.

Table 51-3 summarizes available data from 25 series published from 1978 to 2005 that address lupus nephritis and pregnancy (75 ,85 ,89 ,90 ,93 ,95 ,96 ,97 ,98 ,106 ,112 ,115 ,123 ,125 ,130 ,134 ,141 ,144 ,145 ,146 ,148 ,171 ,172 ,175 ,176 ,177). Although it is not a common practice, percutaneous needle biopsy can be performed during pregnancy under ultrasonic guidance (185).

In all, 918 pregnancies were reported in over 523 women with lupus nephritis. The prevalence of focal or diffuse proliferative nephritis was 40% to 100% (average, 64.6%), and nephrotic syndrome was present in 0% to 69.2% (average, 18%). The rate of flares in pregnancy was from 7.4% to 100% (average, 30.2%). Preeclampsia or severe PIH ranged from 0% to 50%, and hypertension was very common. Transient renal insufficiency occurred in 0% to 37% (up to 50% in active nephritis), and irreversible renal failure in 0% to 21% of patients (Table 51-3).

Flare rates in 115 pregnancies with lupus nephritis activity at conception or during pregnancy, were 48% to 100% (average, 57.9%) (89 ,95 ,96 ,98 ,144 ,145); flares in 256 pregnancies with inactive nephritis at conception were from 7.4% to 32% (average, 15.1%) (75 ,89 ,95 ,96 ,98 ,115 ,144 ,145 ,171).

Similar differences are seen in prevalence of nephrotic syndrome, preeclampsia, transient renal insufficiency, and renal failure in active versus inactive nephritis, with 38.1%, 38.3%, 32.7%, and 8% versus 8.8%, 13.2%, 5%, and 4.1%, respectively (Table 51-3).

Generally, studies of the 1990s and 2000s on SLE nephritis show a trend for planned pregnancies after several months of quiescence or remission, which should eventually improve the maternal and fetal outcome of these pregnancies.

Some of the most notable studies with the most patients are reviewed here.

Devoe and Taylor (85) reported 13 pregnancies in eight women, five with biopsy-proven nephritis and two with severe flares with decreased renal function and preeclampsia. The authors credited serum complement C3 and C4 levels with prognostication of SLE flare. In a subsequent report of 18 pregnancies in 15 women with SLE and renal biopsies prior to conception, decreased renal function, rather than severity of renal biopsy class correlated with abnormal fetal outcome (186).

Lupus Nephritis and Pregnancy in the 1980s

In Houser's report of 18 pregnancies in 11 patients with SLE nephritis, 10 pregnancies in 5 patients with inactive SLE were uneventful, whereas 8 pregnancies in 6 women with active disease included 3 with preeclampsia, severe in 2, and disease flare in a patient who developed class III lupus nephritis (96). All patients were receiving prednisone, and no maternal deaths occurred.

Hayslett and Lynn's questionnaire study of 13 nephrology centers and individual nephrologists reported 47 patients with 65 pregnancies, with focal or diffuse proliferative nephritis in 77% (95). In 9 instances, SLE began during pregnancy (14%), nephritis preceded conception in 80%, and nephritis worsened in 39% of the mothers. Of 25 pregnancies with active renal disease at conception, 12% improved, 48% worsened, and 40% remained unchanged. Pregnancy in patients with active SLE had a hectic course, successful outcome was reduced by 25%, and only 9 of 16 patients with nephrotic syndrome had successful deliveries. When the serum creatinine level was lower than 1.5 mg/dL, 9 of 10 pregnancies resulted in live births, but in 10 pregnancies with serum creatinine above 1.5 mg/dL, fetal loss was 50%. In 4 patients, however, with serum creatinine of 4 mg/dL or higher, pregnancies resulted in live births, indicating that a successful outcome is still possible despite severe renal failure.

Of 16 patients with prior nephritis reported by Zulman et al., 10 (62.5%) had flares during pregnancy (112). In 25% of patients, differentiation of acute presentation of lupus nephritis from toxemia was necessary, and the authors suggested that preeclampsia was a result of lupus nephritis.

Nearly one third of pregnancies reported by Fine et al. (12 of 37) resulted in worse renal function, 5 of them irreversibly (90). No conception occurred in patients with even moderate renal insufficiency. The authors recommended hemodialysis for pregnant patients with a blood urea nitrogen (BUN) of 50 mg/dL or greater and maintenance under that level. Volume removal should be done through isolated ultrafiltration, blood pressure should be supported with albumin, the dialysate should contain glucose and bicarbonate, low-dose heparin should be used, and progesterone should be administered, because the endogenous progesterone is lost in the dialysate.

Jungers et al. reported a retrospective study of 36 patients with 104 pregnancies seen between 1962 and 1980 (98).

Onset of SLE and nephritis occurred during pregnancy or postpartum in 25% of patients. All patients had clinical renal disease, and biopsies showed 5 with mesangial or minimal disease (World Health Organization [WHO] class II and I), 8 with focal proliferative (WHO class III), 6 with diffuse proliferative nephritis (WHO class IV), and two with membranous nephropathy (WHO class V). Flares occurred in 46%, and 8% progressed to irreversible renal failure. A full 66% of patients with active nephritis at conception had a flare; in contrast, only 9% of patients with inactive disease during the 5 months before conception had a flare.

Table 51-3: Course of Lupus Nephritis in Pregnancy

Study	Pregnancies (No.)	Proliferative Nephritis (%)	Nephrotic Syndrome (%)	Flare (%)	Preeclampsia (%)	Transient Renal Insufficiency/Irreversible Renal Failure(%)	
Thomas et al. (106)	13	64	31	55	—	23	
Devoe and Taylor (85)	13	63	—	15	8	15	
1980s							
Houser et al. (96)	18	40	—	28	22	33	
Inactive	10	—	—	20	20	20	
Active	8	—	—	50	38	50	
Hayslett & Lynn (95)	56	77	25	39	—	—	
Inactive	31	43	10	32	6	10/12	
Active	25	67	28	48	—	25/11	
Zulman et al. (112)	19	58	11	63	32	13/10	
Fine et al. (90)	37	—	—	23	—	32/13.5	
Jungers et al. (98)	35	89	3	46	—	/4	
Inactive	11	100	9	9	—	/9	
Active	24	85	17	66	—		
Gimovsky et al. (93)	46	79	—	22	25	21 /16	
Imbasciati et al. (97)	26	58	42	46	—	37 /21	
Mintz et al. (75) Inactive	58	41.8	—	10	0	0	
Bobrie et al. (89)	53	73	—	34	—	/11.4	
Inactive	27	—	—	7.4	—	5	
Active	26	—	—	62	—	—	
1990s							
Wong et al. (125)	29	42	21	16	0	__	
Oviasu et al. (130)	47	66	—	13	0	11	
Packham et al. (172)	64	—	17	48	5	19 /2	
Tincani et al. (123)	9	—	—	33	0	0	
Julkunen et al. (171) Inactive	26	42	4	8	30	0	
Font et al. (175) Nephritis	10	100	0	30	50 (HT)	0	
No nephritis	50	0	0	24	10 (HT)	0	
Huong et al. (176)	25	76	12	32	16	8	
Ramos et al. (177)	21	67	0	42.9	14.3	0	Death
2000-2005							
Cortes-Hernandez (138)	20	83.3	0	40	20(PIH)	5.1/15.1*	0
Huong et al. (141)	32	62.5	15.6	15.6/12.5**	15.6	3.1	3.1
Moroni et al. (144)	70	NS	17.1	40/38.6**	14.3	2.9/4.3	2.1
Known nephritis	57	NS	12.3	26.3	9.8	/3.5	2.6
Nephritis in pregnancy 13	75.0	69.2	100	38.5	23.1/7.7 0		
Tandon et al. (148)	78	57.9	1.3	44.6**	NS	17.3†	0
Soubassi et al. (146)	24	NS	NS	50.0	25.0	NS	0
Rahman et al (145)	55	58.3	NS	12.7	27.2	/1.8	8.3
Inactive	36	NS	NS	8.3	13.9 (PIH)	0	0
Active	19	NS	NS	21.1	46.2 (PIH)	/5.3	25.0
Carmona et al. (134)							
WHO III & IV	42	100	mean 3g/d	19/9.5**	28.1	0	0
II & V	12	0	mean 1.5 g/d	33.3/8.3**	0	0	0
No nephritis	54	0	mean 0.7 g/d	27.8	4.6	0	0

	No. Pregnancies	Percent			
		Nephrotic s.	Flares	Preeclampsia	Transient Renal Insuff/Failure
Active nephritis at conception	115	38.1	57.9	38.3	25.8/4.3
Inactive nephritis at conception	256	8.8	15.1	13.2	5/4.1

HT, hypertension.
*Two of 3 patients with renal failure had prior renal impairment
**Nephritis flares
†20% increase in serum creatinine
NS, not stated

Gimovsky et al. at the University of Southern California reported retrospectively on 39 patients, 19 with SLE nephritis, confirmed by renal biopsy in 15 (79%) (93). The 19 patients had 46 pregnancies, with lupus onset in pregnancy or postpartum in 8 of 39 patients (21%). Hospitalization was required because of flare in 9%, 8%, 14%, and 4% of patients during the three trimesters and postpartum, respectively. Preeclampsia occurred in 10 patients, 6 with nephritis. Of 19 patients, 4 (21%) developed decreased renal function within 2 years of delivery, and 3 required chronic hemodialysis (16%). The nephritis group had a fetal loss of 55%, with 18 of 40 pregnancies resulting in live births.

Imbasciati et al. from the University of Milan, Italy, reported 19 SLE nephritis patients with 26 pregnancies, class IV nephritis in 58%, and nephrotic syndrome in 42% (97). Transient renal insufficiency was seen in 37%, and renal failure in 21%. Four women developed anuric renal failure, 3 among 7 with SLE and nephritis onset during pregnancy, and 2 died, 7.7%. In all but two pregnancies, SLE was active, and by current standards, steroid dosage was low or of short duration, with inadequate control of maternal disease. In 7 flaring patients, mainly postpartum, high-dose steroid (≥50 mg prednisone/day) controlled SLE activity. This study is valuable, as multiple renal biopsies performed in 7 patients showed that 4 patients had progression of a minimal, focal proliferative, or membranous lesion to a diffuse proliferative nephritis, and 2 patients had regression of diffuse proliferative nephritis to focal proliferative in 1 and to minimal lesion in the other patient. Fetal outcome was poor, with 61% live births.

In 1987, Bobrie et al. (89) from Paris added 32 more pregnancies to their previous report (98). Of the 73 pregnancies reported, 14 had SLE onset during pregnancy, postpartum, or postabortion periods (18.4%). Half of these 14 patients had nephrotic syndrome with increased serum creatinine, and all had proliferative glomerulonephritis, diffuse in 11; high-dose steroid therapy improved all but 1 patient, who progressed to renal failure in 2 years. In 53 pregnancies after onset of SLE, nephritis flares occurred in 34% during pregnancy (*n* = 10) or postpartum (*n* = 8), and were more frequent with active SLE at conception (61.5%), than with stable remissions (7.4%). Four patients (11.4%) rapidly progressed to end-stage renal failure despite corticosteroid therapy.

In the prospective study from Mexico City, 58 of 102 pregnancies occurred in patients with known, inactive SLE nephritis, and all but three had previous renal biopsies (75 ,187). Proliferative glomerulonephritis was present in 23 (40%), treated with 30 to 60 mg of prednisone daily (75 ,187). In the 58 pregnancies with SLE nephritis, 6 flares occurred (10.3%) in patients with inactive renal disease for 2 to 5 years, and all responded to increases in prednisone dose. On long-term follow-up of 75 total patients for up to 5 years, renal function deteriorated in 3 of 15 patients with proliferative nephritis, with 1 of 5 deaths from renal disease, 49 months after pregnancy. Of the 44 pregnancies without previous kidney disease, nephritis appeared in 3 patients during pregnancy (6.8%); biopsies showed two with class II and one with class IV glomerulonephritis. All patients were treated with at least 10 mg of prednisone daily during the entire pregnancy. Pregnancy outcome was not different for patients with or without nephritis, and the authors attributed a better fetal outcome to inactive, well-controlled SLE, rather than to the absence of kidney disease.

Lupus Nephritis and Pregnancy in the 1990s

In the prospective study from Hong Kong (125), 6 nephritis flares occurred in 13 patients, with increasing proteinuria and nephrotic syndrome in 3 each. Two of the nephrotic patients remitted within 4 weeks after elective abortion and increased steroids, and the other 4 remitted with only increased doses of prednisone. Eight women with a history of preexisting diffuse proliferative nephritis had successful pregnancies, because of appropriate prednisone therapy per the authors.

In the 25 women with lupus nephritis reported by Oviasu et al. (130), a flare was seen in only 1 of 53 pregnancies, although 6 patients each had increased proteinuria and decreased creatinine clearance, and 9 patients had more than trivial cutaneous lupus. Hypertension was present and controlled in 17 of 47 pregnancies (36%), excluding elective abortions. No one developed renal failure, and cesarean section was necessary in 44% of the 39 completed pregnancies. 11 of the 17 cesarean sections (64.7%) were performed as emergencies). Preterm deliveries were seen in 28%.

Among 64 pregnancies in 41 women with lupus nephritis reported by Packham et al. from Australia (172), proteinuria was stable in 16%, increased in 48% of pregnancies, with nephrotic syndrome in 17%, and was irreversible postpartum in 5% (3 patients). Hypertension was present in 44%, was severe in 13%, and irreversible in 9 patients (14%). Eclampsia occurred in 3 women (5%). Transient renal insufficiency was seen in 19% and renal failure in 1 patient (2%). Prematurity occurred in 19 of 43 live births (44%).

Of 25 pregnancies reported by Tincani et al. (123), 9 occurred in seven women with nephritis. There were three nephritis flares in these patients and de novo nephritis in a fourth.

Julkunen et al. reported 26 pregnancies in 16 patients with lupus nephritis, 42% with focal or diffuse proliferative nephritis, all clinically inactive with normal renal function (171). Two patients flared (8%), one with nephrotic syndrome (4%), 7 of the 23 completed pregnancies were complicated by preeclampsia (30%), and 7 of 23 births (30%) were premature. There was no compromise of renal function.

Carmona et al. from Barcelona reported 10 pregnancies in 9 patients with lupus nephritis, compared to 50 pregnancies in 37 SLE patients without nephritis (115). Flares occurred in 30% of pregnancies, hypertension and preeclampsia were more prevalent in the nephritis patients (50% vs. 11.6%), cesarean sections were more frequent (60% vs. 18%), and neonates were of lower gestational age and birth weight.

Huong Du et al. (176) from Paris reported 25 pregnancies in 16 women with biopsy-proven SLE nephritis, proliferative in 76%. There was proteinuria in 32%, nephrotic syndrome in 12%, flares in 32%, preeclampsia in 16%, transient renal insufficiency in 8%, 1 maternal death (4%), and 15 premature births (60%). A second maternal death occurred 4 years after delivery. Treatment included prednisone, mean 22 mg/day, aspirin and heparin for antiphospholipid syndrome, azathioprine, and, after delivery, cyclophosphamide in 2 patients with renal flares.

Ramos et al. (177) from Mexico City reported 21 pregnant patients with lupus nephritis, proliferative in 67% (mostly class IV), assessed by SLEDAI. Renal flare occurred in 42.9% (first and second trimester), preeclampsia in 14.3%, and fetal loss in 14.3%. No progression of renal disease was noted. Treatment included prednisone (mean 20 mg/day), aspirin, and heparin for antiphospholipid syndrome.

The outcome of SLE nephritis and pregnancy in the 1990s is far better than that of previous decades.

Lupus Nephritis and Pregnancy in 2000 to 2005 (Table 51-3)

Although all articles in this time period include patients with SLE nephritis in pregnancy, one gives adequate details (138) and six series specifically address the subject (134 ,144 ,145 ,146 ,148 ,188).

The above studies addressed 333 pregnancies in 226 women. Fifty-four pregnancies in 54 women without nephritis were used as controls in Carmona et al.'s study (134). In 240 pregnancies proliferative classes of nephritis (WHO III and IV) were most common, 72.8%, ranging from 57.9% to 100%. As would be expected, flares, nephrotic syndrome, preeclampsia, transient renal insufficiency, and irreversible renal failure were more common in pregnancies with active nephritis at conception, or during pregnancy (Table 51-3). Maternal deaths occurred in three studies (141 ,144 ,145). The simultaneous presence of APL further complicated outcomes. The impact on fetal outcome was very substantial, with perinatal (fetal) loss, prematurity, and IUGR/SGA being very prevalent, and more so with active nephritis. Live births ranged from 42.1% to 94%, (average, 71.5%), fetal loss ranged from 6% to 57.9%, prematurity from 31.7% to 77.8%, and IUGR from 4.8% to 37.5%.

In the majority of patients lupus was inactive or quiescent at pregnancy onset, and these patients had a better pregnancy course with fewer flares, less proteinuria, preeclampsia, and a better fetal outcome. With better guidelines to distinguish renal flare from PIH and preeclampsia, there is a clearer picture conveyed by these publications.

Among 103 pregnancies in the series by Cortes-Hernandez, 20 pregnancies occurred in 12 lupus nephritis patients (138). The majority had proliferative nephritis (83.3%), none had nephrotic syndrome, there were 40% flares, and PIH in 25%. In 1 of the 5 pregnancies with hypertension, it was a result of a renal flare. There was transient renal insufficiency in 1 pregnancy (5%), and permanent worsening of renal function in 3 patients (15%), 2 of whom had prior renal insufficiency. The live birth rate was 42% in the nephritis pregnancies, and almost double (76%) without nephritis.

Of 32 pregnancies in the study by Huong (141), 25 were planned; 18 pregnancies were previously reported (117). Almost two thirds had proliferative classes of nephritis (62.5%), 15.6% had nephrotic syndrome, most of the flares were renal flares (15.6% and 12.5%), preeclampsia with features of HELLP syndrome was present in 15.6%, transient renal insufficiency in 3.1% and 1 patient died (3.1%). Flares in this study were halved, compared to their previous report (176). Most patients were treated with prednisone and hydroxychloroquine, and low molecular weight heparin (LMWH) was given to 9 women with prior history of thrombosis or miscarriages.

Moroni et al. reported on 48 women with 70 pregnancies, 57 pregnancies in 38 women with known SLE nephritis, and 13 pregnancies with nephritis developing in pregnancy, group A (144). Some of the patients were previously reported (97). Three quarters of biopsied group A patients (9/12) had proliferative lesions (7 class IV, two class III). In the group with active nephritis there was strikingly greater prevalence of nephrotic syndrome (12.3% vs. 69.2%), flares (26.3% vs. 100%), preeclampsia (9.8% vs. 38.5%), and transient and irreversible renal failure. Of three patients from group A who developed acute renal failure postpartum, 2 were treated with prednisone and azathioprine and 1 with methylprednisolone pulse and oral cyclophosphamide; 2 patients recovered after weeks of peritoneal dialysis and the third developed end-stage renal failure. The only predictor of favorable maternal outcome was quiescence of renal disease. Poor fetal outcome predictors were proteinuria, hypertension, and positive APL.

In a nested case-control study, Tandon et al. from the University of Toronto compared 78 pregnancies in 53 women to 78 nonpregnant patients, with SLE nephritis in both groups (148). The objective of the study was to assess the effect of pregnancy on SLE nephritis. The prevalence of nephritis activity during pregnancy was similar to that of controls (44.6% vs. 41.9%), and similar proportions of pregnant and nonpregnant patients showed a 20% increase in serum creatinine (17.3% vs. 24%).

Mok et al. from Hong Kong published a brief retrospective report on 91 pregnancies in 66 lupus patients (142). These included 33 pregnancies in 27 SLE patients with prior nephritis. Renal disease was quiescent at conception and no information is given about maternal problems. The fetal outcome was excellent with 94% live births, 6% fetal loss, 12.9% prematurity, and 19.4% IUGR.

Soubassi et al. from the University of Athens, Greece, reported 24 pregnancies in 22 patients with SLE nephritis

(146). There were flares with proteinuria in 50%, hypertension in 42%, and preeclampsia in 25%. Fetal outcome included live births in 75%, perinatal loss of 25%, and prematurity in 77.8%. No information is given about the proportion of proliferative nephritis or of nephrotic syndrome.

Rahman et al. from London and Saudi Arabia reported 55 pregnancies in 24 patients, 36 pregnancies during quiescent nephritis (16 patients), and 19 pregnancies with active SLE nephritis at conception (8 patients) (145). Proliferative nephritis was present in 58.3% of the entire group. Most rheumatologists would argue with the authors' definition of active clinical renal disease as serum creatinine of >3 mg/dL and glomerular filtration rate of <65 mL/min/1.73m^2. Pregnancies with active nephritis had more than twofold flares (21.1% vs. 8.3%), more than threefold PIH (46.2% vs. 13.9%), renal failure occurred in 5.3% versus 0%, and 2 mothers died.

Carmona et al. from Barcelona, Spain, published an excellent study on pregnancy in proliferative SLE nephritis (classes III and IV) and compared it to SLE pregnancies with mesangial and membranous nephritis (classes II or V), and with SLE pregnancies without nephritis (134) (Table 51-3): there were 42 pregnancies in 35 women with class III or IV, 12 pregnancies in 10 women with class II or V, and 54 pregnancies in as many women without nephritis. SLE was active at conception in 21.4% of the first, 33.3% of the second, and 11.1% of the third group. Flare rates were similar in the three groups (19%, 33.3%, and 27.8%), and renal flares were 9.5% in the proliferative group and 8.3% in the mesangial-membranous group. Proteinuria increased significantly in conjunction with the renal flares in proliferative nephritis (mean, 3 g/day), moderately, up to 1.5 g/day in the second group, and remained stable at about 0.7 g/day in the group without nephritis. Renal flares were treated with increased prednisone and with azathioprine. Hypertension and preeclampsia were almost exclusively seen in the proliferative group (37.5% and 28.1%) (Table 51-3). Cesarean section was required more frequently in the nephritis groups, 43.7% and 33.3% versus 18.6%. Preterm birth was not different between groups, but birthweight was significantly less in the proliferative nephritis group. The authors concluded that class III and IV lupus nephritis are risk factors for hypertensive disease in pregnancy.

The outcome of 60 pregnancies in 38 SLE patients with kidney transplants was published in a retrospective study by McGrory et al. (179). SLE pregnancies were compared to 374 pregnancies in 247 patients transplanted for other reasons. Most SLE patients were on cyclosporine A (*n* = 39) or azathioprine (*n* = 21) for transplant maintenance. Mycophenolate mofetil (MMF) was discontinued in one patient upon diagnosis of pregnancy. Drug-treated hypertension (45%), and Cesarean section (30%), were significantly less common in the SLE pregnancies, whereas preeclampsia (17%), rejection (5%), and infections (15%) were similar in the non-SLE pregnancies. No maternal SLE flares were reported. Live births were seen in 73%, with 43% preterm births, low birth weight in 53%, and 1 neonatal death, The results of this study are very encouraging for SLE patients with kidney transplants. Of 25 SLE patients with kidney transplants and antiphospholipid antibodies, 15 had clinical thrombotic events, including 3 with late fetal loss (189).

Preeclampsia in Lupus Pregnancy

Preeclampsia, defined as the abrupt onset of hypertension and proteinuria after 24 weeks of gestation, is found in 0.5% to 10% of all pregnancies, and is far more common in the primigravida (190). The clinical manifestations of severe preeclampsia are a result of the presence of systemic endothelial dysfunction and microangiopathy, in which the target organ may be the brain (seizures or eclampsia), the liver (with microangiopathic hemolysis, elevated liver tests, and low platelets; HELLP syndrome), or the kidney (glomerular endotheliosis and proteinuria). It can also be associated with pulmonary edema, intrauterine growth restriction, and oligohydramnios. The risk of preeclampsia is higher in SLE pregnancy (12% to 32%) (88), compared to healthy women (191). Our understanding of the pathophysiology and the risk factors contributing to the development of preeclampsia has dramatically advanced over the past few years. Controlled cohort studies showed that risk factors for preeclampsia and the respective (relative risk values (RR) are, a previous history of preeclampsia (7.19), antiphospholipid antibodies (9.72), preexisting diabetes (3.56), multiple (twin) pregnancy (2.93), nulliparity (2.91), family history (2.90), hypertension (diastolic >80 mm Hg) (1.38), increased body mass index before pregnancy (2.47) or at the first prenatal visit (1.55), and maternal age >40 (1.96, for multiparous women) (192). Individual studies show that risk is also increased with an interval of 10 years or more since a previous pregnancy, autoimmune disease, renal disease, and chronic hypertension.

Although not classically considered a genetic disease, genetic factors contribute to the susceptibility to preeclampsia. Genome-wide scanning of Icelandic families revealed a significant locus on chromosome 2p13 (LOD 4.7) (193). The same locus was recently confirmed in a study of patients from New Zealand and Australia (194). Linkage to HELLP syndrome has been reported with a locus on chromosome 12q (195). Moreover, women with trisomy 13 fetuses have a higher incidence of preeclampsia (196).

Generalized vascular constriction is universally present in preeclampsia and is likely a result of endothelial dysfunction:

- Women with a history of preeclampsia have impaired endothelial-dependent vasorelaxation as measured by brachial artery flow-mediated vasodilatation for up to 3 years after delivery, implying these changes in the maternal circulation maybe more than transient.
- The production of prostacyclin (PGI2, a circulating vasodilator produced by endothelial cells) is decreased in preeclampsia compared to normal pregnancy. Endothelial cells incubated with serum from preeclamptic women produce less prostacyclin in vitro, suggesting the presence of a circulating inhibitory factor.

- Production and urinary excretion of thromboxane A2 (TXA2, a potent vasoconstrictor) and its metabolites is reported to be increased in preeclampsia by some investigators, with decrease in the ratio of prostacyclin to TXA2 metabolites. Low-dose ASA had a favorable effect on the prostanoid ratio (197). TXA2 production also appears to parallel the severity of preeclampsia.
- Studies in human pregnancy have reported decreased nitric oxide production in the setting of preeclampsia.
- Endothelin-1 (ET-1) concentration is increased in sera of patients with preeclampsia and endothelial cells cultured in the presence of plasma from preeclamptic patients show enhanced ET-1 production.

The placenta in preeclampsia is usually abnormal with evidence of hypoperfusion and ischemia, which occurs as a result of defective placental vascular remodeling. During early normal placental development cytotrophoblastic cells of fetal origin attach to the uterine decidua with anchoring villi, that are bathed in maternal blood from the spiral arteries filling the intervillous spaces. These extravillous trophoblasts invade the decidual part of the spiral arteries and replace their endothelial lining. As they do so, they switch their surface phenotype from epithelial cell origin (trophoblastic) into endothelial cell phenotype. As a result, the original small resistance-type vessel spiral arteries are transformed into flaccid, high-caliber capacitance vessels allowing the increase in the placental blood flow that is needed to sustain the fetus throughout the pregnancy. In preeclampsia, this phenotype switch does not occur, and the characteristic placental lesion in severe preeclampsia is diminished endovascular invasion by cytotrophoblasts and failure of uterine spiral arteriolar remodeling. Preeclampsia occurs only in the presence of the placenta, even when there is no fetus (hydatidiform mole) and remits dramatically postpartum when the placenta is evacuated. Recently, gene expression profiling was used to search for candidate factors produced in the placenta that might be responsible for the clinical expression of preeclampsia. Using this approach, it was shown that placental sFlt-1 mRNA (soluble fms-like tyrosine kinase-1) is upregulated in preeclampsia. sFlt-1 is a splice variant of the vascular endothelial growth factor (VEGF) receptor Flt-1 that lacks the cytosolic and transmembrane domains. sFlt-1 acts as a potent VEGF and PlGF (placental growth factor) antagonist by binding these molecules in the circulation. It was recently shown that there is marked rise in circulating sFlt-1 concentration beginning 5 to 6 weeks before the onset of clinical preeclampsia and accompanied by decreases in the circulating VEGF and PlGF. In addition, decreased urinary concentrations of free PlGF during midgestation predict the subsequent development of clinical preeclampsia. The possibility that antagonism of VEGF and PlGF might play a role in preeclampsia has sound physiologic underpinnings. Other than a potent promoter of angiogenesis, VEGF is also known to induce nitric oxide and vasodilatory prostacyclins in endothelial cells. Moreover, exogenous VEGF and PlGF can reverse the antiangiogenic properties of preeclamptic serum as assessed by in vitro angiogenesis assays.

As mentioned, the prevalence of preeclampsia in lupus pregnancies is significantly higher compared to healthy women (8% to 38% vs. 3% to 5%). Preeclampsia is more likely to occur in patients with active lupus (29% vs. 6.4% for inactive disease, $p<0.05$), with lupus nephritis, especially if active (RR, 3) (93 ,96 ,112 ,144 ,145), (Table 51-3), with SLE onset during pregnancy (50%), and with the antiphospholipid syndrome, in the presence or absence of SLE (198 ,199 ,200 ,201). Patients with class III and IV SLE nephritis have a significantly higher prevalence of preeclampsia (28% to 38%) compared to class II or V (11.1%, $p<0.05$) or to lupus controls without nephritis (4.6%, $p<0.05$) (134). Thrombocytopenia at the onset of pregnancy is strongly associated with increased risk of preeclampsia (RR, 3.2), except when the first pregnancy only is considered (135). In addition, preeclampsia in SLE and in antiphospholipid syndrome tends to be severe and of early onset. In a prospective study of 317 women with prior preeclampsia but without SLE or antiphospholipid syndrome, of five antiphospholipid antibodies tested, only antiphosphatidylserine was associated with severe preeclampsia (202). Abnormal uterine artery vascular resistance, as detected by Doppler flow velocity waveform at the 18th week of pregnancy, was associated with significant increase in preeclampsia, compared with normal resistance (11% versus 4%), and with adverse pregnancy outcome (45% versus 28%) (203).

A predictor of development of preeclampsia or IUGR in subjects with APS, particularly with LAC, is bilateral notching of the uterine artery on Doppler examination at around 20 weeks gestation: bilateral notching is associated with a 12- to 14-fold increased likelihood, whereas normal flow is associated with an 80% decreased likelihood of preeclampsia or IUGR (204).

About 25% of lupus patients will develop hypertension and proteinuria in the second half of pregnancy. In case of prior nephritis of any type, hypertension develops in 41% of patients during pregnancy (205).

It is important to distinguish lupus nephritis flares from preeclampsia or PIH because of drastically different therapeutic management. However, in many cases a clear-cut diagnosis remains elusive and the two conditions may also coexist. The following clinical and laboratory findings are useful in distinguishing the two entities:

- Lupus flares are associated with active urine sediment, not just with proteinuria. Active sediment is rarely present in preeclampsia.
- Hypocomplementemia, high titers of anti-DNA antibodies and anti-C1q antibodies are frequently associated with renal flares and are usually normal or elevated in preeclampsia (166 ,167).
- Thrombocytopenia, elevated liver enzymes, and hyperuricemia are more common with preeclampsia.
- Renal flares are frequently associated with other clinical symptoms and signs of SLE.
- After delivery, proteinuria rapidly decreases in preeclampsia, whereas it persists or increases in SLE.

A further cause for potential confusion of preeclampsia with lupus exacerbation is the HELLP syndrome, which may

complicate the course of severe preeclampsia in a minority of patients (80). Hemolysis and thrombocytopenia are a result of disseminated intravascular coagulation (DIC) with microangiopathic hemolytic anemia, rather than autoimmune hemolytic anemia. HELLP syndrome patients have characteristic blood smears with burr cells and schistocytes, elevated liver enzymes in all, and an increased bilirubin level in about half of the patients. The liver dysfunction is attributed to fibrin deposition with obstruction of the hepatic sinusoids, resulting in liver distention, subcapsular hematomas, infarction (11 of 24 pregnancies), and even rupture (147 ,188 ,206). At times the diagnosis of antiphospholipid syndrome was unknown prior to HELLP (188). Treatment of preeclampsia and prompt delivery by Cesarean section is necessary because of grave danger to the mother and fetus (perinatal mortality, 9% to 60%).

The effectiveness of low-dose aspirin (75 to 81 mg/day) in the prevention preeclampsia is controversial, however, a Cochrane database review of 39 randomized trials (30,000 women), and a metaanalysis of 14 studies with over 12,000 high risk women concluded that such therapy is effective in patients at high risk (207 ,306). There was a 15% reduction in preeclampsia, a 14% risk reduction for fetal or neonatal death, and an 8% reduction in preterm birth. In addition, this therapy poses no added risk of bleeding to the mother and fetus.

When assessing proteinuria in lupus pregnancy the possibility of renal vein thrombosis should always be investigated. Proteinuria with severe flank pain and hematuria, especially in the presence of membranous nephropathy, a history of deep vein thrombosis, and positive antiphospholipid antibodies, should raise the possibility of acute renal vein thrombosis, that is easily detected by Doppler ultrasound examination. Chronic renal vein thrombosis has indolent symptoms (chronic flank pain) and no hematuria.

Summary—Lupus Nephritis and Pregnancy

The interrelationship of pregnancy and lupus nephritis can be summarized as follows:

- The need to plan pregnancy after a 6-month period of remission in patients with SLE nephritis cannot be overemphasized: flares and preeclampsia are far less in inactive nephritis.
- Nephritis flares during pregnancy and postpartum can be very severe, with anuric renal failure and even maternal death, or chronic renal failure. Vigilance for early detection and vigorous treatment of flares is required.
- No definitive relationship between the histologic class of lupus nephritis and the severity of flare during pregnancy has been established, but there is a tendency for more severe exacerbations in patients with proliferative nephritis, WHO classes IV and III. Limited information on kidney biopsies before and after SLE pregnancy has shown progression in some patients and regression in others.
- Women with lupus nephritis are prone to preeclampsia, with greater prevalence than in normal pregnancy. This clinical picture should be differentiated from a nephritis flare, because of the possibility of acute anuric or chronic renal failure that may follow a flare, and of the distinctly different therapeutic management indicated.
- Preexisting hypertension is the most common predisposing factor to preeclampsia. Antiphospholipid antibodies also appear important as predisposing factors.
- In the setting of lupus nephritis and prior hypertension, with or without antiphospholipid antibodies, preventive treatment with low-dose aspirin should be initiated at the 10th to 14th week of pregnancy.
- The onset of lupus with nephritis during pregnancy is often associated with a stormy course and acute anuric renal failure, and should be suspected in any young woman with a multisystem presentation that includes rashes, arthritis, and alopecia.
- Renal vein thrombosis should be suspected, detected, and treated, especially in patients with antiphospholipid antibodies and/or membranous nephropathy.
- Hemodialysis should be instituted during lupus pregnancy in patients with BUN levels of 50 mg/dL or greater. Several chronic dialysis patients have been able to conceive and deliver live infants.
- In the presence of active lupus nephritis, especially diffuse proliferative, nephrotic syndrome, moderate to severe hypertension, and a serum creatinine level of 2 mg/dL or greater, pregnancy is possible, but potentially problematic and high-risk.
- Pregnancy in women with SLE and kidney transplants has been successful, although hypertension, preeclampsia, prematurity, and IUGR are not uncommon.

Bottom line: pregnancy with lupus nephritis, even inactive, should be handled as a high-risk pregnancy.

Laboratory Findings, Serologic Markers, and Antiphospholipid Antibodies

When used judiciously and in the context of the patient's clinical picture, certain laboratory tests in lupus pregnancy are useful in predicting disease flare, or potential fetal problems. Monitoring of disease activity is achieved by the serial determination of complement levels, anti-dsDNA antibodies, circulating immune complexes, and urinalysis with microscopic exam. Fetal loss is high in patients with antiphospholipid antibodies, and neonatal lupus erythematosus is associated with anti-Ro/SSA, anti-La/SSB and, rarely, with anti-U1 ribonucleoprotein (RNP) antibodies.

Pregnancy Tests

Urine pregnancy test by radioimmunoassay may be false positive in SLE patients with nephrotic syndrome (208). False-positive urine pregnancy tests occurred in 14 of 140 (10%) nonpregnant lupus patients, including 1 male: 11 of 14 had

renal disease, 8 with nephrotic syndrome and heavy proteinuria, 4 with amenorrhea, and 1 was actually pregnant (209). The false-positive test presents as an atypical ring pattern and reflects a nonimmunologic interference by urine gamma globulins in concentrations of 1.7 to 16.6 mg/mL. Serum radioimmunoassay for beta human chorionic gonadotropin (*beta* HCG) gives false-positive results in only 1 in 10^3 to 1 in 10^4.

Tests for maternal serum alpha-fetoprotein (MSAFP) and hCG are used by obstetricians. High AFP or hCG, whether associated with SLE or not, are followed with serial ultrasound examination for fetal growth and antepartum testing after 34 weeks, because they are predictive of preeclampsia and IUGR (210). These are presumed to be markers of a “leaky” placenta in which the fetal-maternal barrier is damaged by intervillous, decidual, or villous thrombosis.

Pregnancy-Induced Laboratory Test Changes

The sedimentation rate (ESR) increases in normal pregnancy and cannot be relied on for predicting disease activity (109). Average ESR by the Westergren method are 29 mm/hour in the first, 42 mm/hour in the second, and 36 mm/hour in the third trimester. Friedman and Rutherford (91) reported a high incidence of postpartum flares in lupus patients with Westergren ESR ≥100 mm/hour. In addition, creatinine clearance increases, immunoglobulin levels decrease, but are still within normal limits (211), and the hemoglobin decreases; the latter two are attributed to hemodilution.

Complement

Several studies have shown that the C3 complement level rises in normal pregnancy by a mean of 30%, but little, if at all, in SLE (109 ,166 ,212). Failure of C3 to rise, or declining levels in pregnancy have been associated with SLE flare (109 ,112). The C3 level rose by 25% in a control group of normal pregnant women, whereas it only increased by 10% in a group of pregnant SLE patients (212). These findings were confirmed with a larger series; 87 of SLE patients who had a flat or declining C3 level had a significant increase in maternal problems, and in fetal morbidity and mortality (213). Devoe et al. also noted association of a falling C3 level with increased SLE activity in the mother and an increased risk of abortions (85 ,214).

The alternate complement pathway is also activated during lupus flares in pregnancy, with elevated level of Ba (activation product of the alternate pathway) and low hemolytic complement (CH_{50}); the ratio of CH_{50} to Ba was significantly lower in lupus flares than in preeclampsia without SLE (215).

There are further implications of the role of complement activation in antiphospholipid-related fetal loss, to be discussed under Antiphospholipid Antibodies.

Lupus-Induced Laboratory Changes

Antinuclear Antibodies

Antinuclear antibody (ANA) tests have no specificity for disease activity in lupus. Studies of ANA in normal pregnant women have shown a similar prevalence as in the general population, between 1% and 5% (mean, 2.3%) (216 ,217 ,218). A prospective study compared 214 normal pregnant women with 50 age-matched nonpregnant controls and found 11% and 2% ANA positivity, respectively, which was significantly higher in pregnancy (p <0.05) (219). Most positive ANAs were found in the third trimester. None of the ANA-positive subjects were symptomatic or took lupus-inducing drugs, and only two had anti-dsDNA. In an interesting study from Mexico, Garcia-de la Torre et al. (167) found a positive ANA in 6 of 20 women with recurrent fetal loss (30%), in 6 of 40 toxemic patients (15%), and in 2 of 30 normal pregnant women (6.7%). Of the two ANA-positive habitual aborters with anti-dsDNA, one fulfilled four criteria for SLE and three more in this group had one to three criteria. Of the 6 preeclamptic patients with a positive ANA, 3 patients had one, and 1 patient had two SLE criteria. The authors concluded that the high prevalence of ANA in women with recurrent abortion can help identify patients who will eventually develop lupus. We concur that ANA does not appear in pregnancy unless the patient is developing lupus.

Anti-dsDNA, especially the complement-fixing variety, has been recognized as a helpful marker for assessing the activity of lupus and lupus nephritis (220 ,221 ,222) and increasing anti-dsDNA levels or titers predict disease flare (223). IgG-class anti-dsDNA, when present in the mother's plasma, may cross the placenta: Grennan et al. followed four SLE patients with anti-dsDNA through pregnancy and found anti-dsDNA in the cord blood of the neonate whose mother had the highest DNA binding (224). In this baby, the DNA binding capacity fell from 96% to 52% at 2 weeks and to 9% (negative) at 8 weeks of age, which suggests transplacental passage of the autoantibody. Zulman et al. (112), however, did not detect anti-dsDNA in the cord blood of six neonates whose mothers had it. The appearance of complement-fixing anti-dsDNA, or an increase during pregnancy, should alert the physician to the possibility of new onset, or flare of lupus nephritis.

The determination of anti-Ro/SSA and anti-La/SSB antibodies in the pregnant lupus patient is highly recommended because of their link to the neonatal lupus erythematosus syndrome (NLE) (225 ,226) (see Chapter 53). Anti-Ro/SSA is found in 25% to 40% of lupus patients and anti-La/SSB in 10% to 15%. In a prospective study of 100 women positive for anti-Ro with 122 pregnancies, the proportion of children with neonatal lupus was 2 in 122 pregnancies (1.6%) (133). Anti-U1RNP antibody is detected in 40% to 45% of lupus patients and is rarely associated with neonatal lupus (227).

Cryoglobulins

It has been well established that cold-insoluble complexes (mixed cryoglobulins) are present in up to 30% of lupus patients' sera, and represent immune complexes. They are associated with decreased serum complement levels and with evidence of active lupus, especially nephritis (228). We have also found that increased levels of cryoglobulins correlate with disease activity.

Antiphospholipid Antibodies

One of the most important laboratory tests in lupus pregnancy is the determination of antiphospholipid antibodies (APL), the most common of which are anticardiolipin antibody (ACL), lupus anticoagulant (LA or LAC) and anti-ß$_2$-glycoprotein I (anti-ß$_2$GPI) (76 ,229 ,230). Although separate and distinct, these antibodies to negatively charged membrane phospholipids frequently coexist. Recently, anti-ß$_2$GPI has generated a lot of clinical interest, and in terms of mechanisms of fetal loss (231 ,232 ,233). Other antibodies of potential significance for intravascular clotting include anti-thrombin III, anti-protein S, and so on. The literature on APL is voluminous and at times contradictory.

Anticardiolipin antibodies (ACL) react with phospholipids (cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, and others) bound to proteins such as ß$_2$GPI, prothrombin, or annexin V. ACLs can be detected by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (229). (See also Chapter 27).

Lupus anticoagulants (LAC) are antibodies directed against plasma proteins bound to anionic phospholipids (ß$_2$GPI, prothrombin, or annexin V). LAC blocks in vitro assembly of the prothrombinase complex, resulting in prolongation of in vitro clotting assays: the activated partial thromboplastin time (aPTT), the dilute Russell viper venom time (dRVVT), the kaolin plasma clotting time (KCT, Exner test), and rarely the prothrombin time. dRVVT and Exner are preferred tests (234 ,235 ,236).

Antibodies to ß$_2$GPI, a phospholipid-binding inhibitor of coagulation, are found in a large percentage of patients with primary or secondary antiphospholipid syndrome (APS), along with LAC and/or ACL. Antibodies to ß$_2$GPI were the sole antiphospholipid antibody in 20.3% of 133 SLE patients, one fifth of whom had APS features (237), and in 1% to 10% of patients with APS (232 ,238).

Antiphospholipid antibodies have been linked to intravascular clotting, arterial or venous, recurrent fetal loss, livedo reticularis, immune thrombocytopenia, Coombs-positive autoimmune hemolytic anemia, and false-positive tests for syphilis (229 ,235). In a review of 21 studies comprising over 1,000 lupus patients, anticardiolipin antibody was found in 44% (21% to 63%) and LAC in 34% (7% to 73%) (76).

In a recent study of 311 individuals (25 APS, 89 SLE, of whom 23 had thrombotic episodes [SLE/APS], 77 women with unexplained recurrent pregnancy loss, and 120 healthy controls), the SLE patients had 37% positive ACL, 35% anti-ß$_2$GPI, 45% antiprothrombin and 13% antiannexin V (239). The 23 SLE/APS patients were 70% positive for ACL, 57% for anti-ß$_2$GPI, 87% for antiprothrombin and 4% for antiannexin V. Of 56 SLE patients without clotting episodes who were ACL- negative, 5% were positive for anti-ß$_2$GPI, 23% for antiprothrombin, and 9% for antiannexin V. The respective values for 25 APS patients were, 100%, 80%, 60%, and 24%. In a study of 90 SLE and 100 APS patients, anti-ß$_2$GPI and antiphosphatidylserine were the strongest predictors for APS and for arterial thrombosis (240). Antiprotein S antibodies were found in 57 of 184 SLE patients (31%), and were associated with thrombosis and pregnancy morbidity (241).

In addition to the association of APL with recurrent spontaneous abortion, fetal loss, and fetal distress in women with and without lupus, preeclampsia, IUGR, and prematurity have been associated with APL (199 ,200 ,201). Although a study of 424 women asserted that testing for anti-ß$_2$GPI did not identify additional patients at risk for recurrent abortion or fetal loss (241a), there is ample recent evidence that anti-ß$_2$GPI is an important factor in RPL (231 ,232 ,242). Thrombotic events have also been described with a functional or quantitative deficiency of protein C or protein S in association with antiphospholipid antibodies (243).

In 15 retrospective studies of 1,249 pregnancies in SLE and SLE-like illness, there was a mean fetal loss of 37% (14% to 68%) in 479 APL-positive pregnancies. and of 18% (3% to 43%) in 770 APL-negative pregnancies, not a statistically significant difference (127 ,229 ,244 ,245 ,246 ,247 ,248 ,249 ,250 ,251 ,252 ,253 ,254 ,255). In seven prospective studies of 892 lupus pregnancies, however, mean fetal loss was 48% (4% to 100%) in 301 APL-positive pregnancies, and 9% (0% to 20%) in 591 APL-negative pregnancies, a statistically significant difference (256 ,257 ,258 ,259 ,260 ,261 ,262). SLE patients with persistently positive ACL regardless of SLE activity had significantly higher ACL, more thromboses (33% vs. 3%), spontaneous abortions (41% vs. 7%), and lupus anticoagulant (45% vs. 8%), than patients with positive ACL only during active lupus (250).

APL were also reported in a segment of women with RPL but without lupus (4% to 31%) (263), in infertile women (10% to 17%), and in women who resort to assisted reproductive therapy (ART, 14% to 30%) (264 ,265 ,266).

Aside from intravascular clotting, that results in a small placenta with infarcts (258), there are important events triggered by APL. Recent progress in the elucidation of pathogenetic mechanisms in APL-induced fetal loss can be summarized as follows:

In an in vitro system of trophoblast cells that develop into syncytia as manifested by the cytoplasmic presence of hCG, and exhibit invasiveness, trophoblasts express cell membrane anionic phospholipids that bind ß$_2$GPI, the main cationic phospholipid binding protein recognized by the antiphospholipid antibodies. ß$_2$GPI binds to trophoblast in vitro through its fifth domain, as reported for endothelial cells, and can be recognized by anti-ß$_2$GPI antibodies (233). The highest binding occurs when cells show the greatest amount of syncytium formation. Both polyclonal and monoclonal APL, including IgM anti-ß$_2$GPI significantly downregulate hCG secretion and trophoblast invasiveness (267). APL binding to the trophoblast alters the expression of trophoblast adhesion molecules (integrins and cadherins), which can result in inadequate trophoblastic invasion (268). Anti-ß$_2$GPI inhibits in vitro trophoblast differentiation into multinuclear giant cells and IL-3 restores placental functions (269). Low molecular weight heparin significantly reduced the binding of APS IgG to trophoblast cells and restored in vitro placental invasiveness and differentiation (270).

In a mouse model of APL-induced fetal loss, inhibition of the complement cascade in vivo blocked fetal loss and growth retardation, and mice deficient in C3 were resistant to APL-induced fetal injury (271). The same group determined that heparin, low molecular weight or unfractionated, prevented APL-induced fetal loss by inhibiting complement activation in vivo and in vitro (272). They further demonstrated that APL binding to the trophoblast triggers complement activation, followed by rapid increase in decidual and systemic TNF-α, which may become a therapeutic target for APL-induced fetal loss (273).

The above effects of APL, complement and TNF-α on trophoblast cell maturation, differentiation and invasiveness may well interfere with implantation, placentation, and normal vascular perfusion of the developing embryo.

Other Tests

Thrombocytopenia during lupus pregnancy may be the result of association with antiphospholipid antibodies (274), and may signify lupus flare. Thrombocytopenia in the presence of preeclampsia, however, should prompt investigations for the HELLP syndrome (80), which needs to be differentiated from lupus flare. Hyperuricemia should alert clinicians to the possibility of preeclampsia.

Laboratory Monitoring in SLE Pregnancy

Recommendations for laboratory monitoring in SLE pregnancy can be summarized as follows:

- Initial laboratory assessment at diagnosis of pregnancy should include:
  - Complete blood count (CBC) with differential, including platelets
  - Urinalysis with microscopic examination
  - Chemistry panel inclusive of BUN, creatinine, and blood glucose
  - Coombs test
  - Venereal Disease Research Laboratory (VDRL) test, activated partial thromboplastin time (APTT), anticardiolipin, anti-$ß_2$GPI, if available
  - Anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-U1RNP
  - Complement C3 and C4
  - 24-hour urine for protein and creatinine, in the event of nephritis

    A positive screening APTT should be followed by appropriate investigations for the lupus anticoagulant. Highly positive levels of antiphospholipid antibodies should alert the rheumatologist and obstetrician to the possibility of spontaneous abortion or stillbirth, fetal distress, or preeclampsia, and preventive therapy should be initiated Positive anti-Ro/SSA and/or anti-La/SSB and, to a lesser extent, a positive anti-U1RNP should alert the physicians to the possibility of neonatal lupus erythematosus. If the patient is nephrotic or on corticosteroids, serum lipid tests are also indicated. Patients with known nephritis should have frequent monitoring of blood pressure and an initial 24-hour urine collection for protein, creatinine, and creatinine clearance. At the University of Southern California we also obtain quantitative serum cryoglobulin levels as an indicator of circulatting immune complexes.

- Monthly laboratory assessment should include CBC with differential, platelets, urinalysis, chemistry panel (as above), anti-dsDNA, C3, C4, and cryoglobulins or other measures of immune complexes. Patients with known lupus nephritis should also have 24-hour urine collections for protein, creatinine, and creatinine clearance.
- In the event of a hematocrit decrease, Coombs test should be repeated and the peripheral smear reviewed.
- Increasing antibodies to dsDNA, especially if they are complement-fixing, decreasing complement C3 and C4, and increasing immune complexes, indicate active lupus or impending flare in over 80% of patients.

Management during Pregnancy, Delivery, and Postpartum Period

General Principles

The SLE patient should consider strongly planning her pregnancies after a remission of at least 6 months. Once pregnant, she requires special attention by a team of a rheumatologist and a obstetrician-perinatologist, who should have experience in SLE and high-risk pregnancy management, respectively. At the onset of pregnancy, a thorough assessment of system involvement and disease severity and activity should be made. The pregnant woman, her husband or mate, and other family members should be counseled with regard to the pregnancy (see below). During the first half of pregnancy, the woman with SLE under control should be followed every month, with increased frequency of visits during the second half (every 2 to 3 weeks). Aside from laboratory evaluation and monitoring as recommended above, blood pressure should be monitored at every visit, and at home, in patients with known nephritis. Women with nephritis, with or without a history of hypertension, should be considered for low-dose daily aspirin from the 10th or 14th week to week 36 of gestation, for prevention of preeclampsia. Follow-up should be geared toward early detection and aggressive therapy of lupus flares during pregnancy and the postpartum period.

Follow-up of fetal development includes repeated ultrasound evaluation and the modified biophysical profile (275 ,276) (see Chapter 52).

Patients with high levels of antiphospholipid antibodies, and especially those with recurrent pregnancy loss or prior thrombosis, should be considered for heparin treatment protocols that enhance the possibility of live birth (see below). Cytotoxic drugs should be avoided during the first trimester and full doses of prostaglandin inhibitors should be used very sparingly. Steroid preparation for the stress of delivery is needed for patients on steroids for the 2 previous years. Cesarean section should be considered for certain maternal

or fetal indications, such as preeclampsia, HELLP syndrome, maternal avascular necrosis (osteonecrosis) of the hips with inadequate hip abduction, fetal distress, abnormal nonstress test, and the usual obstetric indications (e.g., cephalopelvic disproportion, transverse presentation). A neonatologist should be available at delivery.

During the immediate postpartum period and for the next 2 months, the mother should be watched carefully for disease flare and development of infection at the site of episiotomy or cesarean incision, endometritis, urinary tract infection, or pneumonia. Infection and flare should be treated promptly and aggressively.

Use of Medications during Pregnancy (Table 51-4)

The major drugs used to treat lupus are corticosteroids, nonsteroidal antiinflammatory drugs (NSAIDs, including salicylates), antimalarials, immunosuppressives/cytotoxics, and, in the case of associated antiphospholipid syndrome, anticoagulants. There is a justifiable tendency to use as few drugs as possible during gestation, but a smooth course for mother and fetus might dictate their use. The major concerns about medication use in pregnancy are the pharmacologic effects on the mother and fetus, effects on the length of gestation and labor, and developmental effects on the fetus (intrauterine growth, malformations, and survival). A valuable textbook on the use of drugs in pregnancy and lactation is available (277).

Table 51-4: Medication Use in Lupus Pregnancy and Lactation

Medications Used in Lupus	FDA Category	Lactation Permitted Maternal Dose
Corticosteroids	B	Yes, up to 20 mg/day
Azathioprine	D	No, no data
Cyclophosphamide	D	No, cytopenia in infant
Methotrexate	X	No
Cyclosporine	C	No
Mycophenolate mofetil	C	No data
Leflunomide	X	No
Rituximab	C	No data—Not recommended
Chlorambucil (rarely)	D	No
Antimalarial ([hydroxy]chloroquine)	C	Yes per AAP
Low-dose aspirin	C	Probably yes
Heparin, enoxaparin	B	Yes
Warfarin	D, X_M	Yes
Intravenous immunoglobulin	C_M	Yes
NSAIDs	B, D*	Yes—short-acting NSAIDs
COX-2 inhibitors	C	No data
Misoprostol	X_M	No
Antihypertensive Drugs Allowed in Pregnancy		
Methyldopa	C	Yes
Labetalol	C_M	Yes
Nifedipine	C	Yes

AAP, American Academy of Pediatrics; FDA, Food and Drug Administration; NSAIDs, nonsteroidal anti-inflammatory drugs.
*In third trimester. $_{M,}$ Category per manufacturer.

The Food and Drug Administration (FDA) has categorized drugs in terms of fetal risk:

- Category A: Controlled studies in women fail to show risk to the fetus in the first trimester or later. Possibility of fetal harm seems remote.
- Category B: No controlled studies in pregnant women and no fetal risk in animal studies, or animal studies show adverse effect, not confirmed in controlled studies in women in first trimester and later.
- Category C: Animal studies show teratogenic, embryocidal, or other fetal effects and there are no controlled studies in women, or there are no studies in women and animals. Category C drugs should be given only if benefit outweighs the potential fetal risk.
- Category D: There is evidence of human fetal risk, but benefit from use in pregnant women may be acceptable in spite of risk (i.e., in life-threatening situations or serious disease).
- Category X: Animal or human studies show fetal abnormalities, or there is evidence of fetal risk in humans, or both, AND the risk outweighs any possible benefit.

- Subscript M: The manufacturer has assigned the fetal risk category.
- A website is available, http://www.motherisk.org/, of the Hospital for Sick Children in Toronto, Canada.

Corticosteroids Category B

Despite the lack of controlled or randomized studies, corticosteroids have been established as the mainstay of SLE treatment in acute situations. Active lupus or flares during pregnancy or postpartum should be controlled with aggressive steroid therapy (75 ,120 ,125 ,187). Dose selection depends on the extent and severity of system involvement. Nephritis of the more severe types (diffuse and focal proliferative), neuropsychiatric manifestations, autoimmune hemolytic anemia, thrombocytopenia, and extensive severe vasculitis, cutaneous or visceral, require doses greater than or equal to 1 mg/kg/day of prednisone, and at times, intravenous methylprednisolone pulses of 250 to 1,000 mg per day. Life-threatening lupus manifestations, such as acute pneumonitis or pulmonary hemorrhage, justify intravenous pulse methylprednisolone at 500 to 1,000 mg per day for 3 to 5 days. Pleuropericarditis usually requires 0.5 to 0.8 mg/kg/day, whereas skin rashes and arthritis require 5 to 20 mg of prednisone daily, and/or antimalarials and NSAIDs.

Although Mintz et al. (75) and Wong et al. (125) put their pregnant patients on “prophylactic” steroids, and that may have prevented postpartum flares and maternal deaths, there is no hard evidence to prove that, and there is a tendency away from this practice (115).

Any woman treated with systemic steroids within 2 years of the anticipated delivery should be considered as potentially adrenal-insufficient, and should be given steroid stress coverage (steroid prep) during delivery. The most usual form consists of 100 mg hydrocortisone IV just prior to onset of delivery, and every 8 hours for the first day. During the next day the dose can be reduced to 50 mg every 8 hours and then adjusted to the patient's previous oral dose. If the patient is receiving more than 75 mg of prednisone daily, the appropriate hydrocortisone equivalent should be used in the first 2 days, and then the patient's steroid dose resumed.

Cortisol (278), prednisone (279), prednisolone (279), methylprednisolone (280), betamethasone (281), and dexamethasone (282 ,283) can cross the placenta. With maternal administration of prednisone or prednisolone, fetal blood levels are approximately 10% of the mother's level; with methylprednisolone hemisuccinate, cord levels are 18% to 45% of the mother's, with a large standard deviation; with betamethasone, cord levels are approximately 33% and, with dexamethasone, are similar to the maternal level. Therefore, the use of prednisone or prednisolone to treat the mother is least likely to affect the fetus, methylprednisolone is intermediate and, if steroid therapy of the fetus is indicated, dexamethasone is the appropriate choice (284). There is evidence that placental oxidative enzymes (placental 11ß-hydroxysteroid dehydrogenase type 2 (11ß-HSD2) inactivate in vitro cortisol and prednisolone, but not betamethasone or dexamethasone (278 ,279 ,285 ,286).

In a European database of 11,150 malformed infants, a slight association was observed between first trimester exposure to systemic corticosteroids and the occurrence of cleft lip, or cleft palate, or a combination of the two (OR, 2.59) (287). There was a decreasing trend over time, from 1990 to 2002; the authors expressed concern about dioxin potentiation of steroid effects, as in seen in mice. There are two reports of fetal adrenal failure after maternal steroid treatment, one fatal (288 ,289). Intrauterine growth restriction (IUGR) and low birth weight have been reported with maternal steroid therapy (290). In the large prospective study by Mintz et al. (75), however, it seemed that IUGR was more a function of active maternal SLE than of steroid dose. Several studies have described prematurity in newborns of steroid-treated women, starting in 1967: Hodgman et al. reported 23 infants whose 19 mothers received 7.5 to 22.5 mg of prednisone/day during pregnancy because of SLE (*n* = 16) and rheumatoid arthritis (*n* = 3) (291). There was excessive prematurity in 20 children with first-trimester exposure, with birth weight below the 50th percentile in 73.7% and SGA in 26%. Follow-up at 6 months to 8 years showed normal height, weight, and developmental progress for 22 of 23. Preterm delivery, preterm premature rupture of membranes, and premature rupture of membranes are common in SLE and seem to be multifactorial: disease activity, prednisone use at ≥10 mg/day and ACL are incriminated (178 ,292). On the other hand, dexamethasone or betamethasone therapy for lung maturation has contributed greatly to the survival of small premature infants (293).

Induction or aggravation of maternal diabetes mellitus and hypertension are known risks of steroid therapy. Overall, corticosteroid therapy is the major factor in improved maternal survival in SLE in recent decades, with substantial decrease in maternal mortality.

Salicylates and Other NSAIDs Category B, D (in Third Trimester)

COX-2 Inhibitors Category C

Salicylates and NSAIDs have in common the capacity to interfere with prostaglandin formation through variable inhibition of cyclooxygenases. This inhibition includes prostaglandin action anywhere in the body, including uterine, platelet, and renal, and other prostaglandins. Aspirin and NSAIDs inhibit uterine contractility and prolong labor and gestation (294). Aspirin irreversibly inhibits platelet aggregation, whereas the other NSAIDs have a reversible effect, and both can cause bleeding during delivery (295). Given the immaturity of hepatic enzyme systems in the fetus and newborn, transplacentally delivered drugs may persist much longer. No human teratogenicity exists, at least with low-dose aspirin (277). Of 50,282 mother-child pairs, 35,418 pairs had not taken aspirin, 9,736 had intermediate exposure, and 5,128 had heavy exposure during the first 16 weeks of pregnancy (296). The observed and expected numbers of malformations were similar in the three groups.

Other NSAIDs have, to varying degrees, similar effects to those of aspirin in pregnancy. They readily cross the placenta, potentiate vasoconstriction under conditions of hypoxia, raise systemic vascular resistance, and have profound effects on fetal and neonatal circulation (297). Maternal aspirin intake during pregnancy can cause in utero closure of the fetal ductus arteriosus, with severe heart failure, tricuspid insufficiency, and acidosis, all of which disappeared the day after birth (298). COX-2 inhibitors cause constriction of the fetal lamb ductus arteriosus in vitro and in vivo (299). Maternal indomethacin therapy was considered the cause of primary pulmonary hypertension in a newborn (300). Transient neonatal renal failure and oligohydramnios have also been described (301). Full dose salicylates (almost never used lately), NSAIDs and COX-2 inhibitors should be discontinued 6 to 8 weeks prior to delivery for the reasons outlined above.

Other Indications for Salicylates and Other NSAIDs

The pharmacologic actions of prostaglandin inhibitors can be used to therapeutic advantage. Indomethacin has been successfully used to inhibit premature labor (302), and for postnatal closure of patent ductus arteriosus (303).

Prevention of Preeclampsia

The capacity of low aspirin (ASA) doses (50 to 81 mg/day) to inhibit thromboxane synthesis by platelets, while prostacyclin production by endothelium is unaffected (304), has promising applications in the prevention of preeclampsia, where exaggerated placental production of thromboxane A2 has been invoked (197 ,305). The effectiveness of low-dose aspirin (75 to 81 mg/day) in the prevention of preeclampsia has been controversial. Nonetheless, patients at high risk for preeclampsia, including SLE patients, especially with preexisting nephritis and hypertension and/or APL, benefit from low-dose aspirin: a Cochrane database review of 39 randomized trials (over 30,000 women) concluded that ASA therapy during pregnancy is effective, with 15% reduction in preeclampsia, 14% risk reduction for fetal or neonatal death, and 8% reduction in preterm birth (207). Another metaanalysis od women at high risk for preeclampsia led to similar conclusions (306). In addition, this therapy poses no added risk of bleeding to the mother and fetus.

Intrauterine Growth Restriction

Lu et al. reviewed the evidence for IUGR prevention by low-dose ASA treatment during pregnancy (307). Although a metaanalysis of 13 studies between 1985 and 1994 found an 18% reduction of IUGR in >13,000 pregnancies treated with low-dose ASA, one multicenter trial among healthy, nulliparous women and two large trials among women at increased risk for IUGR and preeclampsia failed to demonstrate any benefit of ASA treatment in preventing IUGR. Nonetheless, there is a trend for decrease in IUGR since 1980: from 31% in the 1980s, to 18.2% in the 1990s, to 18.1% in the 2000s, whereas live births have increased from 72.5% in the 1980s to 82.1 in the 2000s (see Chapter 52 , The Fetus in SLE).

Summary: Salicylates

Large, anti-inflammatory doses of aspirin and NSAIDs are not generally used in lupus patients and should be avoided during the last 2 to 4 weeks of pregnancy for fear of prolonging gestation and labor, increased maternal and fetal bleeding during delivery, and possible premature closure of ductus arteriosus. In susceptible SLE patients, however, low-dose aspirin therapy assists in prevention of preeclampsia, and, in conjunction with heparin, in the prevention of pregnancy loss from APL (308).

Prevention of Fetal Loss with Antiphospholipid Antibodies (Table 51-5)

Overall fetal loss in SLE has been steadily decreasing over the last 55 years, with live births from 72.5% in the 1950s, to 82.1% in the first 5 years of the 2000s (See Chapter 52 , The Fetus). Similar conclusions were reached by Clark et al. in their analysis of papers in the last 40 years (309). A substantial portion of fetal loss in SLE is a result of the presence of antiphospholipid antibodies. Prevention of APL-related recurrent fetal loss has stimulated tremendous interest with numerous investigations and voluminous literature over the last 20 years (262 ,310 ,311 ,312 ,313 ,314 ,315 ,316 ,317 ,318 ,319 ,320 ,321 ,322 ,323 ,324 ,325 ,326 ,327 ,328 ,329 ,330 ,331 ,332 ,333 ,334 ,335 ,336 ,337 ,338 ,339 ,340 ,341 ,342 ,343 ,344). Of the 33 studies on Table 51-5 , 14 were controlled and compared 2 or more therapeutic regimens (310 ,313 ,316 ,318 ,322 ,323 ,325 ,326 ,331 ,333 ,334 ,341 ,342), and most were prospective.

Aspirin alone does not prevent fetal loss (322 ,334). It is now well established that heparin and low-dose aspirin is the treatment of choice (318 ,322 ,326 ,334 ,335). The controlled study of aspirin 81 mg per day versus aspirin plus heparin high dose (up to 30,000 IU per day) showed a definitive advantage of the combination, 44% live births versus 80% (322), and similar benefit was shown by moderate dose of UFH plus ASA versus ASA alone (71% and 42% live births) (334). Comparison of aspirin plus heparin low dose (up to 12,000 IU/day) versus aspirin plus heparin high dose (up to 30,000 IU per day) showed no advantage of the latter (76% vs. 80% live births) (323). Low molecular weight heparin can be given once a day, and showed a decided advantage over historic placebo controls (83.9 vs. 55.9 live births) (326 ,344).

A recent prospective, multicenter, controlled pilot study compared LMWH versus unfractionated heparin (UFH), with aspirin in both arms, and showed that both types of heparin were equally effective, with 84% and 80% live births (331).

Before the establishment of heparin with low-dose aspirin as the definitive treatment, a variety of therapeutic regimens were used, including low-dose aspirin alone (75, 81, or 150 mg/day), combinations of aspirin and 20 to 60 mg/day of prednisone, aspirin plus prednisone plus

platelet antiaggregants, heparin in high or low dose, alone, or with prednisone, or with aspirin, fish oil (eicosapentaenoic acid and docosahexanoic acid), and intravenous gamma globulin (IVIG) alone, with ASA, or with ASA and heparin (313 ,321 ,339 ,341 ,342). Only low-dose aspirin was used (75 mg/day in Europe, 81 mg/day in the United States).

Table 51-5: Outcome of Treatment in Antiphospholipid-Associated Fetal Loss

		No. of Pregnancies		Live Births (%)	
First Author	Therapy Used, mg/d U/d	Before Therapy	After Therapy	Before Therapy	After Therapy
Lubbe (327,328)	Aspirin 75 + prednisone 40-60	28	6	10.7	85.7
Branch (312)	Aspirin 81 + prednisone 40-60	31	8	3.2	62.5
Gatenby (319)	Aspirin 75-150 + prednisone 30-50 or 10-60	145	27	17.2	63
Ordi (332)	Aspirin 50 + prednisone 20	18	9	0	78
Carp (315)	Aspirin 100-300 + Prednisone 10-40 ± heparin 10,000 ± dipyridamole 225	71	27	7	48
Semprini (337)	Heparin + prednisone	27	14	3.7	64
Rosove (335)	Heparin 24,700	29	15	3.4	93.3
Silveira (338)	Aspirin 81 + prednisone 40	32	12	15.6	100
Buchanan (314)	Aspirin 75, or ASA + heparin 10,000	101	87	18.7	63.2
Cowchock (318)	ASA 80 + heparin 20,000	≥2/pt*	12	0	75
	vs. ASA 80 + prednisone 40	≥2 pt*	8	0	75
Out (262)	Prednisone ≥40 ± ASA 40 ± heparin, or ASA or heparin	≥3/pt*	30	NS	73.3
Landy (324)	Prednisone 5-60 + ASA or + heparin, or single drug	126	33	23.8	90.9
Many (330)	ASA ± prednisone 30 + dipyridamole + heparin	102	52 23 23	6.8	51.9 43.5 69.1
Stuart (340)	ASA + prednisone 10-30	24	7	4.2	28.6
Caruso (317)	ASA + prednisone 40	63	28	11.1	82.1
Al Momen (310)	ASA 100	33	9	9.1	55.6
	vs. ASA + prednisone 40-60	80	13	13.8	53.8
	vs. ASA + prednisone + IVIG (failed ASA + prednisone)	44 5	7 0	4.5	57.1
Balasch (311)	ASA + prednisone 15-30	49	21	6.1	90.5
Rossi (336)	Fish oil (EPA + DHA 5.1 g)	≥3/pt*	22	NS	95.5
Carta (316)	low-dose ASA (LDA)	≥2/pt*	15	NS	80
	vs. fish oil	≥2/pt*	15	NS	73.3 p >0.05
Kaaja (321)	IVIg 1 g/kg + ASA 75	14	4	7.1	100
Spinnato (339)	IVIg 0.4 g/kg ± ASA ± heparin	17	5	0	100
Harger (320)	ASA 81 + prednisone 10-60 Individualized, tapered	69	28	8.7	72.0
Kutteh (322)	ASA 81	≥3/pt*	25	NS	44.0
	ys. ASA 81 + heparin 10,000-30,000	≥3/pt*	25	NS	80.0
Kutteh (323)	ASA 81 + heparin low dose (12,000)	≥3/pt*	25	NS	76.0
	vs. ASA 81 + heparin high dose	≥3/pt*	25	NS	80.0
Rai (334)	ASA 75	≥3/pt*	45	NS	42.0
	vs. ASA 75 + heparin 10,000	≥3/pt*	45	NS	71.0
Laskin (326)	Low molecular weight heparin	NS	46	NS	83.9
	vs. historical placebo controls	NS	34	NS	55.9
Backos (344)	ASA + heparin or LMWH	RPL	150	NS	71.0
Noble (331)	ASA 81 mg + LMWH 40 mg/d	≥3/pt*	25	NS	84.0
	vs. ASA 81 mg+UFH 5,000 U bid	≥3/pt*	25	NS	80.0 p>0.05
	Triolo (341) ASA 81 mg+ LMWH	84	19	3.6	84
	vs. IVIg	87	21	2.3	57 p = 0.06
Vaquero (342)	prednisone + ASA	≥2/pt*	29	NS	78.0
	vs. IVIg	≥2/pt*	53	NS	76.0 p>0.05
Laskin (325)	ASA 100 + prednisone 0.5-0.8/kg	≥2/pt*	101	NS	65.0
	vs. placebo	≥2/pt*	101	NS	56.0
Branch (313)	ASA 81 + heparin 15,000 + IVIg.}	31 for all pts	7	25.8	100.0
	vs. ASA 81 + heparin 15,000 + albumin}		9		100.0
Pattison (333)(early fetal loss)	ASA 75	≥3/pt*	20	NS	80.0
	vs. placebo	≥3/pt*	20	NS	85.0

*Fetal losses/patient; NS, not specified

These studies have reported an overall increase in live births (Table 51-5). Fetal loss in the over 1,100 untreated pregnancies was 76.2% to 100%, with only 7.9% mean live births (range, 0% to 23.8%). All treatments have resulted in increase of live births, to a mean of 72.3% (range, 28.6% to 100%).

Most of the studies with prednisone in doses of 40 to 60 mg per day showed effectiveness, but were fraught with steroid complications, including cushingoid features, hypertension, and gestational diabetes. Prednisone has been abandoned as a preventive treatment for recurrent pregnancy loss because of APL.

The suggestion that fish oil was effective in RPL prevention was dispelled by a study of 30 women, 15 each randomized to fish oil, or to low-dose aspirin: there was no significant difference in live births (73.3% vs. 80%, p >0.05) (316). Of five studies on the effectiveness of IVIG, two used patients as their own controls and showed reduction of pregnancy loss, but the number of pregnancies was very small (321 ,339). The three recent studies were controlled: IVIG plus heparin and aspirin compared to heparin plus aspirin plus albumin (313), IVIG versus aspirin plus prednisone (342), and IVIG versus ASA plus LMWH (341). None of these studies proved any superiority of IVIG in pregnancy outcomes (Table 51-5).

The 7th conference of the American College of Chest Physicians on Antithrombotic and Thrombolytic Therapy (345) and the 10th International Congress on Antiphos-pholipid Antibodies (APLA) (308) have developed detailed therapeutic recommendations during pregnancy in APL-positive women, as seen in Table 51-6 . See also definitions of heparin doses in Table 51-6 . The majority of the recommendations are similar:

- With a history of multiple pregnancy losses (two or more early, or one or more late), or pregnancy complications (preeclampsia, IUGR, or abruption), ACCP suggests administration of antepartum minidose, or moderate-dose UFH, or prophylactic LMWH plus low-dose aspirin. The APLA Congress consensus recommends starting treatment as soon as there is a positive pregnancy test, recommends low-dose aspirin plus a slightly higher UFH dose, 7,500 to 10,000 U q12h, and cessation of treatment on the day of, or 6 to 24 hours before, delivery. Heparin is resumed 6 to 8 hours after delivery for 6 to 8 weeks.
- With a history of venous thrombosis, with or without pregnancy loss or complications, patients are usually receiving long-term oral anticoagulation therapy because of the high risk of recurrence. During pregnancy, ACCP recommends low-dose aspirin plus adjusted-dose LMWH or UFH therapy and resumption of long-term oral anticoagulation therapy postpartum. As the half-life of LMWH is shorter in pregnancy, twice daily dosing is preferable, at least in the initial treatment phase. The APLA Congress also recommends ASA, weight-adjusted LMWH q 12h, even higher doses with history of prior arterial thrombosis, stopping therapy on the day of delivery (6 to 24 hours before). Resumption of oral anticoagulants is recommended 6 to 8 hours after delivery, or LMWH for 1 to 12 weeks postpartum. With history of severe thromboembolism or thrombotic strokes, warfarin may be used from gestational week 14 to 34 (after organogenesis is complete).
- Women with persistent APLs and no prior venous thromboembolism or pregnancy loss, should be considered at increased risk for the development of venous thrombosis and, perhaps, pregnancy loss. ACCP suggests one of the following approaches for these women: surveillance, minidose heparin, prophylactic LMWH, and/or low-dose aspirin, 75 to 162 mg/day. The APL Congress suggests low-dose aspirin or surveillance.

Table 51-6: Treatment Recommendations in Antiphospholipid-Positive Pregnant Women for Prevention of Fetal Loss and Thrombosis

Clinical Situation	American College of Chest Physicians 7th Conference (345)	10th International APLA Congress Consensus Conference (308)
A. Previous pregnancy losses (≥2 early, or ≥1 late) or complications (preeclampsia, IUGR, abruption)	Low-dose aspirin plus Minidose unfractionated heparin (5000 U q12h), or moderate dose UFH, or prophylactic LMWH	Low-dose aspirin plus LMWH-enoxaparin 1 mg/kg/day, or dalteparin 5000 U/day or nadroparin Ca++ 3800 U/day, or UFH 7,500–10,000 U q12h Stop 6–24 hours before delivery, resume heparin 6–8 hours after delivery for 6–8 weeks
B. Previous intravascular thrombosis (with or without pregnancy loss or complications)	Low-dose aspirin plus adjusted dose LMWH or UFH and resumption of oral anticoagulation postpartum	Low-dose aspirin plus LMWH-enoxaparin 1 mg/kg/q12h, or dalteparin 5,000 U q12h, or nadroparin Ca++ 3800 U q12h Stop 6–24 hours before delivery. Resume oral anticoagulation 6–8 hours after delivery, or continue LMWH in postpartum, for 1–12 weeks UFH for emergencies, higher doses for history of severe/arterial thrombosis, warfarin at 14–34 wks.
C. Persistent positive APL without thrombosis or pregnancy loss or complications	Surveillance, or minidose UFH, or prophylactic LMWH and/or low-dose aspirin	Low-dose aspirin or surveillance
D. Persistent positive APL with (ovulation induction and) in vitro fertilization	Not addressed	LMWH or UFH during “high estrogen levels” (ovulation induction) Stop heparin during ovum pick up No consensus during pregnancy: Low-dose aspirin +/- LMWH for 10+ weeks

UFH: Unfractionated heparin; U=Units; SC: Subcutaneously; q: Every; h, hours.
LMWH: Low molecular weight heparin
Minidose UFH: UFH 5,000 U SC q12 hours
Moderate-dose UFH: UFH SC q12 hours in doses adjusted to target an anti-Xa level of 0.1 to 0.3 U/ml
Adjusted-dose UFH: UFH SC q12 hours in doses adjusted to target a mid-interval aPTT into the therapeutic range
Prophylactic LMWH: e.g., dalteparin 5,000 U SC q24h, or enoxaparin 40 mg q24h (or 1 mg/kg q24 h)
Intermediate dose LMWH: e.g., dalteparin 5,000 U q12h, or enoxaparin 40 mg SC q12h
Adjusted dose LMWH: weight-adjusted, full-treatment doses of LMWH once or twice daily (e.g., dalteparin 200 U/kg, or tinzaparin 175 U/kg, or dalteparin 100 U/kg q12h.

- A topic addressed by the APL Congress, but not by the ACCP, is the group of infertile women with persistent APL who resort to assisted reproductive therapy (ART) with ovulation induction, and in vitro fertilization (308). Some of these women develop SLE, or APS or thromboembolism during the ART. The APL Congress recommends LMWH or UFH after gonadotropin stimulation, during high estrogen levels, and discontinuation when ova are harvested, so as to avoid hemorrhage. There is no definitive recommendation during pregnancy, with some patients treated with low-dose aspirin and others with addition of LMWH for at least 10 weeks.

Women with positive APL, hypertension, and lupus nephritis should be given low-dose aspirin, even without previous pregnancy losses, because of heightened probability of preeclampsia.

Unfractionated heparin and the LMWHs are category B drugs, do not cross the placenta, and are not harmful to the fetus. Both, however, can cause osteoporosis and thrombocytopenia in the pregnant woman. There is evidence that LMWHs have a lower risk for development of spinal osteoporotic fractures than UFH (3% vs. 15%) (346). In a randomized trial, bone mineral density was significantly lower in women receiving UFH, whereas women on dalteparin did not differ from controls (347). The need for concomitant steroid therapy can further increase the chance of developing osteoporosis. Calcium intake of 1.5 g/day and exercise against gravity are recommended as preventive measures against

osteoporosis. Heparin-induced thrombocytopenia (HIT) is IgG-mediated and occurs in 3% of nonpregnant women 5 to 15 days after starting UFH. LMWHs cause less HIT (348). The heparinoid danaparoid sodium can be used if LMWH also causes HIT (349). LMW heparins are costlier than UFH.

Although warfarin has been used after organogenesis in mothers with prior thrombosis and recurrent fetal loss, it may be associated with malformations and fetal warfarin syndrome (277). Warfarin is FDA category D, XM (Table 51-4).

In a longitudinal study of SLE and APS women, in 9/15 with APS and prior fetal loss, ACL became negative during pregnancy and the women had live births (350). It should be noted that not all pregnancies in mothers with antiphospholipid antibodies are doomed; 4 patients had uncomplicated pregnancies and delivered at term (351).

Summary: Prevention of Fetal Loss Due to Antiphospholipid Antibodies

- Women with prior fetal losses and pregnancy complications related to antiphospholipid antibodies can be treated effectively, with substantial reduction in fetal loss.
- Guidelines for treatment are improving and categories of patients better delineated.
- Heparin with low-dose ASA are the mainstay of therapy and should begin when the urine pregnancy test becomes positive, should be continued until delivery, and sometimes into the postpartum period.
- LMW heparins show superiority over unfractionated heparin in terms of avoidance of osteoporosis, have less HIT, and lesser frequency of administration, but are costlier.
- Corticosteroids should only be given for lupus activity, not to suppress APL (318 ,325).

Antimalarial Drugs: FDA Category C

Antimalarial drugs have been used by pregnant women for years as prophylaxis in large-scale malaria eradication programs in Africa and Asia, with a good safety record (352). Even if the patient discontinues antimalarials early in pregnancy, there are deposits in the liver and other organs from which the drug is slowly excreted (353), with a half-life of 2 months. Hydroxychloroquine (HCQ) is the major antimalarial drug used in rheumatic diseases in countries where available. HCQ crosses the placenta with cord blood concentration nearly identical to the mother's (354).

There is a single report of retinal degeneration in two infants after malaria prophylaxis in the mother (355). No fetal malformations specific to HCQ per se have been reported. Most of the admonitions against the use of antimalarials during pregnancy cite a report of a woman with discoid lupus who intermittently took 500 mg of chloroquine daily, a dose over twice that recommended (356). Four of her seven pregnancies, conceived while on chloroquine, resulted in three children with congenital anomalies and one spontaneous abortion.

Discontinuation of HCQ carries a risk for flare: in a randomized, double-blind study, lupus patients on. placebo had a 2.5-fold risk for flare compared to HCQ patients, and the risk for a severe flare was 6.1-fold (357).

A total of 492 lupus pregnancies have been reported in mothers on HCQ, at times during the first trimester, without any fetal malformations in excess of the rate for the general population (358 ,359 ,360 ,361 ,362 ,363 ,364).

In 36 pregnancies among 33 women with SLE who continued hydroxychloroquine during pregnancy, there were no teratogenic effects (358). In 21 pregnancies where mothers took antimalarials for a mean of 32 months before pregnancy, there were no congenital malformations or developmental problems in the children through a mean follow-up of 5.3 years (361). The same authors reviewed 215 pregnancies with first-trimester antimalarial exposure, with only 7 cases of congenital malformations (3.3%), which is similar to the general population. In a double-blind, randomized, placebo-controlled study of 20 pregnant lupus patients, flares and toxemia occurred only in the placebo group (3 each), and there were no congenital abnormalities in the infants (362). Sixteen more lupus patients on HCQ throughout pregnancy delivered children without any ocular or aural deficits (364). A controlled study of 133 pregnancies on HCQ for at least 6 months before pregnancy was reported from a University center in Paris (359). Of the 90 mothers, 69 had SLE, and 20 of them SLE/APS. In 122 of the pregnancies the maternal dose of HCQ was 400 mg per day, in 11 pregnancies, 200 mg per day, and additional medications included prednisone and aspirin 100 mg in over 80%, heparin (26%) and azathioprine (2%). Of the 117 live births in mothers on HCQ, were present in 3 (2.6%) and in 4 of 70 control pregnancies (5.7%). The mean malformation rate in the European registry is 2.3%. Follow-up of the children until 26 months of age failed to show any vision, hearing, or developmental problems. Of 40 babies born to mothers on HCQ, 24, including 13 who were breastfed, were followed up during early infancy and had normal visual function and neurodevelopmental outcome (363). Similarly, 21 children with 7.2 months' gestational exposure to HCQ or chloroquine, had no ophthalmic abnormalities on follow-up (360). A survey of 52 lupus experts from North America and the United Kingdom showed that 69% continue antimalarial treatment sometimes, often, or always in pregnancy, and 63% continue HCQ in the postpartum period and advise breastfeeding (365). The physicians' recommendation to continue antimalarials increased with the number of pregnant lupus patients seen, and no fetal toxicity was observed.

Patients should be informed about the antimalarial deposits in the liver, the safety record of HCQ, and the paucity of congenital anomalies, when deciding about its use during pregnancy. In our experience, most patients opt to discontinue as many medications as possible during gestation.

Immunosuppressive-Cytotoxic Drugs (Table 51-4)

The most commonly used immunosuppressive drugs in SLE are azathioprine, cyclophosphamide, methotrexate (all three are FDA category D), mycophenolate mofetil (FDA category C), and cyclosporine (FDA category C). Nitrogen

mustard (FDA category D) and chlorambucil (FDA category D) are rarely, if ever, used. The first three are known to induce fetal malformations in animals and have shown potential for human teratogenesis (277). Use of these drugs during fetal organogenesis (the first trimester) has the greatest potential for causing fetal demise or malformation.

Among lupus, vasculitis, and rheumatoid arthritis patients on cytotoxic drugs, there is very little occurrence of fetal malformations. As a rule, patients are counseled to use effective contraception prior to starting immunosuppressive medication. A textbook and several excellent reviews on the subject have been published (277 ,366 ,367 ,368 ,369). Very often newborns have IUGR, low birthweight, and prematurity.

Azathioprine Category D

Azathioprine crosses the placenta, but the fetus lacks the enzyme inosinate phosphorylase, which converts azathioprine to active metabolites (mercaptopurine); hence, only traces of mercaptopurine are found in cord blood (370). The drug may cause decrease in thymic shadow size, lymphocyte count, and IgG and IgM in the neonate (371), and neonatal chromosomal abnormalities persisting for up to a year.

Of 974 pregnancies reviewed by Polifka and Friedman, 799 concerned mothers with renal transplants, 53 with SLE, 1 with mixed connective-tissue disease, and 121 with other transplants and other diseases (367). Fetal malformations were seen in 35 of 974, 3.6%, not unlike the general population prevalence of 3% to 4%, and there was no specific pattern. Prematurity was seen in 16.7% and IUGR was relatively common. In their review, Prevot et al. note prematurity of 40% to 52% and IUGR of 19% to 40% (368). Pregnancies in women with renal transplants, however, are high-risk pregnancies, often complicated by hypertension and fetal morbidity (372 ,373). Congenital anomalies were noted in 9 of 273 (3.3%) and 2 of 42 (4.8%) infants fathered by men on azathioprine for renal and cardiac transplants respectively (374 ,375).

Several additional reports of normal neonates born to lupus and renal transplant patients who received azathioprine and prednisone have appeared (90 ,95 ,101 ,376 ,377).

Ramsey-Goldman et al. reported on 23 lupus pregnancies on azathioprine, cyclophosphamide, or methotrexate (376). Adverse pregnancy outcomes and neonatal complications were no different in 519 pregnancies before SLE, 117 pregnancies after SLE on no immunosuppressives, and 23 pregnancies on immunosuppressive therapy.

Azathioprine interacts with warfarin. Either they should not be used concomitantly, or be used together with extreme caution (378). In summary, azathioprine is relatively safe during pregnancy, but the dose should be reduced to half in the third trimester, otherwise the neonate develops hypogammaglobulinemia.

Cyclophosphamide Category D

Cyclophosphamide (CTX) with prednisone or methylprednisolone has been the accepted therapeutic standard for severe SLE nephritis since the 1980s (379). CTX is highly teratogenic in experimental animals and can induce malformations in the human fetus. In addition, it can induce gonadal failure in men and women. Daily oral cyclophosphamide causes amenorrhea within a year, usually with permanent ovarian failure (71%), and monthly intravenous “pulse” cyclophosphamide can also cause amenorrhea (45%), depending on the dose (380). The risk of amenorrhea depends on the patient's age and total dose: in a National Institutes of Health (NIH) study of 39 women, amenorrhea was greatest in women older than 31 years of age, 62%, versus 12% in women younger than 25 years of age, and 27% at 26 to 30 years of age (381). Patients receiving seven doses of IV cyclophosphamide had a 12% chance of amenorrhea, versus 39% in patients on long-term therapy (15 or more doses) (381). Similar findings were reported in three more studies, from Greece, France, and Korea: of 67 women on long-term IV CTX (1.5 years total) 31.3% developed amenorrhea, the major factor being age ≥ 32. Important risk factors in younger women were SLE duration over 5 years, and the presence of anti-Ro and anti-U1RNP antibodies (382). Of 84 women treated with IV CTX for SLE (n = 56) and other diseases, mainly vasculitis (n = 28), 27.3% developed amenorrhea, which was sustained in 22.6%, with age being the major factor (383). Of 67 women with proliferative SLE nephritis treated with IV CTX, amenorrhea occurred in 37.3%, and became sustained in 14.9%. Age, high damage index, and high cumulative dose of cyclophosphamide were risk factors for permanent ovarian failure (384). The last two studies reported pregnancies after IV CTX. It has been suggested that the administration of monthly IV cyclophosphamide be timed during menses, when the ovarian follicles are quiescent. Preservation of ovarian function during CTX therapy has been achieved by treating concurrently with monthly injections of 3.75 mg gonadotropin-releasing agonist (GnRH-a, Lupron) (385). This treatment was successfully used in women with lymphoma. Of 75 patients with lymphoma, leukemia, or SLE undergoing chemotherapy, 93.3% maintained ovarian function, whereas only 46.3% of 82 patients on chemotherapy alone did (386).

First trimester exposure to cyclophosphamide has resulted in a fetal malformation complex termed cyclophosphamide embryopathy: it consists of growth deficiency, hypoplasia of calvarium and facial bones, hypotelorism, short palpebral fissures, supraorbital ridge hypoplasia, flat nasal bridge, microtia (small ears), cleft palate, and hypoplastic or absent thumb, radius and toes, variously present in the 8 newborns reported (387 ,388). Five of 8 newborns survived, 1 died, 1 pregnancy was terminated, and the fate of 1 was unknown. One pregnancy with cyclophosphamide late in the first trimester, and 4 pregnancies with chemotherapy including cyclophosphamide beyond the first trimester had a normal outcome (389). At the Los Angeles County University of Southern California Medical Center, three of our lupus patients on monthly IV cyclophosphamide for SLE nephritis became pregnant; cyclophosphamide was immediately stopped on all. One woman proceeded with a normal pregnancy and had a normal infant at term. The

second patient had a first-trimester spontaneous abortion, but conceived two more times, after cessation of cyclophosphamide therapy, and, despite a mild exacerbation during both pregnancies, carried to term and had two healthy infants. At this time, 17 and 15 years later, the children have developed normally and are healthy. The third patient received IV CTX inadvertently, had a normal newborn who is healthy and developing well to date, 1 year later. It is virtually impossible to foretell any risk of oncogenesis among such children. I have recommended IV pulse CTX during two pregnancies after the first trimester for a colleague's patient who developed acute oliguric/anuric renal failure, with improvement in the mother and without ill effects to the children so far. A primigravida with SLE nephritis unresponsive to steroids was given cyclophosphamide without apparent problems in the newborn (390). Cyclophosphamide was also given during pregnancy to four patients with severe SLE: in two, CTX was given inadvertently in the first trimester and both miscarried. The other two patients were treated for severe nephritis and thrombocytopenia with microangiopathic anemia at week 20 and 22, and both had fetal demise, 1 day and 7 days after CTX (391). In summary, IV CTX should only be given during pregnancy if the mother's life is endangered by severe SLE.

Mycophenolate Mofetil Category C

Mycophenolate mofetil (MMF) (FDA category C) is an inosine monophosphate dehydrogenase inhibitor that inhibits purine synthesis and is getting established as an effective therapy for lupus nephritis (392). Three instances of fetal malformations have been reported with MMF: of 14 pregnancies in 10 women with kidney transplants, there were 6 spontaneous abortions and 8 live births (393). A newborn with exposure to MMF, tacrolimus, and prednisone since the 7th week of gestation, had hypoplastic finger- and toenails and shortened fifth fingers bilaterally (394). Another newborn, conceived while the mother was on the same drugs, had cleft lip and palate and an ear deformity. A third case was reported from Paris, with first trimester exposure to MMF (500 mg/day), tacrolimus and prednisone (395). At 13 weeks the pregnancy was diagnosed and azathioprine 50 mg/day substituted for MMF. Major malformations were detected and the pregnancy was terminated at 22 weeks. The fetus had a large cleft lip and palate, hypertelorism, micrognathia, microtia, atresia of the external auditory canal, agenesis of the corpus callosum, and a left pelvic ectopic kidney. In a subsequent pregnancy, the patient was treated with azathioprine, tacrolimus, and prednisone and delivered a normal premature infant. Effective contraception is imperative in patients undergoing MMF therapy.

Methotrexate (MTX) Category X

Methotrexate, a methyl-derivative of aminopterin, reversibly inhibits dihydrofolate reductase, thus interfering with purine synthesis, and also has anti-inflammatory effects. It is used in rheumatoid arthritis as a first-line disease modifying drug in low weekly doses (7.5 to 25 mg), and also in SLE, poly/dermatolmyositis and other systemic rheumatic diseases. It has also been used as an abortifacient. Methotrexate induces multiple animal and human malformations, especially with exposure from the 4th to 12th week of gestation. The MTX-induced malformations are known collectively as “aminopterin syndrome” and consist of CNS and skeletal abnormalities (spina bifida, mental retardation, hydrocephalus, anencephaly, synostosis of lambdoid sutures, micrognathia, high or cleft palate, short extremities, hypertelorism, syndactyly, absent digits, club foot, large fontanelles, wide-set nasal bridge, and dextrocardia) (396). It has been suggested that there may be a common mechanism through apoptosis for the fetal malformations seen with CTX, MTX, and cytarabine (388). In the review by Lloyd, of 71 fetal exposures to MTX, 42 were in the first trimester and 10 of these (23.8%) resulted in physical malformations, with 4 from mothers on <20 mg MTX per week (396). Two children had later developmental abnormalities after third trimester exposure. Because MTX is found in the liver up to 116 days after the last dose, it is highly recommended that no conception be attempted before 5 months have elapsed from discontinuation, and that folinic acid, 10 mg per week, rather than folic acid, be taken throughout pregnancy in women exposed to MTX. Fertility after MTX therapy is unimpaired, with 97% conception rate 1 year after cessation of MTX. Of ten pregnancies in eight women with rheumatoid arthritis who conceived while on 7.5 mg MTX per week, three had spontaneous and two, elective abortions (397). All except one stopped methotrexate within the first trimester, and most received folate supplements. The five remaining pregnancies resulted in full-term normal infants.

Cyclosporine A Category C

Cyclosporine A (CSA) is a calcineurin inhibitor and has been widely used to prevent rejection in organ transplantation; it can cross the placenta, and is excreted in milk. Knowledge about cyclosporine in pregnancy is derived mostly from renal transplant recipients. By 1998, in 405 pregnancies under cyclosporine treatment, there were no excessive fetal malformations, or any specific pattern in those observed (398). On follow-up of 175 children of 133 women with renal transplants maintained on CSA through pregnancy, developmental delay was found in 0.5% of 20 children 1 year of age, in 6.3% of 84 children 1 to 5 years old, and in 9.7% of 71 children 5 to 12 years old (399). Overall, 16% of the children had delays or needed educational support. Given the high prevalence of prematurity and IUGR in this group (at times exceeding 50%), developmental delays may not be a result of CSA.

Nitrogen mustard has been used for the treatment of lupus nephritis in the past. In a report of 250 patients with nephritis, 18 of 44 women who had at least one course of nitrogen mustard had 11 successful pregnancies (159).

The decision to continue cytotoxic drugs during pregnancy depends on the need for disease control, and should be made jointly with the patient while weighing the potential

risks versus benefits. If continuation of pregnancy under cytotoxic therapy is desired, it is wise to have an amniocentesis done and the karyotype determined.

Leflunomide Category X

Leflunomide is only occasionally used in SLE (400), is a competitive inhibitor of dihydroorotate dehydrogenase, an enzyme necessary for pyrimidine synthesis, and has a very long half-life because of enterohepatic circulation, approximately 14 to 15 days. It is a known embryotoxic drug, and, because of its long half-life, pregnancy should not be attempted just after discontinuation. The manufacturer recommends cholestyramine treatment, 8 g three times a day for 11 days, with confirmation of blood levels less than 0.02 mg/L after treatment and 2 weeks later (401).

Rituximab Category C

Rituximab is a chimeric monoclonal antibody against CD20, which is expressed by B lymphocytes from the pre-beta cell to the mature B cell stage. It can effect long term B cell depletion, has been used successfully in lymphomas and in phase I/II studies of severe lupus, including nephritis, with or without cyclophosphamide (402 ,403). There are two case reports of pregnant women treated with rituximab during pregnancy for non-Hodgkin's lymphoma (404 ,405). The first had aggressive $CD20^+$ lymphoma and was treated with rituximab 375 mg/m^2, doxorubicin, vincristine, and prednisone for 4 cycles, starting in the 21st week of pregnancy. In the 35th week, she delivered by Cesarean section. Her child had normal B cell counts and CD19/20 by 4 months of age (404). The second patient, also with non-Hodgkin's lymphoma, conceived after the first dose of rituximab and decided to continue the pregnancy. She delivered vaginally at the 40th week. The child had granulocytopenia until 4 months, initially low IgM and IgA, but immunoglobulins, immune function, and development were normal by 18 months of age (405).

Miscellaneous Therapeutic Measures

Plasmapheresis

Plasmapheresis can be safely performed during pregnancy and has been used successfully in severe preeclampsia/eclampsia, with refractory HELLP syndrome that does not improve after delivery in women with APS and/or SLE (406 ,407 ,408 ,409 ,410), in severe lupus during pregnancy (411 ,412 ,413 ,414), for prevention of recurrent fetal loss in women with APL (415 ,416 ,417 ,418), and for prevention of congenital heart block due to anti-Ro and anti-La (419 ,420 ,421 ,422 ,423). Severe lupus manifestations improved with plasmapheresis (and steroid) include vasculitis, with decreased steroid requirement (412), extremely severe flare with myositis (414), and diffuse alveolar hemorrhage with oliguric renal failure (2 patients), in combination with continuous venovenous hemofiltration, steroids, and cyclophosphamide in 1 woman (411). In 1 patient, plasmapheresis failed to reverse thrombocytopenia, with subsequent fetal demise (413). Two patients with recurrent pregnancy loss and APL were given plasmapheresis. In 1 patient, dramatic reduction of APL was associated with successful pregnancy outcome (415). In the other, who had a multisystem disease with myositis, plasmapheresis was given because of fetal distress. The authors believed that they gained 2 more weeks of gestation, during which further fetal maturation occurred, which allowed fetal survival after Cesarean section at 29 weeks (416). Immunoadsorbent plasmapheresis with dextran sulfate, low-dose prednisone and low-dose aspirin in 9 pregnancies decreased lupus anticoagulant and anticardiolipin antibodies, with successful delivery in eight of nine lupus pregnancies (417 ,418). Prevention of congenital heart block because of anti-Ro and or anti-La was accomplished in 6 pregnancies by plasmapheresis, dexamethasone or prednisone (419 ,420 ,423), plus azathioprine (421 ,422). Plasmapheresis was started as early as the 15th week of gestation, and dexamethasone from the 7th or 10th week (422 ,423). An excellent review on plasmapheresis in pregnancy has been published (424).

Intravenous Immunoglobulin Therapy Category CM

Ten papers have reported 34 women with four to nine fetal losses each, positive anticardiolipin antibodies, lupus anticoagulant, and preeclampsia in three (310 ,321 ,425 ,426 ,427 ,428 ,429 ,430 ,431 ,432). Treatment with intravenous immunoglobulin (IVIg), at times with the addition of low-dose aspirin, heparin, and steroids, resulted in live births. Immunoglobulin was given at 400 to 1,000 mg/kg/day for 2 to 5 days per month. IVIg suppressed APL levels after each infusion (428). In an in vitro study, IVIg neutralized lupus anticoagulant activity in 10 of 11 patient sera (433). The mechanism of IVIg action is probably a result of anti-idiotypic antibodies against antiphospholipid antibodies (434) and other autoantibodies. In addition, IVIg has been used in severe clinical SLE manifestations (severe thrombocytopenia, pulmonary alveolar hemorrhage, transverse myelitis, and perhaps anuric renal failure) refractory to high-dose steroids. Three recent controlled studies in women with APL and recurrent pregnancy loss compared IVIg plus heparin and aspirin to heparin plus aspirin plus albumin (313), IVIg versus aspirin plus prednisone (342) and IVIg versus ASA plus LMWH (341). None of these studies proved any superiority of IVIg in pregnancy outcomes, as noted previously. However, this expensive treatment is worth considering in severe, life-threatening SLE, as noted above.

Herbs and Alternative "Medicines"

We all live in societies of great cultural diversity and should be keenly aware that our patients may be taking substances that may be harmful to themselves and their fetus, although patients are often convinced that "herbs are harmless." Some examples are: Ginger, used for morning sickness, may cause abortion, mutations of the fetus, or increased risk of bleeding. Blue cohosh tea causes uterine contractions and is used

to help induce labor, but it has been associated with myocardial infarction of the fetus. Species of birthwort are also used in pregnancy, even though they contain known nephrotoxins and mutagens. Hellebore is used for nausea and to regulate menstruation, hemlock as a sedative or for cramps and spasms, and tragacanth for immune stimulation, yet all are known teratogens in animals. These and other supplements should generally be avoided in pregnant patients.

In recent years, the potential dangers of herbs have become apparent with thousands of adverse events reported by users of ephedra supplements for weight loss, liver problems from kava use, and renal failure in African folk medicine users. For further information, see http://www.naturalstandard.com/.

Recommendations for Therapy in Lupus Pregnancy

- The best assurance for a successful pregnancy is control of the mother's lupus. Maternal disease should be assessed carefully for activity and severity months before and at the onset of pregnancy, which should ideally be planned after several (approximately 6) months of remission. The mother should be watched carefully for flares.
- Maternal flares should be diagnosed early and treated aggressively with appropriate steroid dose. Unless there is a dire emergency, i.e., acute anuric renal failure, or pulmonary alveolar hemorrhage, cytotoxic drugs such as cyclophosphamide, MMF, and methotrexate should be avoided during the first trimester. Azathioprine in reduced doses, or cyclosporine, can be used during pregnancy. Mothers with nephritis at risk for hypertension and preeclampsia should be treated with low-dose aspirin (60 to 81 mg/day) until the 36th week of gestation.
- The mother's status in terms of antiphospholipid antibody and anti-Ro/SSA, anti-La/SSB positivity should be known: the former, because of predisposition to fetal loss, IUGR and preeclampsia, and the latter two because of the small risk for congenital heart block in the child (see also Chapters 52 and 53).
- Joint follow-up by the rheumatologist and high-risk obstetrician should be done every month for the first half of pregnancy, and more frequently thereafter (every 1 to 3 weeks), with blood pressure check, careful exam, repeat of CBC, urine, chemistry, quantitation of proteinuria in patients with nephritis, complement C3 and C4 levels, anti-dsDNA, and a measure of circulating immune complexes on a monthly basis. Patients with known prior problem pregnancies should be followed more often.
- In the event of highly positive antiphospholipid antibodies (positive LAC, high IgG ACL, LAC plus IgG ACL, anti-β_2GPI), with a history of intravascular thrombosis or prior fetal loss, the patient should be enrolled in an effective antithrombotic protocol (LMWH with low-dose aspirin). Unless there is concomitant lupus activity, high-dose prednisone for APL alone is not indicated.

Breastfeeding (Table 51-4)

All drugs are excreted in human milk, usually in trace but variable amounts (352,435–437a). Factors influencing drug concentrations derived from milk in the infant have been delineated by Brooks and Needs (435). Maternal factors include milk fat and protein concentrations, milk pH, mammary blood flow, and maternal drug metabolism (e.g., absorption, protein binding, and plasma clearance). Drug-related factors include molecular weight, lipid solubility, pKa, elimination half-life, pharmacokinetics, dose amount, and interval. Infant factors include volume of milk consumed, feeding intervals relative to maternal drug intake, absorption, and metabolic and deconjugating ability of the infant.

An index has been proposed to calculate infant exposure to drugs in breast milk, which takes into account milk to maternal plasma drug concentration and drug clearance in the infant (438).

After a single dose of 5 mg of prednisolone, 0.07% to 0.23% of the dose was found in maternal milk (437a). With a maternal dose of 7.5 mg per day, a child drinking 1 L of milk daily would receive 0.028 mg of prednisolone (0.33%) (436). Long-term treatment of the mother with 10 to 80 mg/day of prednisolone produces milk concentrations 5% to 25% of those in serum (437 ,439). The milk/plasma ratio increases with increasing serum concentrations and it has been calculated that, at a maternal dose of 80 mg/day, the infant would be exposed to less than 0.1% of the maternal dose (437 ,440). The peak plasma level after oral intake is attained at 1.1 ± 0.7 hours; therefore, the exposure of the infant can be minimized by appropriate timing of nursing. No untoward effects in nursing infants have been reported (437 ,441), and maternal doses of up to 30 mg/day are probably safe.

Cyclophosphamide is found in substantial concentrations in human breast milk (442) and neutropenia and leukopenia were reported in two infants whose mothers used cyclophosphamide while breastfeeding; thus, nursing is contraindicated in a mother who requires CTX. The milk level of methotrexate in a woman with choriocarcinoma of the uterus was 8% of the plasma level (443). With small weekly doses, such as those used in rheumatic diseases, there may be a greater measure of safety, but data are lacking. Only small amounts of azathioprine have been detected in breast milk (444). Encouraging data are available for cyclosporine: in seven lactating transplanted mothers on cyclosporine, drug concentration in breast milk was similar to maternal trough level, but was below the detection limit in the infants, and their creatinine levels were normal (445).

Overall, a great deal of good judgment and caution should be exercised. Until data such as those on cyclosporine are available, the need for maternal cytotoxic drug therapy would preclude breast-feeding.

Antimalarials are also found in small amounts in human milk (446 ,447). Nation et al. (446) have calculated that the infant would be exposed to about 2% of the maternal daily dose of hydroxychloroquine. Although there are very few

problems with malaria prophylaxis in nursing mothers, doses used in lupus could expose the child to the risk of retinopathy.

In general, NSAIDs are weak acids and achieve low concentrations in the acidic pH of milk. After a single aspirin dose of 450 to 650 mg, 0.1% to 21% reaches the infant over a 24-hour period (448). Peak salicylate concentrations in milk occur about 2 hours after peak serum levels (449). However, if the mother takes anti-inflammatory doses, in view of the immature neonatal metabolic processes, the infant may develop acidosis and bleeding diathesis. Furthermore, the infant can absorb free salicylic acid from the cleavage of salicylphenolic glucuronide in the milk (450). Trace amounts of naproxen, piroxicam, ibuprofen, and diclofenac have been reported in milk. Some of the NSAIDs have enterohepatic circulation (e.g., indomethacin, sulindac), and are not recommended during lactation. With a maternal daily dose of 200 mg celecoxib, the infant's daily dose would be approximately 0.3% of the mother's, deemed to be unlikely to cause untoward effects (451). For the position of the American Academy of Pediatrics on drugs in milk, see (452).

Contraception

Uncontrolled and anecdotal reports have suggested that older oral contraceptives (OC) containing larger doses of estrogens cause SLE flares (50 ,453). In a study of 20 women with SLE nephritis by Jungers et al., the use of preparations containing 30 or 50 µg of ethinyl estradiol was associated with SLE flares in 43% within 3 months of beginning oral contraceptives, whereas 11 patients on progestational oral contraceptives did not flare during a 30-month period (98). Nevertheless, many patients tolerate small-dose estrogen OC (“mini-pills”) without adverse effects (454). In retrospective studies by Julkunen et al. (455 ,456) and Buyon (457), substantial numbers of lupus patients are able to tolerate modern-day OC or hormone replacement, with flares in 8% and 13% respectively and no thromboembolism. Newer OC contain <35 µg of ethinyl estradiol, and are preferable to 50 µg in the older preparations. Exceptions are women with antiphospholipid syndrome and prior thromboembolic episodes, or severe migraines, because thromboembolic phenomena have been associated with estrogen contraceptives (458).

Two important studies were recently published: in a prospective double-blind randomized placebo-controlled multicenter study, 183 women with inactive (76%) or stable-active (24%) SLE were assigned to either combination OC or placebo (OC-SELENA study). Rates of severe flare were similar, 7.7% in OC patients versus 7.6% in placebo patients (459). The 12-month rates of severe flare (0.084, OC group, and 0.087, placebo group) and of mild or moderate flares (1.40, OC and 1.44, placebo) were also similar, There were two clotting episodes in the OC group and three in the placebo group.

In a single-blind study from Mexico City, 162 women with SLE were randomly assigned to combined OC, a progestin-only pill, or a copper intrauterine device (IUD) for 1 year (460). Disease activity remained mild and stable in all groups throughout the trial, and there were no significant differences in global or maximum disease activity, incidence or probability of flares, or medication use. Flare rates and severe flare rates per patient-year were similar in the three groups: 0.86 and 0.049 in the combined OC group, 1.14 and 0.114 in the progestin group, 0.91 and 0.046 in the IUD group. There were two thrombotic events in each of the hormone groups. The conclusion from these seminal studies is that combination OC can be well tolerated in women with inactive or stable active SLE.

In a controlled study with progestogens, using either oral levonorgestrel 0.03 mg daily or norethisterone enanthate 200 mg IM every 3 months, there was no increase in flare rate compared to controls (6/122 patient/months and 4/48 patient/months respectively) (170). Menstrual irregularity and spotting were common complaints among the patients. Commercially available progestational agents include oral norethindrone (norethisterone), norgestrel, levonorgestrel, ethynodiol diacetate, and lynestrenol (461). Intramuscular depot medroxyprogesterone acetate (Depo-Provera), given every 12 weeks, is effective and does not confer increased risk of thrombosis, but may cause irregular bleeding, weight gain, and decreased bone density with protracted use. If long-term contraception (up to 5 years) is desired, a subdermal progestin implant (levonorgestrel) may be considered, as long as the implantation site is carefully watched for infection (462). Menstrual irregularity for 6 to 12 months from onset of use is common, and bleeding, amenorrhea, and, rarely, ectopic pregnancy may occur.

Mechanical barrier methods, such as the diaphragm, cervical cap, female condom, and male condom with spermicide cream or jelly, although considered cumbersome by some, are safe and effective. Male condoms are not very reliable and may rupture. Intrauterine devices are associated with dysmenorrhea, more frequent local infections (84 ,460), risk of menorrhagia, endometritis, and perforation, and should be used with caution. The copper IUD lasts for 10 years. An IUD with levonorgestrel (Mirena) is available and lasts for 5 years.

Hormone Replacement Therapy (HRT)

An important multicenter double-blind randomized placebo-controlled study of hormone replacement during menopause (SELENA study) was recently published: 351 postmenopausal women with inactive (81.5%) or stable-active SLE (18.5%) were randomized to combination HRT (0.625 mg estrogen plus 5mg progesterone for 12 days per month) or placebo for 1 year (463). The risk for severe flares was similar in the HRT and placebo groups, 7.5% and 4.5% respectively (12-month incidence rates 0.081 and 0.049). Mild and moderate flares were increased in the HRT group, with a significantly greater incidence rate: 1.14 flares/person-year for HRT versus 0.86 for placebo. There was one death, one stroke, and three clotting episodes in the HRT group, and one thrombotic episode in the placebo group. Women with positive APL antibodies or history of thrombosis were excluded from this trial. Although HRT has been discredited as cardioprotective (464 ,465), there are potential indications for premature

menopause management in SLE patients, and short-term treatment of postmenopausal bone loss.

Lupus and Assisted Reproduction

Ovulation induction (OI) is the first phase in assisted reproductive therapy (ART), followed by in vitro fertilization (IVF) and embryo transfer (ET) in the uterus. Stimulation of ovulation is done with a variety of hormonal or nonhormonal manipulations and results in tremendously high plasma estradiol levels. Several types of problems related to OI have been described: flares in patients with known lupus, de novo development of lupus, development of APS, and thrombotic episodes in patients with or without APS. To further complicate matters, ovarian hyperstimulation syndrome (OHSS), which may occur in 1.4% to 2.3% of women undergoing ART (466), can mimic a lupus flare. OHSS appears to be mediated by a variety of cytokines, including VEGF, and is distinguished into three grades: grade 1 consists of ovarian enlargement to 5 cm; grade 2, of ovarian size of 5 to 12 cm, nausea, vomiting, diarrhea, and abdominal distention; and grade 3, of massive ovarian size (>12 cm), ascites, pleural effusions, edema, hypotension, electrolyte imbalance, and hypercoagulability with phlebothrombosis (467). Thrombotic episodes often occur in the upper extremities and jugular veins (468).

The prevalence of SLE in 136 women undergoing OI-IVF was determined as 1.5% by questionnaire, ANA, anti-dsDNA and clinical assessment (469).

A total of 8 patients without prior SLE (1 with Raynaud phenomenon) developed lupus, 2 with SLE and APS, after OI (470 ,471 ,472 ,473). The development of SLE occurred after 1 to 27 cycles of OI. One of the patients developed rapidly progressive glomerulonephritis (470).

In 12 patients, 11 with known SLE and 1 with discoid LE, flares developed, ranging from arthritis, malar rash, alopecia, and myalgia, to myositis, pericarditis, vasculitis, seizures, and transverse myelitis with death (56 ,474 ,475). The last patient had also APS and died after a second attempt at conception with OI (56). One of the patients with SLE developed superior vena cava and left renal vein thrombosis (474). Of 7 SLE patients reported from New York, 3 had flares and 2 OHSS (475). The same paper reported 10 patients with APL syndrome and 2 with APL positivity undergoing OI while on antithrombotic therapy: 1 patient developed osteopenia secondary to heparin, but no thrombosis.

New diagnosis of APS was made in 8 patients undergoing OI, with femoropopliteal thrombosis and pulmonary embolus in 1 of 5 patients from Paris (472), and 1 each with middle cerebral artery stroke and OHSS (476), intracardiac thrombosis (477), and catastrophic APS (478).

Wechsler et al. (479) proposed the following guidelines for SLE patients desirous of ART:

- SLE remission without need for aggressive therapy for at least 12 months.
- Absence of systemic hypertension, significant renal failure, pulmonary hypertension, frank heart valve disease, or major vascular past history.
- Antiestrogens should be used (clomiphene and tamoxifen), followed by pulsatile GnRH, if the above fails. Gonadotropins have the greatest risk for OHSS.

The same group reported on 21 women, 19 of whom fulfilled 4, and two fulfilled 3 criteria for SLE, and 9 had concomitant APS (472). Of 18 pregnancies accomplished, 9 resulted in live births. Planned OI procedures resulted in 6 live births of 7 pregnancies, whereas 75% of pregnancies ended with fetal loss when the diagnosis was unknown to the gynecologists. Pregnancy rates (25%) and SLE flares (27%) were higher with gonadotropin or GNRHa treatment, and lower with clomiphene (4% and 6% respectively). There was no OHSS in this report.

A protocol for the prevention of OHSS has been proposed (480).

Although APL have been found with greater frequency among women failing IVF, and some have been treated with heparin and aspirin, the Practice Committee of the American Society for Reproductive Medicine has examined seven prospective studies and concluded that there was no association between APL and IVF failure, that testing for APL is not indicated, and that treatment is not justified (481).

Family Planning and Counseling

The patient with SLE and her partner must be assured that she is just as fertile and capable of having children as any other woman; however, she has an increased likelihood of a high-risk pregnancy. She should ideally plan her pregnancy after a sustained remission of several months. She, her partner, and the physician should assess her functional limitations and explore her emotional motivation prior to undertaking pregnancy and the responsibilities of raising a child. Her socioeconomic setting and husband/mate relationship are no less important. The rheumatologist and obstetrician should counsel the patient and family about chances of flare, fetal loss, prematurity, and IUGR, and prepare her to cooperate with the rigorous follow-up necessary. Only in the event of severe renal, myocardial, or pulmonary compromise should elective abortion be considered. The patient and husband should be cautioned, however, about the possibility of flare after elective abortion.

Couples should be made aware of the following points:

- Women with lupus have normal fertility, therefore, there is a need for family planning, just as in individuals without lupus.
- The best time to plan a pregnancy is after a 6- to 12-month remission or at least quiescence of SLE, even though there is no guarantee that the disease will remain inactive. With conception after remission, the chance of a flare is 10% or less.
- The probability of a flare varies and depends on the severity and activity of disease. The milder the disease, and the less active, the lesser the chance of a flare during pregnancy.
- Hypertension and preeclampsia are more common in SLE with nephritis, with antiphospholipid antibodies, and particularly in patients conceiving with active disease.

- Increased pregnancy loss, especially with active lupus, nephritis, and antiphospholipid antibodies can usually be prevented with heparin and low-dose aspirin. Details and side effects of this treatment should be explained.
- The methods for monitoring fetal growth and cardiac health need to be clearly explained (see also Chapters 52 and 53).
- The potential necessity of and indications for cesarean section should be outlined beforehand (preeclampsia, fetal distress, abnormal nonstress test, maternal aseptic necrosis of the hips with inadequate hip abduction, and usual obstetric indications, including cephalopelvic disproportion, transverse presentation, and others).
- Prematurity may be as high as 60% in women with active SLE, and intrauterine growth restriction may reach 30% of premature deliveries. The need for proper care of the newborn in adequate intensive care units and afterward should be stressed.
- Women positive for anti-Ro/SSA and/or anti-La/SSB should be aware of the small risk (about 1.6–2%) of congenital heart block in the child, the need for early detection, and for in utero treatment.
- Prednisone treatment may increase prematurity and has a small risk of cleft lip and palate, whereas cytotoxic drugs during the first trimester, should they be needed for severe flares, carry a risk of congenital malformations
- With close monitoring and aggressive treatment during pregnancy and the postpartum period, no long-term worsening of SLE should occur.

In the poststeroid era, the early diagnosis and appropriate therapy of lupus have led to tremendously increased survival rates of SLE patients—up to 97% for 5 years, 93% for 10 years, and 83% for 15 years (162). Similarly, with increased awareness of the potential problems for the mother and fetus, meticulous multidisciplinary follow-up, and effective disease control, most women with lupus can and do achieve motherhood.

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Chapter 52

The Fetus in Systemic Lupus Erythematosus

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A variety of fetal and neonatal problems is associated with lupus. This chapter will deal with fetal loss, its pathogenesis and treatment, prematurity, intrauterine growth restriction (IUGR), their causes, and management recommendations. For a definition of terms used, please see Chapter 51 . Over the last 55 years there has been an enormous interest in the subject with a plethora of studies documenting that practice has evolved toward earlier disease detection and more aggressive therapy. In the series antedating 1980, the outcome of pregnancy in patients with systemic lupus erythematosus (SLE) was characterized by higher fetal loss than the general population, with increase in spontaneous abortions and stillbirths. There had been little change in the 30 years between 1950 and 1980 (1 ,2 ,3 ,4) (Table 52-1). However, in the 1980s, 1990s, and 2000s, there is better understanding of the causes of fetal loss and better fetal survival through improved pregnancy management (5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 , 41 ,42 ,43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53).

Fetal Outcome in Systemic Lupus Erythematosus

A pregnancy may result in live birth, full-term, or premature (preterm) delivery, or in fetal loss. A neonate may be of normal weight for age, or small for gestational age, the latter also known as IUGR. Fetal loss may be a result of spontaneous abortion (before the 20th week of pregnancy), or of intrauterine fetal death (stillbirth, after the 20th week of gestation).

The factors responsible for overall fetal loss in lupus include antiphospholipid (APL) antibodies, disease activity, and lupus nephritis, discussed later.

It is increasingly recognized that the death of a fetus of normal appearance on ultrasound after 10 weeks' gestation has a much stronger association with autoimmune mechanisms than earlier loss. The common very early pregnancy loss is most often chromosomal or hormonal in nature. Therefore, the International Congress on Antiphospholipid Antibodies (APL Congress) included both pre-embryonic, embryonic losses, and the fetal-neonatal complications in their 1999 criteria, distinguishing three categories of pregnancy loss (54):

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
- One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe preeclampsia or severe placental insufficiency
- Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation

In practice, this means that we look for evidence of APL antibodies, or subclinical SLE or APL syndrome (APS) as a cause of pregnancy loss in the above settings.

The older data dividing spontaneous abortions and stillbirths by 20 weeks' gestation are still needed for comparison and trends, especially for regional and national statistical comparisons.

Spontaneous Abortions

The prevalence of spontaneous abortions (SABs) in SLE pregnancy (Table 52-1) was reported as 11.6% for the decade 1950 to 1959, 19.5% for the years 1960 to 1969, and 19.4% for the period 1970 to 1979. All these figures are derived from retrospective studies. In most studies that compare pregnancy outcome before and after lupus onset, fetal loss was found to be increased even before disease onset (2 ,3 ,4 ,5 ,6 ,7 ,8). SABs ranged from 14% to 35%, with general population rates reported as 7% to 12.5%. Full-term deliveries ranged from 64% to 86% (average 72.5%).

After onset of SLE, there was usually an increase in SABs, reported in 4% to 40%. In the study by Fraga et al. (3), 23.1% of 183 pregnancies before SLE onset ended in SABs, with 12.5% in 288 control pregnancies (4). After SLE onset, SABs rose to 40.5%. An exception is a study from Greece, where no increased fetal loss prior to disease onset was found (8).

Between 1980 and 1989, 17 reports on 724 lupus pregnancies appeared (4,5,8–22), three of them were prospective (16,18,22), and five addressed SLE nephritis and pregnancy (4,5,12,15,20). The mean prevalence of SABs in 543 pregnancies (excluding most of the nephritis pregnancies), was 13.7% (Table 52-1). The highest prevalence was 30% in a patient questionnaire study from Vancouver (17), whereas the lowest rates are 0% and 6% (8,9). In the prospective study from Mexico City by Mintz, the frequency of SABs was 16.6% (17 of

102 pregnancies), significantly higher than 6.7% in their controls (18).

Table 52-1: Fetal Outcome of Systemic Lupus Erythematosus Pregnancies, Exclusive of Elective Abortions

		Percent					
		Pregnancy (Fetal) loss					
Decade/Time Period	No. of Pregnancies	Spontaneous Abortions	Perinatal loss/IUFD + NND	Total	Live Births	Premature Births	IUGR/SGA
1950–1959	155	11.6	10.3	27.5	72.5	before 1980:	—
1960–1969	307	19.5	6.8	27.0	73.0	15.3	—
1970–1979	505	19.4	8.5	27.9	72.1	—	—
1980–1989	543 (724)[†]	13.7	7.4	22.3	75.1	21.9	31.0
1990–1999	1652 (1780)[‡]	14.8	4.6	19.1	80.4	36.4	18.2
2000–2005	1464 (1859)[a]	14.6	4.7	17.9	82.1	23.2	18.1
Studies of the 1980s	**No. of Pregnancies**	**Percent**					
Tozman (9)	18	6	6	11	89	11	—
Houser (10)	17	8	0	18	82	21	—
Zulman (11)	24	8	4	12	88	4	—
Fine (13)	45	7	22	29	71	31	32
Varner (14)	34	9	6	15	85	10	35
Gimovsky (4)	65	35	11	46	54	22	34
Lockshin (16)*,**	25	—	—	44	56	33	—
Stein (17)	54	30	7	37	63	—	—
Mintz (18)*,**	102	17	5	23	77	63	23
Meehan (19)	18	17	0	17	83	17	—
Siamopoulou-Mavridou (8)	14	0	14	14	86	7	—
McHugh (21)	47	—	—	34	66	—	—
Lockshin (22)*	80	—	—	24	76	—	—
Studies of the 1990s							
Nossent (23)	39	10	5	15	75	19	—
Wong (24)*,**	19	11	0	11	89	47	12
Pistiner (25)	528	18	—	18	82	—	—
Petri (28)*,**	74	—	—	15	85	45	—
Tincani (29)*	25	16	0	16	84	20	27
TambyRaja (30)*	50	8	2	10	90	—	—
Julkunen (31)	105	19	2	21	79	—	—
Petri (32)	157	—	—	27	73	24	—
Derksen (33)*	35	23	3	26	74	—	—
Huong (34)*	94	16	3	19	81	51	—
Lima (35)*	106	6.6	9.4	16	84	42.7	31
Huong (36)*	60	16.7	3.3	20	80	60.4	—
Johns (37)	40	30	7.5	37.5	62.5	36	—
Rahman (38)*	121	28.1	2.5	30.6	69.4	24.4	7.1
Carmona (39)*	56	5.4	8.9	14.3	85.7	20.8	9.4
Sittiwangkul (40)	44	13.6	6.8	20.4	79.6	38.6	20
Kobayashi (41)	75	8	2.7	10.7	89.3	16.7	21.2
Huong (42)	24	8	8	16	84	64	NS
Studies of 2000–2005							
Georgiou (43)**	49	15	2	17	83	7.8	4.1
De Bandt (44)	52	25	3.8	28.8	73.1	48.7	28.2
Yasmeen (45)	555	NS	3.5	3.5	96.5	21	6.7
Brucato (46)	146[b]	9.5 <10 wk	5.4 >10 wk	15.0	85.0	22.2	0-2.3
Cortes-Hernandez (47)	95	15.8	12.6	28.4	71.6	27.9	35.3
Nephritis	19	26.3	31.6	57.9	42.1	37.5	37.5
Tan (48)	26	0	7.7	7.7	92.3	7.7	19.2
Mok (49)	91	NS	NS	12.1	87.9	16.3	17.5
Nephritis	33	NS	NS	6	94.0	12.9	19.4
Chandran (50)	41	29.3	12.2	41.5	58.5	4.2	4.2
Inactive	23	8.7	8.7	17.4	82.6	NS	0
Active	18	55.6	16.7	72.2	27.8	NS	9.1
Molad (51)	29	20.7	0	20.7	79.3	17.4	50.0
Chakravarty (52)	61[c]	9.4	3.2	12.7	90.6	53.7	9.0
Clowse (53)	267	7.1	7.1	14.2	85.8	36.2	22.5
Low activity	210	7.1	4.8	11.9	88.1	29.7	20.8
High activity	57	7.0	15.8	22.8	77.2	63.6	29.5
Carmona (62)	52	17.3	0	17.3	82.7	18.6	NS

*Prospective series.
**Controlled series
NS, not specified.
[†]227 pregnancies are discussed under Lupus Nephritis, Table 52.2
[‡]128 pregnancies
[a]395 pregnancies
[b]147 embrya (1 twin pregnancy)
[c]64 embrya (3 twin pregnancies)

Of 21 studies from the 1990s (23 ,24 ,25 ,26 ,27 ,28 ,29 ,30 ,31 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42 ,56), 10 are prospective (24 ,28 ,29 ,30 ,33 ,34 ,35 ,36 ,38 ,39), 2 are prospective and controlled (24 ,28), and the rest retrospective, with a total of 1,780 reported pregnancies. Three studies specifically addressed SLE nephritis and pregnancy and are discussed later (26 ,27 ,56). Spontaneous abortions ranged from a low of 5.4% (39) to a high of 30% (37), with a mean of 14.8% (Table 52-1). The reasons for the variations are not apparent, but, aside from lupus activity and APL antibodies, may include socioeconomic, educational, and cultural diversity parameters. Overall pregnancy loss is lower than previous decades, 19.1%, with a corresponding increase in live births to 80.4%.

Of the 18 studies from 2000 to 2005, reporting 1859 pregnancies, 11 address SLE in general (43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53), 6 address pregnancies with nephritis (57 ,58 ,59 ,60 ,61 ,62), and 1, SLE pregnancies after renal transplantation (63). In the first 11 series the trend of the 1990s continues: pregnancy loss is the lowest ever, 17.9%, with 14.6% SABs, 4.7% perinatal loss and further increase in live births (82.1%) (43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53). SAB rates varied widely and ranged from a low of 7.1% in the Johns Hopkins Lupus Cohort (53), to a high of 29.3% in a study from India (50). In the latter study, women with active SLE during pregnancy lost over half of their fetuses to SABs (55.6%) (Table 52-1). Women with nephritis had high losses as well and will be discussed later.

Overall, the last 25 years show a reduction and then stabilization of SABs below 15%, which is an improvement from the 19.5% rates of the 1960s and 1970s, and much closer to normal population values.

Perinatal Death (Table 52-1)

Perinatal death is the sum of stillbirth (intrauterine fetal death) and neonatal death, approximately 10/1000 in the general population. Intrauterine fetal death (IUFD) or stillbirth is the death of the fetus after the 20th week of gestation. The rate is approximately 5 per 1,000 in the general population. Neonatal death is defined as death occurring in the first 28 days after birth. The background rate is 5 per 1,000. In lupus, IUFD is the major component of perinatal loss. Perinatal loss was 10-fold that of the general population in the 1950s (10.3%), and has been decreasing over the last 55 years to 6.8% in the 1960s, 8.5% in the 1970s, 7.4% in the 1980s, 4.3% in the 1990s, and 4.7% in 2000 to 2005 (Table 52-1).

Of the 21 studies of the 1990s, reporting 1,780 pregnancies, perinatal loss was the lowest ever, 4.6%, excluding most nephritis patients (1,652 pregnancies, 23–25,28–42), and ranged from 0% (24 ,28) to 12% in women with lupus nephritis and APL antibodies (27). In the 11 studies from 2000 to 2005, perinatal loss was seen in 4.7% of 1466 pregnancies (43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53), essentially the same as in the 1990s. There were no stillbirths or neonatal deaths reported in a study from Israel (51), whereas the highest rate was encountered in severely active SLE, 16.7% and 15.8% (50 ,53) and in lupus nephritis, 31.6% and 38.5% (47 ,58).

Neonatal deaths are excessive in SLE pregnancies, with the highest prevalence of 20% in the 1965 study by Estes and Larson (2), and of 25% in the 1978 study by Thomas et al. (55). In the 1950s there were 5.8% neonatal deaths (9 of 155 uninterrupted pregnancies), in the 1960s, 0.7% (2 of 307), and in the 1970s, 0 of 505 pregnancies (1). Among the 16 series published in the 1980s, there were 15 neonatal deaths in 724 pregnancies (2.07%) (4 ,11 ,12 ,16 ,18). In the 21 reports from the 1990s, there were 18 neonatal deaths in 1,780 pregnancies (1.01%) (26 ,30 ,31 ,33 ,39). Some of these were due to extreme prematurity, or to congenital heart block (39 ,44). In 2000 to 2005, there were 21 neonatal deaths in 1859 pregnancies, 1.13%, over double the general population rate (44 ,45 ,50 ,52 ,58 ,61). Most neonatal deaths occur in very premature and growth-restricted neonates.

Pregnancy (fetal) loss is the sum of spontaneous abortions and perinatal deaths. Excluding elective (induced, therapeutic) abortions, the prevalence of pregnancy loss in lupus patients remained stable in the three decades between 1950 and 1980, at 27% to 27.9%. From 1980 to 1989, fetal loss prevalence was 22.3%, and six series showed, for the first time, a decrease to under 20% (8 ,9 ,10 ,11 ,14 ,19). This trend continued into the 1990s, with the mean pregnancy loss at 19.1%, with 12 of 20 series showing loss under 20% (23 ,24 ,25 ,26 ,28 ,29 ,30 ,34 ,35 ,39 ,41 ,42). In the last 5 years, pregnancy loss decreased even further to 17.9%, with most studies (8 of 12) under 20% (43 ,45 ,46 ,48 ,49 ,52 ,53 ,62) (Table 52-1). An analysis of pregnancy outcomes over 40 years (1960 to 2000) in 5-year increments has also shown that pregnancy loss has decreased from 43% in 1960 to 1965 to 17% in 2000 to 2003 (68). The decrease is attributed to better disease management and perinatal monitoring.

Etiology of Pregnancy Loss

The activity of maternal lupus, of SLE nephritis, and APL antibodies have been incriminated as etiologic factors for total perinatal loss in lupus.

Activity of Lupus

In early pregnancy studies, lupus activity was determined by experienced clinicians; more recently, it is assessed by validated lupus activity instruments (Systemic Lupus Erythematosus Disease Activity Index [SLEDAI], Systemic Lupus Activity Measure (SLAM), Lupus Activity Index [LAI], British Isles Lupus Assessment Group [BILAG], European Consensus Lupus Activity Measure [ECLAM]) (65 ,66). In recognition of differential diagnostic dilemmas between SLE activity versus preeclampsia, which have certain symptoms and findings in common (headache, hypertension, proteinuria, seizures, red blood cells in urine), and versus pregnancy-associated problems (fatigue, edema, alopecia), certain modifications have been developed in the above activity measures for use in pregnancy (67 ,68).

In certain studies, pregnancy loss seems independent of lupus activity, for example, in the prospective study by

Mintz et al. there was no significant difference between spontaneous abortions or stillbirths in active (13.7% and 6%) versus inactive lupus (19.6% and 3.9%) (18), and, in their study of 108 pregnancies, Lima et al. concluded that flares during pregnancy did not increase the risk of fetal loss (35). On the other hand, a prospective study from Johns Hopkins has assessed definitively the impact of lupus activity on all aspects of fetal outcome (53) (Table 52-1). Among 267 pregnancies there were 210 low-activity, and 57 high-activity pregnancies, as defined by a physician's estimate of lupus activity. With high activity in the first trimester, spontaneous abortions tripled, 29% versus 9% in low-activity pregnancies. Perinatal mortality in the high-activity pregnancies was also threefold that of the low-activity group, 15.8% versus 4.8%. Similarly, a study from India found inactive lupus pregnancies to have a much lesser rate of SABs, perinatal death, and pregnancy loss than active lupus pregnancies (8.7% vs. 55.6%, 8.7% vs. 16.7%, and 17.4% vs. 72.2%) (Table 52-1) (50).

Lupus Nephritis (Table 52-2)

Several studies associate severe nephritis flares with adverse pregnancy outcomes, including pregnancy loss. In the 1980s, 5 studies addressed SLE nephritis and pregnancy (4 ,5 ,12 ,15 ,20). Overall spontaneous abortions were 22% in these 227 pregnancies, versus 13.7% in the remaining 543 pregnancies (Tables 52-1 and 52-2). It should be noted that there were nephritis patients among the 543 pregnancies, without adequate data to distinguish their outcome (8 ,9 ,10 ,11 ,13 ,14 ,16 ,17 ,18 ,19 ,21 ,22). Perinatal loss was similar, at 7.3% and 7.4%, and pregnancy loss was higher with nephritis 29.5%, than without, 22.3%. Gimovsky et al. reported 40% spontaneous abortions and 12.5% IUFDs in women with lupus nephritis, with 28% and 2.5% respectively without (4). These patients were socioeconomically disadvantaged, with a great proportion of illegal aliens (as much as 60% to 70%). In the Hayslett study, of 55 pregnancies with active lupus or nephritis at conception, live births were at 56% with nephrotic syndrome, 64% with active SLE, and 88% with quiescent SLE (12). Fetal loss was seen in one of 10 pregnancies with serum creatinine less than 1.5 mg/dL, and in 50% with creatinine more than 1.5 mg/dL. Hence, in the 1980s, severe lupus nephritis tended to be associated with greater fetal loss and pregnancy was discouraged.

In the 1990s, three studies with 128 pregnancies addressed the role of active nephritis in the outcome of pregnancy: Overall, SABs were 10.1%, perinatal loss was 4.7% with a pregnancy loss of 14.7% (26 ,27 ,56). Of 47 pregnancies in 25 women with mostly stable lupus nephritis, overall fetal loss was 19%, but was double in hypertensive patients than normotensive patients (29% vs. 13%), and all SABs occurred with nephritis class III, IV and V (26). In the above study, there was no determination of APL antibodies. In the study of 57 pregnancies with lupus nephritis by Packham et al., fetal loss was slightly greater with class III and IV glomerulonephritis, than with membranous nephropathy. Nevertheless, the majority of fetal loss (3 of 5 SABs, all 5 stillbirths, and 2 of 5 neonatal deaths) occurred in mothers with the lupus anticoagulant (LAC) (27).

Five more studies from the 1990s merit attention: In a study of 121 pregnancies, active nephritis predicted fetal loss (13.4% live births with active nephritis, vs. 33% with inactive nephritis), and hypertension predicted prematurity and IUGR (23.8% preterm and IUGR babies with hypertensive mothers, vs. 6.6% with normotensive mothers) (38). In this study, fetal loss with and without APL antibodies did not differ. In a study from Spain, hypertension occurred in 5 of 10 pregnancies with nephritis versus 11.6% of 43 pregnancies without nephritis, and nephritis was significantly associated with lower gestational age at delivery, 35.9 versus 37.3 weeks (39). Three additional studies related poor fetal outcome to nephritis flares (34 ,37 ,40), and one study linked small-for-date neonates to hypocomplementemia, even without flare (41).

In 2000 to 2005, 6 studies specifically addressed SLE nephritis and pregnancy (57 ,58 ,59 ,60 ,61 ,62), 2 SLE pregnancy studies gave pertinent data on the nephritis patients included (47 ,49), and 1 study reported on lupus pregnancies after renal transplant (63). These 9 studies include 395 pregnancies, where the SAB rate is 21.6%, versus 14.6% in patients without nephritis (Table 52-1), perinatal loss is 7% (vs. 4.7%) with all pregnancy loss 27.7% versus 17.9%. Active nephritis or new-onset nephritis during pregnancy, proteinuria, hypertension, and hypocomplementemia had the highest pregnancy loss with 57.9%, 38.5%, and 52.6% (47 ,58 ,61) (Table 52-2). On the other hand, well controlled, quiescent nephritis at conception had a very low pregnancy loss, 6% (49). In a study of 101 pregnancies from Barcelona, pregnancy loss was similar in 38 pregnancies with class III and IV nephritis (18.4%), 11 pregnancies with class II and V nephritis (18.2%), and 52 pregnancies without nephritis (17.3%) (62). Hypertension, preeclampsia, and the need for cesarean section (43.7%) were significantly more frequent in pregnancies with class III and IV, and mean birthweight was lower by more than 500 grams, whereas cesarean section was needed in only 18.6% in the group without nephritis (62). Pregnancies with inactive lupus nephritis had the best outcome, with up to 94% live births (49).

In the 54 SLE pregnancies after kidney transplantation, pregnancy loss was 20.5%, hypertension was present in 45% of pregnancies, and cesarean sections were needed in 30% (63). Overall, the pregnancy loss of SLE patients was similar to kidney transplant patients without lupus.

Antiphospholipid Antibodies

Since Nilsson et al. in 1975, Firkin et al., and Soulier and Boffa (69 ,70 ,71) suggested a link between the lupus anticoagulant (LAC) and recurrent abortions, a tremendous body of knowledge has been amassed.

The spectrum of obstetric problems associated with APL includes the following (72):

- Recurrent pregnancy loss, especially second or third trimester loss

Table 52-2: Fetal Outcome in Lupus Nephritis, Exclusive of Elective Abortions

		Percent					
		Pregnancy (Fetal) Loss					
Study	Number of Pregnancies	Spontaneous Abortions	Perinatal Loss/IUFD+NND	Total	Live Births	Premature Births	IUGR/SGA
1980s	227	22.0	7.3	29.5	70.5	17.5	10.0
1990s	128	10.1	4.7	14.7	85.3	28.1	13.7
2000–2005	395	21.6	7.0	27.7	72.5	42.3	16.8
Studies in the 1980s							
Hayslett (12)	55	15	9	25	75	5	—
Jungers (5)	35	14	3	17	83	11	NS
Bobrie (20)	67	16	4	20	80	12	—
Imbasciati (15)	24	25	8	33	67	42	10
Gimovsky (4)	46	40	12.5	52.5	47.5	NS	NS
Studies in the 1990s							
Oviasu (26)	47	17	2	19	81	21	10
Packham (27)	57	9	12	21	79	33	—
Julkunen (56)	24	4.2	0	4.2	95.8	30.4	17.4
Studies in 2000–2005							
Cortes-Hernandez (47)	19	26.3	31.6	57.9	42.1	37.5	37.5
Huong (57)	32	21.9	6.3	28.1	71.9	73.9	21.7
Moroni (58)	64*	25	10.9	35.9	64.1	31.7	4.8
Known nephritis	51**	30.8	3.8	34.6	65.4	30.3	NS
Nephritis in preg	13	0	38.5	38.5	61.5	37.5	NS
Mok (49)	33	NS	NS	6	94	12.9	19.4
Tandon (59)	65	24.6	4.6	29.2	70.8	NS	NS
Soubassi (60)	24	16.7	8.3	25	75	77.8	NS
Rahman (61)	55	27.3	10.9	38.2	63.6	48.6	20
Inactive	36	25	5.6	30.6	69.4	40	18.5
Active	19	31.6	21.1	52.6	47.4	70	23.1
Carmona (62)							
WHO III and IV	38	15.8	2.6	18.4	81.6	34.6	—
WHO II and V	11	18.2	0	18.2	81.8	26.6	—
No nephritis	52	17.3	0	17.3	82.7	18.6	—
McGrory (63)	54	18.5	2	20.5	81.5	43.0	39,14y

* 65 embrya (1 twin pregnancy).
**52 embrya (1 twin pregnancy).
†Babies in III and IV were smaller than babies with no maternal nephritis x, mothers had SLE with kidney transplants y, birth weight <2,500 g and birth weight <1,500 g, respectively

- Fetal growth restriction, especially severe, early onset
- Oligohydramnios, otherwise unexplained
- Placental abruption
- Placental infarction
- High midpregnancy maternal serum alpha-fetoprotein (MSAFP) and human chorionic gonadotropin (hCG), otherwise unexplained
- Pregnancy-induced hypertension (PIH), especially severe and of early onset
- Pregnancy-related thrombosis, venous or arterial

Any of the above problems, especially if severe, of early onset, and otherwise unexplained, should prompt investigation for APL, even if the clinical picture does not point to lupus and the history does not include intravascular thrombosis.

Table 52-3 shows the fetal outcome in SLE and SLE-like illness in the presence or absence of antiphospholipid antibodies. In 15 retrospective studies of 1,249 pregnancies, there were 479 pregnancies in APL-positive women, and 770 pregnancies in APL-negative women (31 ,73 ,74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83 ,84 ,85). In the APL-positive pregnancies, there was a mean fetal loss of 37% (range, 14% to 68%). In the APL-negative pregnancies, there was a mean loss of 18% (range, 3% to 43%) and the difference was not statistically significant. Kutteh et al. have determined the prevalence of multiple abortions in lupus (2 or more) as 27% among anticardiolipin (ACL)-positive women and in only 3% of ACL-negative women (85). These authors showed that the percentage of fetal loss correlated with the level of ACL antibody: at 10 phospholipid units, fetal loss was 10%; at 10 to 20, 20%; at 21 to 80, 32%, and at 80 units, 60% (85). In their study of 70 lupus nephritis pregnancies, Moroni et al. reported 76% fetal loss in APL-positive, vs. 13% fetal loss in APL-negative pregnancies, an odds ratio of 17.8 (58).

Table 52-3: Fetal Outcome and Maternal Antiphospholipid Antibodies in Systemic Lupus Erythematosus (SLE) and SLE-Like Illness

Author	No. APL Positive	% Fetal Loss	No. APL Negative	% Fetal Loss
Retrospective Studies				
Boey (73)	26	35	27	19
Elias (74)	16	25	—	—
Colaco (75)	17	18	32	3
Harris (76)	80	16	38	3
Petri (77)	7	14	48	10
Fort (78)	9	22	13	31
Kalunian (79)	19	68	24	42
Gharavi (80)	22	32	—	—
Deleze (81)	53	57	145	25
Ishii (82)	29	41	14	7
Vianna (83)	13	54	80	18
Ramsey-Goldman (84)	81	51	174	43
Kutteh (85)	31	39	94	11
Julkunen (31)	56	30	41	12
Mean		37%		18%
Prospective Studies				
Lockshin (86)	11	46	12	0
Lockshin (87)	13	77	37	5
Hanly (88)	4	100	7	0
Englert (89)	19	53	15	14
Koskela (90)	53	4	91	5
Out (91)	20	35	93	19
Abu-Shakra (92)	181	19	336	20
Mean		48%		9%

APL, antiphospholipid antibodies.

In seven prospective studies of 892 lupus pregnancies, 301 occurred in APL-positive, and 591 in APL-negative women. In the APL-positive pregnancies, mean fetal loss was 48% (range, 4% to 100%), and in the APL-negative, 9% (range, 0% to 20%) (86 ,87 ,88 ,89 ,90 ,91 ,92). Despite the large range, the significance of positive APL as a cause of fetal loss seems irrefutable. APL positivity has been linked more to midpregnancy and late fetal loss (27 ,77 ,93 ,94), but is also emerging as an important factor in early loss (95).

Risk factors for fetal loss include the LAC (27 ,91), high-level IgG anticardiolipin, alone (76 ,81 ,85 ,86 ,89 ,93), or in combination with the LAC (96 ,97), and a history of prior fetal loss (76 ,84 ,86). In lupus patients with both LAC and ACL (about 16% of 349 lupus patients), fetal loss was the highest, at 89% (97). APL levels may fluctuate, especially when low positive (82 ,98 ,99 ,100 ,101).

Another antiphospholipid, antibody to $ß_2$-glycoprotein I ($ß_2$GPI), is often found in association with ACL antibody and uncommonly alone. Although initially it was thought that testing for anti-$ß_2$GPI did not identify additional patients at risk for recurrent abortion or fetal loss (102), several recent studies support the value of testing for anti-$ß_2$GPI, suggesting that as many as 10% of patients with features of APS may not be identified with LAC and ACL alone (103 ,104 ,105).

High APL level was predictive of fetal distress, manifested by abnormal fetal heart deceleration and preceding fetal death (86). Two subsequent studies further confirmed the association of high levels of IgG ACL, or lupus anticoagulant with fetal death: with IgG ACL of over 40 GPL international units, the proportion of fetal deaths was 46% to 65%, and with 0 to 39 GPL it was 17% to 19% (98); similarly, in women with high-positive APL (lupus anticoagulant, or more than 19 IgG binding units of ACL), fetal death was 27%, whereas with negative APL, IgM anticardiolipin only, or low-positive IgG ACL (<20 binding units), fetal death was 3% to 8% (99).

“Unexplained” high MSAFP, or hCG are considered markers of a “leaky” placenta in which the fetal-maternal barrier is damaged by intervillous, decidual, or villous thrombosis. Two studies have addressed the significance of elevated MSAFP levels: in 13 of 60 pregnancies with APL antibodies, elevated second-trimester MSAFP in the absence of fetal anomalies was associated with significantly increased fetal death and perinatal loss over pregnancies with normal maternal AFP (62% vs. 6%, and 77% vs. 15%, respectively) (106). MSAFP was prospectively determined in 54 lupus, and 1,001 control pregnancies at the 16th to 31st week of gestation (107): elevated MSAFP was significantly more frequent in lupus pregnancies than in controls (7.4% vs. 2.6%), and was associated with high ACL, preterm delivery, and higher prednisone dose. Patients with “unexplained” high MSAFP or hCG, whether associated with SLE or not, are followed with serial ultrasound examination for fetal growth and antepartum testing after 34 weeks of gestation.

The pathogenesis of APL-related fetal loss involves vascular insufficiency, intravascular clotting of the placenta, and fetal hypoxia, has been discussed in Chapter 51 , and will be briefly reviewed later.

Other potential causes of intravascular clotting include interference with the natural anticoagulant system of protein C and protein S, factor V Leiden mutation (G1691), alone or in combination with APL (108). Decrease in free protein S occurs in nephrotic syndrome (109). IgG anti-protein S antibodies were found in 31% of 184 SLE patients, more frequently with venous thrombosis, prematurity, preeclampsia, and IUGR (110). There was no interference, however, with plasma free protein S, hence the antibody significance is unclear at this time.

Antiphospholipid Antibodies and Fetal Loss without Lupus and with in vitro Fertilization-Embryo Transfer

Investigators have been searching for causes of fetal loss without lupus or APS, of infertility, and failure of in vitro fertilization. APL was found in 5.3% of 7,278 normal obstetrical patients, 20% of 2,226 women with recurrent fetal loss, 24% of 3,343 women undergoing in vitro fertilization, and 37% of 1,579 women with SLE (72).

Five large studies tested 6,631 normal pregnant women, mostly consecutive patients in obstetric services, for APL (111 ,112 ,113 ,114 ,115). Lupus anticoagulant was rather rare, 0.27% to 0.07% and overall prevalence of positive APL varied from 1.25% (of >5 SD IgG ACL) (113) to 24.4% (115). Fetal loss in these patients varied from 0% (112) to 50% in women with high-positive IgG ACL (113). APL-positive pregnancies had a 60% prevalence of preterm birth (111) and there was IUGR in 18% of neonates whose mothers had prolonged APTT (114). Routine testing for APL in the absence of recurrent fetal loss, SLE, or APS is not recommended (112).

The prevalence of APL was determined in 1,698 women with pregnancy loss and had a wide range. In women with 3 or more fetal losses, APL ranged from 5.5% to 31% (77 ,91 ,116 ,117 ,118 ,119 ,120 ,121); with 2 or more, it was 10.7% and 15% (118 ,122); with <2 losses, 4.8% (116), and with one loss, APL were present in 0% to 11% (77 ,123 ,124). Eroglu and Scopelitis reported strictly on first trimester losses (120). Control subjects had 0% to 10% APL and no fetal loss (117 ,119 ,120).

Of 41 infertile women 17% had APL in addition to other autoantibodies, versus 6% of 351 normal pregnant women (125).

Several studies have addressed APL in women with implantation failure in the process of in vitro fertilization-embryo transfer (IVF-ET). Overall prevalence of APL in women failing IVF is 18% to 34% and some investigators have instituted treatment with heparin and aspirin, or intravenous immunoglobulin (126 ,127 ,128 ,129 ,130). A controlled, nonrandomized study showed the prevalence of APL in 191 women undergoing IVF as 18.8%, 5.5% in 200 normal controls, 26% in 200 women with recurrent pregnancy loss, and 32% in 200 women with SLE (128). There was no difference in pregnancy rates between the heparin and ASA-treated IVF group versus the group on standard treatment for IVF (25% vs. 19.4%). A randomized controlled trial also showed that heparin and aspirin did not improve pregnancy or implantation rates for patients undergoing IVF (129).

The Practice Committee of the American Society for Reproductive Medicine has addressed whether the presence of APL affects the outcome of IVF-ET, by examining 16 studies, 7 of which have data on IVF patients with or without APL with no treatment for APL. The clinical pregnancy and live birth rates were comparable, 57% and 46% in APL-positive, and 49.2% and 42.9% in APL-negative women. Therefore, the American Society for Reproductive Medicine has concluded that APL do not affect IVF success, testing for APL is not indicated, and treatment is not justified in this situation (131).

Treatment for Prevention of Antiphospholipid-Related Fetal Loss

Since 1983, several publications have addressed the prevention of fetal loss associated with APL antibodies. In 34 studies reporting 10 or more pregnancies, there has been improved fetal outcome (91 ,132 ,133 ,134 ,135 ,136 ,137 ,138 ,139 ,140 ,141 ,142 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,150 ,151 ,152 ,153 ,154 ,155 ,156 ,157 ,158 ,159 ,160 ,161 ,162 ,163 ,164 ,165). Fetal loss in the over 1,310 untreated pregnancies was 76.2% to 100%, with only 7.9% mean live births (range, 0% to 23.8%). All treatments have resulted in some increase of live births, to a mean of 70.8% (range, 28.6% to 100%). A total of over 1,403 pregnancies were treated: the initial therapy with low-dose aspirin (75 to 100 mg/day) and 40 to 60 mg of prednisone per day for 1 to 2 months was effective but is no longer used because of serious maternal side effects, including diabetes mellitus and hypertension. The most effective treatment regimen is heparin, low molecular weight (LMWH) or unfractionated (UFH), plus low-dose aspirin, LDA (139 ,141 ,142 ,143 ,144 ,153 ,154 ,155 ,156 ,157 ,163 ,164). For details, please see Table 51.5 in the preceding chapter.

Comparison of low-dose (up to 12,000 IU/day) versus high-dose UFH (up to 30,000 IU per day) showed no advantage of the latter (76% vs. 80% live births) (154). LMWH is safe in pregnancy, can be given once a day and showed a decided advantage over historical placebo controls (83.9 vs. 55.9 live births (156)). A recent prospective, controlled study of 50 women with APS suggests that the LMWH enoxaparin is equivalent in safety and efficacy to unfractionated heparin (160). Low-dose enoxaparin (40 mg/day) was equally effective as high-dose (40 mg BID) in 51 women with APL and recurrent pregnancy loss, without heparin-induced thrombocytopenia or bleeding (164). Heparin and LMWH are category B drugs, do not cross the placenta, and are not harmful to the fetus, LWMH causes less heparin-induced thrombocytopenia (HIT) and osteoporosis than UFH. Low-dose aspirin therapy alone is not nearly as effective as the aspirin-heparin combination (42% to 44% live births, versus 71% to 80%, respectively) (153 ,154 ,155).

The efficacy of intravenous immunoglobulin (IVIG) has been reported in three prospective, controlled studies (158 ,161 ,162). Heparin plus LDA and IVIG was compared to heparin plus LDA and albumin in 14 patients: live births were 100% with both regimens (158). In another study, 40 women with APS were randomized to heparin and LDA versus IVIG as primary treatment. The live birth rate was higher (84%) in the heparin group than in the IVIG group (57%) (161). In the third study, of 82 recurrent aborters, 29 were treated with prednisone and LDA and 53 with IVIG: there was no difference in live births, at 78% and 76%, but birth weight was higher in the IVIG group (162).

A possible role for IVIG may still be found in failures of the ASA and heparin regimen. Of interest is a three-phase case-control study of 687 APL-positive women undergoing IVF-ET (130), concerning IVIG in addition to heparin and aspirin. When the women underwent IVF under heparin and ASA treatment, there were 46% live births in treated IVF cycles, versus 17% in untreated cycles. Of 322 women with a single APL subtype treated with ASA and heparin, there were 17% live births with IVF in those with antiphosphatidylethanolamine (anti-PE) or antiphosphatidylserine (anti-PS), versus 43% in women with other APL specificities. Of the women with anti-PE and anti-PS, 121 who did not achieve live births despite ASA and heparin, were given these plus IVIg: this treatment resulted in a 41% birth rate (130). This study suggests that, in specific situations, addition of IVIg to ASA and heparin may provide further pregnancy success. The conclusion is supported by a recent review (165).

A pilot study of 22 pregnancies treated with fish oil (eicosapentaenoic acid and docosahexanoic acid, 5.1 gram/day) resulted in 95.5% live births (149), but a subsequent prospective controlled study of 30 APS pregnancies showed that fish oil was as effective as aspirin (live births 73.3% vs. 80% respectively) (159).

A study on early fetal loss associated with APL compared aspirin 75 mg per day versus placebo and found no difference, with 80% and 85% live births, respectively (166).

There are two sets of treatment recommendations for prevention of fetal loss in APL-positive women, developed by the 7th conference of the American College of Chest Physicians (ACCP) on Antithrombotic and Thrombolytic Therapy (167), and the 10th International Congress on Antiphospholipid Antibodies (APL Congress) (168). Most recommendations are similar and all details can be seen in Table 51.6 of the preceding chapter.

Essentially, as soon as pregnancy is confirmed, women with prior APL-related pregnancy complications, but no thrombosis, should start subcutaneous (SC) enoxaparin 0.5 mg/kg (or other LMWH) daily, calcium supplementation at 1.5 g daily, and axial weight-bearing exercise, or unfractionated heparin, 5,000 to 10,000 U subcutaneously twice daily, calcium, and exercise.

For women who have clotted previously, SC enoxaparin 1 mg/kg every 12 hours, or adjusted dose of unfractionated heparin subcutaneously; all regimens are accompanied by daily low-dose aspirin.

For more details see Pregnancy Management for Optimal Fetal Survival , below, and the corresponding section in Chapter 51 .

In summary, there is a substantial prevalence of spontaneous abortion, stillbirth (IUFD), and overall pregnancy loss in lupus patients, which is linked mainly to active maternal lupus, active severe nephritis, and APL antibodies. There is a diminution of fetal loss during the 1990s, and 2000s, accompanied by significant increase in live births in these high-risk pregnancies, through better SLE management and treatment with antiplatelet and anticoagulant agents.

Anti-Ro/SSA Antibodies

It is well known that anti-Ro/SSA antibodies are responsible for complete congenital heart block in children of positive mothers (169). Additionally, four studies have implicated anti-Ro positivity in fetal loss (170 ,171 ,172 ,173), and two have found no such a relationship (46 ,174). In a retrospective study of 50 anti-Ro positive women, 20 with SLE, 34 had

84 pregnancies with 28% fetal loss, which was far more pronounced in African-American women, 71% (171). No information was given about the APL status of these patients. In a retrospective cohort study, recurrent pregnancy loss was associated with anti-Ro in women with Sjögren syndrome or rheumatoid arthritis (23.7%), but not with SLE (172). The same investigators found that antibodies to Ro52, Ro60, p57, and thyroglobulin, were independent predictors of recurrent pregnancy loss in women with autoimmune diseases (173). Two more studies failed to find a relationship between anti-Ro positivity and pregnancy loss in 47 SLE women (174) and in 100 women with a variety of autoimmune diseases, including 53 with lupus (46). The last study was prospective. Apart from congenital heart block, the effect of anti-Ro on fetal death is unclear.

Other Observations

In the study by Nossent and Swaak, the male-to-female ratio of offspring of lupus patients (male babies per 100 female babies) was 72.7 before, and 78.9 after onset of SLE, lower than the normal of 105 for the Dutch population (23). This implies greater fetal loss of male offspring. In an attempt to explain the high female-to-male ratio in SLE, Oleinick examined the family histories of 198 lupus patients and their 581 siblings. He found that the ratio of male to total siblings born within 4 years of the birth of lupus patients was lower and suggested that excessive male fetal loss may explain, in part, the female preponderance in SLE (175). In a later investigation, the author found that there was no excess mortality risk in early life for male siblings or offspring of lupus patients (176).

Elective (Induced, Therapeutic) Abortions

Elective (induced or therapeutic) abortions in lupus pregnancies are reported as high as 34% from Hong Kong (24), 25% from Toronto (9), 21% from Vancouver (17), and as low as 0% from Mexico City (18). In the U.S. series, the prevalence ranges from 10% to 24% (14 ,16). These worldwide variations are probably a result of different local legislation regarding elective abortion, patient attitudes and socioeconomic setting, and physician attitudes, sophistication, and level of comfort with lupus pregnancy.

Elective abortion may be followed by severe SLE flare, fails to induce remission, and, in earlier studies, was at times followed by death of the mother (13 ,177 ,178). More recently, the experience has been that patients tolerate the procedure, and disease activity improves with corticosteroid or other treatment. Pistiner et al. reported 106 elective abortions in 634 pregnancies (16.7%) among 227 women with lupus, without ill effects (25). Vigorous steroid treatment, rather than induced abortion, is medically indicated for suppression of lupus flares during pregnancy. If an elective abortion is indicated for psychologic or social reasons, a careful evaluation of SLE activity should be undertaken, and steroid dosage adjusted accordingly before the procedure.

Prematurity (Preterm Birth) (Table 52-1)

Prematurity or preterm birth is defined as birth before the 37th week of gestation and prematurity rate is the percentage of premature infants among all neonates (live births). Preterm birth in lupus women was virtually fivefold that of control women in a study of 555 SLE deliveries, 21% in SLE, versus 4.2% in controls (45). Before 1980, the prematurity rate was 15.3%, but preterm births were not always reported (Table 52-1). The lowest rate was 6% (179), and the highest, 30% (180).

Ten of the 13 series in the 1980s, with 362 pregnancies (excluding nephritis, see below) contained adequate data on prematurity (4 ,8 ,9 ,10 ,11 ,13 ,14 ,16 ,18 ,19): 21.9% of 362 pregnancies resulted in preterm birth, which ranged from a low of 4% (11) to a high of 63% (18).

In the 1990s, 14 of 20 series contained information on prematurity (23 ,24 ,28 ,29 ,32 ,34 ,35 ,36 ,37 ,38 ,39 ,40 ,41 ,42): preterm birth ranged from a low of 16.7% (41) to a high of 64% (42), with a mean of 36.4%.

In the twelve series of 2000 to 2005, excluding the eight studies on nephritis, prematurity data are given on all 1,464 pregnancies (the twelfth study concerns 52 pregnancies without SLE nephritis used as controls in (62)): preterm births have a wide range, from 4.2% (50) to 63.6% in patients with high SLE activity (53), with the mean at 23.2% (Table 52-1).

Given the large numbers of pregnancies reported in the 1990s and the first half of the 2000s, the reduction of preterm deliveries from 36.4% to 23.2% is very credible and a most welcome improvement in the outcome of lupus pregnancies.

Etiology of Preterm Births-Maternal Lupus Activity, Nephritis, Hypertension, Corticosteroids, Preterm Premature Rupture of Membranes, and Antiphospholipid Antibodies

The etiology of premature birth is multifactorial, and many of the invoked factors coexist. Several authors have associated preterm births with active maternal lupus, lupus nephritis, APL antibodies, preeclampsia, steroid therapy, and preterm premature rupture of membranes.

Lupus activity during conception and/or during pregnancy was held responsible for premature births in several studies (2 ,5 ,10 ,20 ,28 ,29 ,34 ,43 ,47 ,49 ,52 ,53 ,181 ,182). A recent study of 267 SLE pregnancies over 16 years from Johns Hopkins showed definitively the impact of lupus activity in pregnancy on fetal outcome (53): 28 of 44 live births (63.6%) were premature in high-activity pregnancies, compared to 55 of 185 (29.7%) preterm births in low-activity patients. Similar findings were reported earlier by the same group (28), by Georgiou et al. (43), Mok et al. (49), and Chakravarty et al. (52). If hypocomplementemia and steroid therapy are considered surrogates for increased SLE activity, then more studies ascribe premature births to

lupus activity (41 ,51 ,182). Certain authors have considered prematurity unrelated to SLE activity (12 ,18 ,23 ,24 ,31).

Maternal hypertension was cited as a factor for prematurity in several studies (18 ,28 ,32 ,34 ,38 ,47 ,51 ,60). In a study of 121 pregnancies, hypertension predicted prematurity and IUGR (23.8% preterm and IUGR babies with hypertensive mothers, vs. 6.6% with normotensive mothers) (38). Proteinuria was associated with prematurity in three studies (49 ,58 ,60).

Nephritis, especially when active or newly diagnosed during pregnancy, has been associated with preterm births (Table 52-2). Of the ten preterm births in Oviasu's series, nine occurred in patients with the more severe forms of nephritis (26). In a study from Spain, hypertension occurred in 5 of 10 pregnancies with nephritis versus 11.6% of 43 pregnancies without nephritis, and nephritis was significantly associated with lower gestational age at delivery, 35.9 vs. 37.3 weeks (39). During the last 5 years, premature births in 395 pregnancies with nephritis were 42.3% versus 23.2% in the overall lupus series, with a range from 12.9% (49) to 77.8% (60) (Tables 52-1 and 52-2). Pregnancies with active nephritis had almost twice the preterm deliveries than pregnancies with inactive nephritis, 70% versus 40% (61). Patients with proliferative nephritis, WHO classes III and IV, had almost twice the premature birth rate of women without SLE nephritis, 34.6% versus 18.6%, although the difference was not statistically significant (62). Furthermore, the relationship between prematurity and SLE nephritis activity appears to be mediated through superimposed preeclampsia, which necessitates prompt delivery for maternal well-being.

In 66 lupus pregnancies reported by Johnson et al., preterm premature rupture of membranes (PPROM) occurred in 39% of premature deliveries, and PROM in 30.3% of term deliveries, and was the major cause of prematurity (181). PPROM was responsible for 16 of 29 preterm births (55.2%) in a recent study (52). It should be clear that PPROM precedes spontaneous preterm birth, as distinguished from induced preterm birth, by medical means or by cesarean section.

The role of steroid therapy alone in the cause of prematurity has been clarified by Laskin et al. (156): of 202 women with unexplained recurrent fetal loss, positive autoantibodies and no lupus, 101 were randomized to received ASA 100 mg/day and steroid, 0.5 to 0.8/mg/kg/day, and the remaining 101 patients, placebo. In the steroid treatment group, preterm delivery was significantly greater, 62%, compared to 12% in the placebo group. Similar findings were reported by Silver et al. (183).

Several reports have found increased preterm births in pregnancies of APL-positive women, including studies from the last 5 years (36 ,47 ,58 ,60 ,111 ,146 ,182 ,184 ,201). Clark et al. found ACL IgG in 55.5% of preterm deliveries versus 19.4% in term deliveries, a significant difference (182). Cortes-Hernandez et al. noted the association of prematurity with any APL antibody, including ACL, LAC, and anti-β_2GPI, in addition to maternal hypertension and steroid treatment (47). In two studies, there was no correlation of preterm birth with maternal APL (27 ,31).

In summary, although there is no complete unanimity in the reported series, premature births are commonplace in lupus and are associated with maternal disease activity, hypertension, nephritis, preeclampsia, preterm premature rupture of membranes, steroid therapy, and antiphospholipid antibodies.

Intrauterine Growth Restriction

Both birth weight and fetal weight are normally a function of the gestational age of the newborn or fetus, and growth curves have been developed to show this relationship (185). When weight is below the norm for gestational age, the condition has been alternatively called IUGR, fetal growth restriction (FGR), intrauterine malnutrition, or small newborn for gestational age (SGA). By convention, IUGR means that weight is below the tenth percentile for gestational age. Very often, premature babies have low birth weight or IUGR.

Not all reports of SLE pregnancy have this information. From 1980 to 1989, four studies offered IUGR information (4 ,13 ,14 ,18): overall IUGR was present in 31% of pregnancies, with a range of 23% to 35%. In the 1990s, 7 of 20 studies found overall 18.2% IUGR in 446 pregnancies (range, 7.1% to 31%) (24 ,29 ,35 ,38 ,39 ,40 ,41). Of the twelve studies from 2000 to 2005, IUGR data are given in 11 (43 ,44 ,45 ,46 ,47 ,48 ,49 ,50 ,51 ,52 ,53): IUGR ranged from 4.1% (43) to 50% (51), with a mean of 18.1%, almost identical to that of the 1990s.

IUGR is often associated with preterm birth: 66% of premature neonates had IUGR, compared to 28% of term neonates (4). In the study by Mintz et al., 30% of preterm newborns had IUGR, compared to only 14% of term neonates (18). This difference is statistically significant, and was associated with better survival of the term neonates. In the study by Le-Thi-Huong, IUGR correlated with pregnancy of short duration, hypertension, and low serum C3 or C4 (34).

Several publications point out the association of IUGR and low birth weight with maternal APL antibodies (51 ,72 ,138 ,139 ,140 ,146 ,186). Caruso et al. found that birth weight was significantly lower when three tests were positive for APL (LAC, ACL, and Venereal Disease Research Laboratories [VDRL]) (146). In a study of 22 patients with IUGR and 43 healthy pregnant controls, ACL was significantly higher in the former, especially with severe IUGR (186). Out et al. found significantly lower birth weight in the APL-positive patients, but no difference in prevalence of IUGR between APL-positive and APL-negative women (24% vs. 21%) (91).

Treatment of APL-related recurrent pregnancy loss with antithrombotic therapy is associated with increased birth weight and reversal of IUGR: when Semprini et al. treated 14 recurrent aborters with heparin and prednisone, achieving a 64% rate of live births compared to 3.7% before treatment, the prevalence of low birth weight was reduced from 44% to 12% (138). Wallenburg and Rotmans treated 24 women with prior IUGR with low-dose aspirin and dipyridamole after 16 weeks' gestation, with reduction of fetal growth restriction to 13% in treated versus 61% in untreated pregnancies (187).

Other associations with IUGR include SLE activity 6 months before and at conception (49), nephritis (48), hypertension, low C4 and maternal age older than 35 years (47), hypertension, pregnancy-induced hypertension (PIH) (61), and proliferative lupus nephritis, classes III and IV (62).

In summary, APL antibodies have emerged as an important factor in poor fetal outcome, including pregnancy loss, preterm birth, and growth restriction.

Live Births

The overall proportion of live births in systemic lupus pregnancies has varied over the last 55 years, is increasing, and can be seen on Table 52-1 . In the 1950s mean live births were 72.5%, in the 1960s, 73%, in the 1970s, 72.1%, in the 1980s, 75.1%, in the 1990s, 80.4%, and, in 2000 to 2005, 82.1%. Clark et al. have analyzed meticulously live birth and preterm birth data in lupus pregnancies from 1960 to 2003, grouped in 5-year periods and weighted according to sample size (68). The authors showed that pregnancy loss has diminished very significantly, from 43% in 1960 to 1965 to 17% in 2000 to 2003 (see Table 52-1). Because of inadequate data on induced vs. spontaneous preterm birth, the authors could not group studies, but showed a trend for decrease in preterm births from 37.3% in 1980 to 32% in 2003 (68).

Live births vary widely, even in the last 5 years, depending on lupus activity, origin of the study, socioeconomic setting of patients, and other factors.

The rate of cesarean sections in lupus births varies from 18% to 44% and to 89% (26 ,29 ,45 ,62 ,188). In the study of 555 lupus pregnancies, cesarean sections were needed in 38.2% of these patients versus 19.7% of controls (45). The major indications for cesarean section are fetal distress and maternal preeclampsia.

Pathogenesis of Adverse Fetal Outcome

The pathogenetic mechanism of APL-associated fetal outcome is multifaceted, involves expression of β_2GPI in the placenta, attachment of APL, involvement of the complement cascade, interference with annexin V, inhibition of trophoblast differentiation and invasiveness, and is discussed in the previous chapter (Chapter 51). We will discuss here certain data about placental immunopathology in SLE and APS.

Placental Studies

There are at least 17 reports concerning placentae of patients with lupus or APS (88 ,190 ,191 ,192 ,193 ,194 ,195 ,196 ,197 ,198 ,199 ,200 ,201 ,202 ,203 ,204 ,205). The largest studies were reported by Out et al. (195), Magid et al. (200), Ogishima et al. (201), and Van Horn et al, (205) comprising 47, 40, 47, and 81 placentae, respectively. The majority of these studies concern patients with SLE and APL or just with APL syndrome (APS).

Common findings in most studies include low placental weight, infarction, thrombosis, ischemic-hypoxic change, hemorrhage, decidual vasculopathy with fibrinoid change and atherosis, and chronic villitis. Magid et al. distinguished the following clinical outcomes in SLE without APL: abortions, prematurity, and fetal growth restriction (IUGR), and in SLE with APL, there was fetal death in addition to the above (200). Placental changes were qualitatively similar: decreased weight, infarction, ischemic-hypoxic change, decidual vasculopathy, decidual and fetal thrombi, and chronic villitis of implied unknown etiology. Extensive infarction (>20%) of placenta and diffuse, moderate or marked ischemic-hypoxic change were significantly more frequent with APL-positive placentae. Placental changes and adverse fetal events were not deemed to correlate with maternal SLE activity. Similar changes were described in the studies by Out et al. (195), Van Horn et al. (205), and Nayar et al. (203). The last study reported acute atherosis in a placenta from a first trimester abortion. Ogishima et al. noted that the most severe placental infarctions, decidual vasculopathy, thrombosis, and fetal death were associated with double APL positivity for ACL and LAC (201). In a controlled study, placental histopathology was similar in 44 antiphospholipid and 37 antiphospholipid-like syndrome specimens (negative APL tests, similar clinical pregnancy problems) (205).

IgG is normally found in trophoblast basement membranes (189). Granular IgG, C3, and fibrinogen deposits on placental vessels and stroma were found in several studies of SLE placentae (190 ,191 ,194 ,197 ,202). In one patient with lupus nephritis and high DNA binding, anti-DNA was eluted from the placenta (190), and another placental eluate had antinuclear antibody (ANA) activity. Large IgG, massive vascular IgM, and C3 deposits were found in decidual vasculopathy areas (191). Guzman et al. described ANA-like nuclear staining by IgG in five placentae, C3 in two, and IgG deposits along the amniotic membranes in a lupus band-like pattern (194). Erlendsson's two patients with SLE, LAC, ACL, and false-positive VDRL had pregnancies with severe IUGR and fetal death with very small, infarcted placentae with immunoglobulin deposits and necrotizing arteritis; after treatment with low-dose ASA and 40 mg prednisone/day, they had successful pregnancies with minimal placental changes on biopsy (196). In La Rosa et al.'s study, β_2GPI was found increased on the trophoblast surfaces of patients with persistently high APL titers, along with IgG deposition (197). This is consistent with recent findings about the mechanism of APL-related fetal loss, that trophoblast cell membrane anionic phospholipids bind β_2GPI, which in turn binds anti-β_2GPI antibodies (206).

Two studies reported elution of APL from placentae of women with recurrent fetal loss (198 ,199). Chamley et al. eluted IgG ACL, ANA, and β_2GPI from placentae of four women with high serum ACL (198); β_2GPI was localized in syncytiotrophoblast. Katano et al. eluted five types of APL from seven placentae of patients with IgG APL (three also had IgM APL) and a history of at least two fetal losses (199). One patient had SLE, and all were treated with prednisolone and aspirin. IgG APL were eluted from four of seven placentae, most commonly antiphosphatidyl inositol and antiphosphatidyl serine. Cord blood was negative for

APL. Fetal outcome was one IUFD, four IUGR, and two neonates of normal weight. Infarcts and fibrinoid deposits were present in six of seven placentae, degeneration necrosis in four, and thrombosis in three. The number of pathologic findings seemed to correlate inversely with placental and fetal birth weight (199).

In placentae from SLE and APL syndrome, the distribution of cell-adhesion molecules was the same as in controls, and included intercellular adhesion molecule-1 (ICAM-1) in placental vascular endothelium and the placental villous stroma, platelet endothelial cell adhesion molecule (PECAM) in the placental vascular endothelium, whereas P-selectin was mildly expressed in the stem vessel endothelium only (204).

The deposition of immunoglobulins, complement, anti-DNA, and ANA in the placenta, and in areas of vasculopathy/vasculitis, and chronic villitis suggest that bland clotting is not the only mechanism of placental hypoperfusion and ischemia, and, hence, fetal ischemia, and that an immune-mediated inflammatory mechanism is be at play as well.

Pregnancy Management for Optimal Fetal Survival

The value of multidisciplinary care, careful maternal and fetal monitoring, and judicious use of surgical delivery were amply demonstrated in the studies from Mexico City, where fetal wastage was reduced by almost half, from 40.5% in the retrospective, to 22.5% in the prospective study (3 ,18). Modern perinatology (high-risk obstetrics) and neonatology offer tremendous advantages in fetal surveillance. Fetal ultrasound, Doppler studies of blood flow of the uterine and umbilical arteries (146 ,188 ,207 ,208 ,209 ,210 ,211), and fetal echocardiography (213 ,214), have added to conventional antepartum testing for fetal well-being—the modified biophysical profile (212 ,215). Early in the pregnancy, fetal ultrasound is used for accurate gestational dating, and for assessment of the growth of the fetus. Throughout pregnancy, ultrasound is used to assess fetal growth. Evaluation and monitoring of the uterine and umbilical arterial blood flow by Doppler velocimetry or waveforms has an important place in the early (146), and the third trimester evaluation of lupus pregnancies (211).

The diagnostic accuracy of the Doppler is limited to bilateral uterine artery notches at 22 to 24 weeks, especially in the subgroup of women with positive lupus anticoagulant. Bilateral notching of the uterine artery Doppler at 22 to 24 weeks is a useful screening test in predicting preeclampsia and SGA infants: bilateral notching was associated with a 12- to 14-fold increased likelihood of preeclampsia or IUGR, whereas normal flow was associated with an 80% decreased likelihood of the same outcome (211).

The biophysical profile consists of real-time ultrasonography, during which the fetal tone, movements, breathing movements, and amniotic fluid volume are scored; a nonstress test follows and a score of zero to 10 is assigned, 10 being optimal (212). At our institution, the modified biophysical profile is performed (216), which includes the nonstress test and amniotic fluid index, with the full biophysical profile as a back-up (215).

Fetal echocardiography is important for the follow up of bradycardia or heart block, which is almost always detected on simple auscultation; the mainstay of antepartum fetal heart testing is the nonstress test. During normal intrauterine fetal movement, the fetal heart accelerates. Failure to accelerate constitutes a nonreactive, or abnormal nonstress test. The earlier the gestational age, the greater the percentage of nonreactive nonstress tests: nonstress test is most meaningful after the 28th week of gestation. Fetal heart rate decelerations during the nonstress test are associated with fetal distress during labor (213), and nonperiodic fetal heart decelerations at 20 to 28 weeks may identify the fetus at risk for intrauterine death (217). The contraction stress test is cumbersome and has been abandoned.

Recommendations

The following are recommendations for management of lupus pregnancy to optimize fetal survival and diminish loss.

Mother

- As recommended in Chapter 51 , maternal disease should be assessed carefully for activity and severity at the outset of pregnancy, which ideally should be planned after 6 to 12 months of remission. The mother should be watched carefully for SLE flares.
- Maternal flares should be diagnosed early and treated aggressively, depending upon severity, with appropriate steroid dose and route (i.e., intravenous large doses for severe flares. Unless there is a dire emergency, i.e., acute anuric renal failure, cytotoxic drugs should be avoided during the first trimester. Mothers with nephritis at risk for hypertension and preeclampsia should be treated with low-dose aspirin (60 to 81 mg/day) until the 36th week of gestation, or until 1 week prior to delivery. The risk of abnormal bleeding is theoretical, but discontinuation may remove impediments to regional anesthesia.
- The mother's status in terms of APL, and anti-Ro/SSA, anti-La/SSB positivity should be known; the former, because of predisposition to fetal loss, preeclampsia and IUGR, and the latter two because of the small risk for congenital heart block in the child.
- Joint follow-up by the rheumatologist and obstetrician-perinatologist should be done every month for the first half of pregnancy, and more frequently thereafter (every 1 to 3 weeks), with repeat of CBC, urine, chemistry, quantitation of proteinuria in patients with nephritis, complement C3 and C4 levels, anti-dsDNA, and a measure of circulating immune complexes every 1 to 3 months depending on disease activity. Blood pressure should be monitored very carefully.
- In the event of high-positive APL antibody, especially of the IgG class, or with a “double positive” for APL (both

ACL and LAC positive, or/plus anti-β2GPI-positive), the option of an antithrombotic agent should be discussed with the patient, even in the absence of a prior thrombus or pregnancy complication. See also Chapter 51 and Table 51.6 for antithrombotic therapy recommendations (167 ,168).

Unless there is concomitant lupus activity, high-dose prednisone for APS is not recommended.

- Steroid stress doses should be given at the time of delivery, be it vaginal or by cesarean, if steroids have been employed during pregnancy.

Fetus

- Accurate gestational dating is important because of the frequent IUGR and premature births in SLE. Menstrual dating should be confirmed by ultrasonography at the first prenatal visit.
- Ultrasound evaluation of the growth of the fetus should be performed monthly after 20 weeks' gestation. At the baseline growth scan, uterine artery Doppler is used to identify the fetus at high risk for IUGR and superimposed preeclampsia. Delayed growth indicates an increased risk of fetal death in utero and should prompt a search for evidence of increased disease activity, or developing superimposed PIH. Doppler of the umbilical artery and middle cerebral artery is employed liberally at the time of the ultrasound for fetal growth to detect placental dysfunction and central redistribution of fetal blood flow associated with growth restriction.
- In the event of positive anti-Ro/SSA and/or anti-La/SSB, fetal heart tones should be checked for 1 minute at every visit after the 16th week of gestation. Any abnormal heart rhythm should prompt a detailed fetal echocardiography to rule out developing heart block.
- Antepartum testing for fetal well-being should be initiated between 24 and 34 weeks' gestation. In patients at highest risk, such as those with a history of fetal compromise, active SLE, or positive for both ACL and LAC, testing should be initiated near the cusp of viability, at 24 weeks. In women with quiescent lupus who are APL and anti-Ro/SSA negative, testing may start at about 34 weeks with the modified biophysical profile (216). Umbilical artery Doppler is used whenever fetal growth restriction is suspected or low amniotic fluid volume is encountered. Decreased amniotic fluid may presage IUGR or cord accidents leading to stillbirth.
- An abnormal fetal heart tracing or an amniotic fluid index less than 5 cm is almost always an indication for prompt delivery, usually by cesarean. When delivery is planned before 34 weeks' gestation, long-acting steroids (dexamethasone or betamethasone) should be administered to enhance fetal maturity, if the delivery can be safely delayed (217).

Other drugs should be used with great care. Maternal thiazide diuretic therapy is generally safe in late pregnancy and has rarely been associated with severe neonatal thrombocytopenia (218). Angiotensin-converting enzyme (ACE) inhibitors should not be used in pregnancy because fetal and neonatal hypotension, anuria, and even renal failure may occur (218). For more information on antirheumatic drugs in pregnancy and lactation, see the section on use of medications in Chapter 51 . As mentioned in that chapter, prednisone, prednisolone, and methylprednisolone are oxidized by placental enzymes, whereas dexamethasone and betamethasone are not; the latter are utilized to treat the fetus in utero.

Under dire circumstances, unconventional therapeutic interventions may be appropriate during pregnancy such as intravenous cyclophosphamide for maternal anuric renal failure, or intravenous gamma globulin for severe maternal SLE.

It is reassuring to note that in over 5,330 lupus pregnancies reported in the last 55 years (Table 52-1), there were very few cases of congenital malformations, with the exception of congenital heart block (see Chapter 53). These figures include infants whose mothers were on cytotoxic medications, mainly azathioprine and cyclophosphamide. Details are discussed in Chapter 51 .

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Chapter 53
Neonatal Lupus

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Introduction

Although physicians counseling prospective mothers with SLE appropriately focus attention on the clinical status of the mother, accumulating evidence supports that injury to the developing fetus and neonate can occur independently of maternal health. Following the seminal observation in the late 1970s that sera from nearly all mothers of children with isolated congenital heart block (CHB) contain specific autoantibodies (1 ,2), this disease, previously of interest only to the disciples of cardiology, became an important model of passively acquired autoimmunity. Over the next three decades the association solidified. Identification of heart block (first-, second-, or third-degree) before or at birth, in the absence of structural abnormalities, predicts in more than 85% of cases that the mother will have autoantibodies to SSA/Ro and/or SSB/La ribonucleoproteins, regardless of whether she has SLE, Sjögren syndrome (SS), or is totally asymptomatic (3 ,4). Cardiac injury occurs in a previously normal fetus and is presumed to arise from the transplacental passage of maternal IgG autoantibodies that result from an autoimmune process in the mother (5). Autoimmune-associated CHB does not occur randomly during fetal development but rather is most often detected between 16 and 24 weeks of gestation (6). In some cases there may be an associated myocarditis/endocarditis (7 ,8). One of the most intriguing aspects of CHB is that it is an injury unique to some phase(s) of development. Despite the presence of identical antibodies in the maternal circulation, conduction defects have not been reported in the maternal heart, with a single recent exception (9). Because CHB carries a substantial mortality [~20% (10 ,11)] and morbidity [100% eventual need for permanent pacing (12)], understanding the biology of disease and bringing this knowledge to the clinic is critical. Other abnormalities affecting the skin, liver, and blood elements are also linked with anti-SSA/Ro-SSB/La antibodies in the maternal and fetal circulation and are now grouped under the heading of neonatal lupus syndromes (NLS) (13 ,14 ,15 ,16). Neonatal lupus was so termed because the cutaneous lesions of the neonate resembled those seen in SLE (15 ,16). Disease in the offspring parallels the presence of maternal antibodies in the fetal and neonatal circulation and disappears, except for CHB, with the clearance of the maternal antibodies by the sixth to eighth month of postnatal life. The transient hematologic abnormalities and skin disease of the neonate reflect the effect of passively acquired autoantibodies on those organ systems that have the capacity of continual regeneration. In contrast, these regenerative processes apparently do not occur in cardiac tissue; to date, third-degree block is irreversible.

NLS, albeit infrequently encountered by those caring for pregnant patients with SLE, presents a unique challenge to the clinical team of rheumatologist, perinatologist, neonatologist, pediatric cardiologist, and dermatologist. For the basic immunologist, the study of these syndromes may yield important insights into the mechanism by which an autoantibody directly or indirectly causes tissue injury, whereas the embryologist may uncover relevant milestones in fetal antigen expression. The study of NLS exemplifies not only translational research, which inherently draws upon clinical observations and explores them in the laboratory, but “integrational” research which attempts to fit critical clinical and basic observations together, even those seemingly at odds.

Passively Acquired Heart Block: an Irreversible Manifestation of NLS

Pathogenesis: Laying the Groundwork for Discovery

This model of passively acquired autoimmunity offers an exceptional opportunity to examine the effector arm of immunity and define the pathogenicity of an autoantibody in mediating tissue injury. A molecular scenario in which maternal anti-SSA/Ro-SSB/La antibodies convincingly contribute to the pathogenesis of cardiac scarring has yet to be formulated. One difficulty in identification of a pathogenic effect of an autoantibody is accounting for the heterogeneity of that effect. CHB is a stellar example in that not only is the injury seemingly rare, but the degree of injury varied, with the spectrum inclusive of clinically inconsequential first-degree block as well as third-degree block and an associated cardiomyopathy that is often fatal.

The necessity of anti-SSA/Ro-SSB/La antibodies is supported by their presence in more than 85% of mothers whose fetuses are identified with conduction abnormalities in a structurally normal heart (4). However, when Brucato et al. (9) prospectively evaluated 118 pregnancies in 100 patients with anti-SSA/Ro antibodies, the frequency of CHB in a fetus was only 1.7%. Although recurrence rates exceed the 2% risk

for a mother who has never had an affected child by five- to tenfold, the risk is not 100%. Moreover, the concordance rate in genetically identical twins is also not 100%. Accordingly, it is likely that antibody specificity alone cannot account for cardiac injury and that fetal factor(s) and/or the in utero environment must amplify the effects of the antibody, which may be necessary but insufficient to cause disease. Notably, one mother in the series reported by Brucato et al. (9), who gave birth to two healthy children, developed complete heart block herself, raising the possibility that her heart had acquired “fetal factors.” Clearly, this is a unique situation and one that needs to be further studied, because it is likely to contribute important clues to pathogenesis.

Another challenging aspect of the pathogenicity of this disease is that the candidate target antigens are normally sequestered intracellularly. This suggests several possibilities: (a) the proposed target is not correct, (b) there is a cross-reactivity of the true target with an antigen normally found on the cardiac surface, and/or (c) the target becomes available to maternal antibody following a change in the cell that results in translocation to the membrane.

The sections that follow address several of the challenges posed by trying to fit the clinical clues with pathogenesis.

Cardiac Histopathology

Histopathologic studies constitute a major basis for formulating hypotheses regarding the pathogenesis of CHB. It appears logical to assume that the time of death relative to initial immune attack may influence the pathologic findings. Evidence of a cellular infiltrate might be present if death occurs close to the time a bradyarrhythmia is first detected, but calcifications may be the sole pathologic finding if death has occurred months later. An inflammatory component is supported by the finding of a mononuclear cell infiltration in the myocardium of a fetus dying in utero at 18 weeks of gestation (17) and the demonstration of patchy lymphoid aggregates throughout the myocardium of an infant delivered at 30 weeks and dying in the immediate postnatal period (18). Moreover, immunofluorescent studies have shown deposition of IgG, complement (including C1q, C4, C3d, C6, and C9), and fibrin (18 ,19). The first cardiac lesion may be a global pancarditis with inflammation of the pericardium, myocardium, and endocardium, resulting in subsequent fibrosis of the conducting system clinically manifest as permanent heart block. Litsey et al. identified IgG deposits in the epicardial, myocardial, and endocardial tissue of the right atrium on postmortem analysis of a neonate with CHB (19). Although published literature on serial echocardiograms in mothers at high risk of a pregnancy complicated by CHB is limited (albeit the PRIDE study, discussed below, will address this), it has been the general experience that the first clinically apparent abnormality in cardiac function is bradycardia, and only very rarely myocarditis (i.e., effusions, ventricular dysfunction). This implies that early inflammation is not clinically detectable and/or that atrioventricular (AV) nodal injury occurs independent of an inflammatory pancarditis.

Specific vulnerability of the conducting system is unexplained (20). Ho et al. described the histopathology of seven hearts with CHB and associated maternal antibodies to the SSA/Ro polypeptide. In all of these hearts there was atrial-axis discontinuity: the AV node was replaced by varying degrees of fibrosis or fatty tissue (20). The distribution of the distal conducting system was normal.

In five cases of CHB compiled by Carter there was disruption of the AV conduction system by a process of uncertain cause (21). In all five instances the presence of microscopic crystalline structures was associated with the conduction system and with fibrous structures of the heart. These deposits have been designated as products of connective tissue degeneration resulting from an intrauterine inflammatory process. Hogg has reported hematoxylin bodies in the AV node (22). Further support for an inflammatory process is demonstrated by the findings of calcification along ventricular portions of the conducting system and area of the sinoatrial (SA) node (19 ,23). The diffuse fibroelastosis reported in some of these affected babies is considered to result from dilatation of the cardiac chambers secondary to the compensatory increased stroke volume present in CHB (22). However, Nield et al. (7) have recently reported 13 CHB patients with endocardial fibroelastosis (EFE), six diagnosed in utero and seven in the postnatal period, despite ventricular pacing of all but one infant. EFE is associated with significant mortality and morbidity: nine (70%) of these 13 patients died, and two (15%) required heart transplants.

Given the importance of histologic data to infer pathogenic mechanisms, medical records of all families enrolled in the U.S. Research Registry for Neonatal Lupus (RRNL; established in September 1994) were reviewed to determine the incidence and timing of death, with emphasis on the pathologic findings in the affected fetal hearts (24). Complete autopsy reports were available in 11 cases. The mean time from detection of CHB to autopsy was 11 weeks. Although in three cases there were various lesions of the tricuspid valve, the pathologic descriptions were more suggestive of an imposed injury than a true developmental defect. These included nodularity, dysplasia, hypoplasia and fusion of valve leaflets, and fibrosis. The pulmonary valve was abnormal in two: one was described as stenotic dysplastic, and the other nodular and dysplastic. Aortic valve insufficiency and stenosis and hypoplasia of the mitral valve leaflet were observed in one. Endocardial fibroelastosis of the right and left ventricles (RV, LV), with or without calcification, was present in seven. Chronic changes in the myocardium were documented in ten, and included biventricular hypertrophy and increased RV and LV walls, thickened but hypoplastic RV, and hyperchromatic nuclei of the myocytes. Abnormalities of the AV node or vicinity were noted in eight with involution, fibrosis, fatty infiltration or calcification. However, in two the AV node per se appeared normal: in one there was calcification in adjacent tissue, and in another there was an atrophic His bundle with replacement by dense focally calcified fibrous tissue and scarring of the left and right bundle branches. Although previously unappreciated, autopsies obtained from the RRNL revealed a high incidence of valvular

abnormalities. Although there were sufficient changes in the AV node to account for CHB in most cases, clinical conduction abnormalities may have been secondary to a functional exit block in a normal-appearing node. SA nodal disease expands the spectrum of conduction dysfunction (as addressed below in the discussion of arrhythmogenicity and perturbation of L-type calcium channels).

These studies leave little doubt that the signature lesion of autoantibody-associated CHB is fibrosis, which can clearly extend beyond the conduction system. Consequently, the cascade leading to fibrosis is a major focus of investigation.

CHB Occurs in an Anatomically Developed Heart and Coincides with Placental Transport

The presence of maternal immunoglobulins in the fetal circulation is directly related to the normal physiology of antibody traffic across the placenta (25). Maternal antibodies interact with Fc receptors on the trophoblastic cell surface in a specific transport process. Each Fc receptor has a different ligand specificity and affinity for the IgG subclasses but all receptors bind IgG1 and IgG3 with greater affinity than IgG2 or IgG4 (26). IgG1, IgG2 and IgG3 are transported relatively early with detectable levels noted at 6 to 11 weeks of gestation. In contrast, IgG4 is present in the fetal circulation after 19 weeks (27). The resultant fetal concentrations of total IgG are marginally detectable in the first trimester (<100 mg/dL) and remain low until after 17 weeks, at which time they steadily increase, reaching 400 mg/dL by 24 weeks and 800 mg/dL by 32 weeks as placental transfer becomes more efficient (28). IgM and IgA antibodies do not cross the placenta, suggesting that CHB may occur in offspring of mothers whose antibodies are of IgG1 and IgG3 subclasses, whereas those whose antibodies are predominantly IgG2 and IgG4 are protected. However, no significant differences in subclass distribution have been observed between mothers with anti-SSA/Ro and/or -SSB/La antibodies who had pregnancies complicated by CHB or those who had normal pregnancies (29). For both groups of mothers, IgG1 antibodies were significantly increased over the other three subclasses in the anti-52kD and 60kD SSA/Ro responses. IgG1 and IgG3 were the major subclasses represented in the 48kD SS/La responses. All subclasses, including IgG2 and IgG4, were observed in one-third to one-half of the maternal serum with anti-52kD and 48kD responses. In contrast, anti-60kD antibodies were, with rare exception, confined to IgG1. Accordingly, the IgG subclasses of anti-48kD SSB/La, 52kD, or 60kD SSA/Ro antibodies do not account for the susceptibility of one fetus versus another for the development of CHB.

The stage of cardiac ontogeny that coincides with the initiation of transplacental passage of maternal autoantibodies into the fetal circulation may influence the extent of tissue injury and permanent dysfunction. The human heart attains most of its adult characteristics by 6 to 8 weeks of gestation (30). Parasympathetic innervation of the heart occurs very early in fetal development whereas sympathetic innervation develops much later and is completed some months after birth. The SA node can be recognized in the first trimester and by 10 weeks of fetal age attains its own artery. Landmarks of the three internodal pathways from SA to AV nodes appear in the second month of gestation, although the septal course of these pathways does not become fully developed until the closing of the foramen ovale cordis, shortly after birth. The AV node arises separately from the bundle of His and is joined to it at 8 weeks. The human His bundle undergoes extensive postnatal remodeling to achieve its adult form. Clearly the fetal conduction system has reached functional maturity before maternal antibodies gain access to the fetal circulation.

To support a pathogenic role of maternal autoantibodies in the development of CHB, the onset of bradycardia would be predicted to coincide with heightened placental transport and occur in a previously normal heart. As expected if the hypothesis is correct, Deng et al. reported the lack of immunoglobulin deposition in cardiac tissue from 10-week fetuses of two mothers with anti-SSA/Ro antibodies (31). There is likely to be a “window of vulnerability” when maternal antibodies gain access to the fetal circulation and recognize an antigen unique to the developing heart that is absent or otherwise inaccessible in the maternal heart.

Review of data obtained from the RRNL revealed that in 71 (82%) of 87 fetuses, bradycardia was identified before 30 weeks of pregnancy (11). Detection was most frequently clustered between 20 and 24 weeks. Fourteen (16%) cases were first identified in the third trimester, five of which were noted at the time of delivery. The median time of in utero detection was 23 weeks. Of the 85 fetuses diagnosed with CHB during pregnancy, 15 were born between 1970 and 1987, eight of whom were diagnosed before 30 weeks of gestation. Seventy pregnancies occurred between 1988 and 1997, in which 63 were diagnosed with CHB prior to 30 weeks (P <0.003, earlier detection of CHB in pregnancies after 1988 compared to pregnancies before 1988).

Classification of Heart Block

There still exists some confusion regarding the assignment of “congenital” heart block. Webster defines congenital as that “existing at or dating from birth.” Data from Hübscher et al. strongly suggest that postnatally “acquired” heart block (even without structural abnormalities) is not associated with maternal antibodies to SSA/Ro or SSB/La (32) and is probably best categorized as a separate entity.

Again, the issue of “complete” versus “incomplete” heart block warrants clarification. Initially, heart block was operationally defined as third-degree or congenital complete heart block (CCHB) although it was hypothesized that heart block might progress through various stages. Of 187 children in the RRNL with CHB associated with anti-Ro/La antibodies in the mother, nine had a prolonged PR interval on EKG at birth, four of whom progressed to more advanced AV block (33). A child whose younger sibling had third-degree block was

diagnosed with first-degree block at age 10 years at the time of surgery for a broken wrist. Two children diagnosed in utero with second-degree block were treated with dexamethasone and reversed to normal sinus rhythm by birth, but ultimately progressed to third-degree block. Four children had second-degree block at birth: of these, two progressed to third-degree block. Accordingly, the general abbreviation “CCHB” is not precise and CHB (congenital heart block) has been adopted.

When CHB is associated with major structural abnormalities such as transposition of the great vessels, maternal autoantibodies are generally not present. This defect of cardiogenesis occurs prior to the 10th week of gestation and results in the disruption of the development of the AV conducting system. Such cases should be considered as a distinct classification of CHB secondary to abnormal cardiac embryogenesis and not likely the result of passively acquired autoimmunity. Examination of a heart from a child with this type of CHB, whose maternal serum did not contain autoantibodies, revealed nodoventricular discontinuity, with the AV node well formed and normally situated (34). Similarly, mass lesions developing in the conducting system, such as mesotheliomas of the AV node, would constitute another distinct type of CHB.

Arrhythmogenicity of Maternal Autoantibodies and Perturbation of L-Type Calcium Channels

Two earlier publications, both in animal models, indirectly invoked arrhythmogenic effects of anti-SSA/Ro-SSB/La antibodies. Alexander et al. reported that superfusion of newborn rabbit ventricular papillary muscles with IgG-enriched fractions from sera containing anti-SSA/Ro-SSB/La antibodies specifically reduced the plateau phase of the action potential consistent with an alteration of calcium influx (35). Garcia et al., using isolated adult rabbit hearts, showed that IgG fractions from women with anti-SSA/Ro-SSB/La antibodies induced conduction abnormalities and reduced the peak slow inward calcium current (I_{Ca}) in patch-clamp experiments of isolated rabbit ventricular myocytes (36). Because conduction in the AV node is essentially dependent on calcium electrogenesis, AV block would be expected to result from treatments leading to reduction of the I_{Ca} in ventricular myocytes. The L-type calcium channel is mainly responsible for I_{Ca} in ventricular myocytes and for the propagation of the action potential in the AV node.

These intriguing publications led to a collaborative effort with Dr. Mohamed Boutjdir (Brooklyn VA Medical Center, Brooklyn, NY) in which attention focused on the human fetal heart (37). To assess the effect of IgG fractions and affinity-purified antibodies on conduction and heart rate, electrocardiogram (EKG) recordings were obtained from whole human fetal hearts, aged 18 to 24 weeks. Baseline EKGs were recorded after a stabilization period of 30 to 45 minutes. Perfusion of the heart for 27 minutes with purified anti-52kD SSA/Ro antibodies from three mothers of children with CHB resulted in bradycardia associated with widening of the QRS complex that could represent bundle branch block or an intraventricular defect in the conducting system. The average increase in R-R and P-P interval corresponded to 32% and 30%, respectively. At 33 minutes of perfusion, complete AV block was diagnosed with the presence of only P waves and missing QRS complexes. Reperfusion of the heart with antibody-free Tyrode solution for 48 minutes resulted in partial and slow recovery. In contrast, IgG from four control mothers did not have any measurable effect on AV conduction. These findings were further characterized by studying the effects of IgG fractions and affinity-purified anti-52kD SSA/Ro antibodies on whole cell L-type I_{Ca} recorded by the patch clamp technique (38). IgG from two CHB mothers, but not from three control mothers, inhibited peak I_{Ca} at all voltages tested. The average inhibition at 0 mV was 59%. Similarly, affinity-purified anti-52kD SSA/Ro antibodies from three CHB mothers inhibited peak I_{Ca} by 56% at 0 mV. Accordingly, inhibition of I_{Ca} by the autoantibodies in isolated myocytes further supports the contribution of Ca^{2+} channels to the conduction abnormalities observed in the whole heart.

The biophysical properties by which the autoantibodies inhibited whole cell I_{Ca} were then investigated at the single channel level using the cell-attached configuration of the patch-clamp method (38). Barium currents were recorded through Ca^{2+} channels as described (39). Bath application of affinity-purified anti-52kD SSA/Ro antibody from two CHB mothers produced a significant decrease in the Ca^{2+} channel activity and the ensemble average current. The ensemble average currents decreased from 0.23 pA to 0.13 (-43%, $P < 0.02$). Similar inhibition was obtained with IgG from two CHB mothers, but no significant effect was observed with IgG from three control mothers. Analysis of single channel kinetics indicated that this inhibition was the result of shorter open times and longer closed times, which could also explain the basis of the whole cell I_{Ca} inhibition by the autoantibody. The effect of the affinity-purified antibody and IgG from CHB mothers was less pronounced in the cell-attached than the whole-cell recordings, suggesting involvement of a diffusible cytosolic constituent in mediating the response to autoantibodies. Boutjdir et al. have extended this work and recently reported a direct interaction of maternal anti-SSA/Ro-SSB/La antibodies with the pore-forming α(1)-subunit of Ca channels using transfected *Xenopus* oocytes (40), a finding of interest that remains to be confirmed.

Given the data in the rabbit and human heart, it is tempting to conclude that inhibition of L-type Ca^{2+} channels explains the pathogenicity of anti-SSA/Ro (perhaps anti-SSB/La) antibodies in the development of CHB. Several facts are highly supportive of this conclusion. AV nodal electrogenesis is dependent on L-type Ca^{2+} currents. Ca^{2+} channel density is lower and sarcoplasmic reticulum less abundant in fetal compared to adult cardiac cells, increasing the dependency on transsarcolemmal Ca^{2+} entry (41). Prolonged exposure of fetal Ca^{2+} channels to the maternal anti-SSA/Ro-SSB/La antibodies may lead to internalization and degradation of the channel, cell death, and ultimately fibrosis. Inhibition of

ventricular Ca^{2+} channels may result in decreased contraction and congestive failure. Alternatively, antibody binding to the channel may result in opsonization and phagocytosis by macrophages with inflammatory/fibrotic sequelae (similar to the apoptosis theory described above).

SA nodal electrogenesis is also dependent on L-type Ca^{2+} currents (42). Interestingly, detailed evaluation of autopsies done on children in the RRNL revealed pathology in some cases at the SA node (as described earlier in this chapter) (24). Mazel et al. observed sinus bradycardia in a murine model of passive immunity with anti-SSA/Ro antibodies (43). Highly relevant to these histologic and functional observations is Brucato's identification of sinus bradycardia in four of 24 EKGs from otherwise healthy newborns born to the cohort of mothers with SSA/Ro antibodies (9). Of 187 cases in the RRNL (33), atrial rates from postnatal EKGs were available for 40 neonates; the mean rate was 137 bpm ± 20 standard deviations (SD), range 75 to 200. The single slow rate of 75 bpm was obtained during sleep and increased to 140 bpm when awake. In an additional child, the records stated sinus bradycardia; however, no EKG was available and subsequent records were not sent to the RRNL. In both our review and the study by Brucato (9) the sinus bradycardia was not permanent. Although this suggests that the nature of the initial insult and/or the subsequent reparative processes may be different for the SA and AV nodes, normal atrial rates may reflect the functioning of other pacer foci in the atria.

Accounting for Accessibility of Intracellular Antigens to Circulating Maternal Autoantibodies

A mechanism whereby antibodies might interrupt critical intracellular events in fetal cardiomyocytes or specialized Purkinje cells is largely unknown. As previously stated by Tan, “the question is whether autoantibody reacts with intrinsic antigens to perturb the biologic function of normal cells” (44). For anti-SSA/Ro and -SSB/La antibodies to be causal in the development of NLS, three basic requirements should be satisfied. First, the candidate antigens must be present in the target fetal tissues; second, the cognate maternal autoantibodies must be present in the fetal circulation; and third, these antigens must be accessible to the maternal antibodies.

Earlier studies have firmly demonstrated reactivity of anti-SSA/Ro antibodies with fetal cardiac tissues, including the conduction system (31 ,45 ,46). The preferential vulnerability of the fetal versus the adult heart is addressed by a study demonstrating that a 23-week fetal heart contained a greater quantity of SSA/Ro per mg protein than 18-to-22-week hearts or an adult heart (47). The presence of SSA/Ro and SSB/La antigens in the fetal heart is well established and therefore satisfies the first requirement. Similarly the second requirement has been fulfilled by studies demonstrating anti-SSA/Ro-SSB/La antibodies in the fetal circulation as assessed by measurements in cord blood (29 ,48).

The third requirement, accessibility, has been more difficult to establish. It has been suggested that autoantibodies can penetrate living cells, subsequently alter function, and cause cell death (49), but this notion still remains controversial. Alternatively, if the antibody cannot cross the cell membrane, then is the antigen trafficked to the cell surface? Finally, anti-SSA/Ro-SSB/La antibodies could crossreact with other surface cardiac antigens.

Several lines of evidence have been advanced to support the possibility that otherwise sequestered intracellular autoantigens can be expressed on the cell surface. Baboonian et al. have demonstrated the sequential expression of the SSB/La antigen from the nucleus through the cytoplasm and ultimately on the cell surface of HEp-2 cells infected with adenovirus (50). There is now accumulating data supporting surface expression of SSA/Ro in keratinocytes, after exposure to ultraviolet light (51 ,52 ,53) or following incubation with TNF-α (54).

Reichlin et al. have bolstered support for the accessibility of the candidate antigens to the respective maternal antibodies by the finding of antibodies to native 60kD SSA/Ro and denatured 52kD SSA/Ro in acid eluates of a heart from a fetus with CHB who died at 34 weeks' gestation (55). The enrichment was apparently selective, because these antibodies were not detected in eluates from the brain, kidney, or skin. Furthermore, Horsfall et al. have demonstrated maternal IgG-bearing anti-SSB/La idiotypes on the surface of fetal myocardial fibers on autopsy of a neonate with CHB (56).

Apoptosis has been traditionally conceptualized from an immunologic point of view as either a means of maintaining B- and T-cell tolerance (57 ,58) or as a mechanism for providing accessibility of intracellular antigens to induce an immune response (59). Casciola-Rosen et al. have demonstrated that autoantigens are clustered in two distinct populations of surface blebs on keratinocytes (59). The larger blebs, so-called apoptotic bodies derived from the apoptotic nucleus, contain both SSA/Ro and SSB/La proteins with SSB/La detected at the cell surface surrounding large blebs in the later stages of apoptosis. The 52kD protein was not specifically identified, but rather deduced, because evaluation was done with a patient serum considered “monospecific” for 52kD SSA/Ro antibodies. The smaller blebs, arising from fragmented rough endoplasmic reticulum and ribosomes, contain SSA/Ro presumably of cytoplasmic origin. SSB/La was not contained in these blebs.

Apoptosis may be relevant in the pathogenesis of NLS. It is a selective process of physiologic cell deletion in embryogenesis and normal tissue turnover, and plays an important role in shaping morphological and functional maturity (60). Apoptosis is a process that affects scattered single cells rather than tracts of contiguous cells. In the normal adult myocardium, apoptosis has been observed only rarely (61 ,62). In contrast, apoptosis does occur during the development of the heart. In the 1970s, Pexeider extensively characterized the temporal and spatial distribution of cell death in the hearts of chicken, rat and human embryos (63). Major foci included the AV cushions and their zones of fusion, the bulbar cushions and their zones of fusion, and the aortic

and pulmonary valves. Albeit much of the cell death was noted in nonmyocytes, a focus of myocyte death was apparent in the muscular interventricular septum as it grew toward the AV cushions in midgestation. Takeda et al. demonstrated apoptosis in midgestational rat hearts using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL), an in situ technique that detects DNA strand breaks in tissue sections (64). Although not coincident with the precise timing of CHB, it has also been suggested that apoptosis contributes to the postnatal morphogenesis of the SA node, AV node, and His bundle (65). Perhaps a novel view of apoptosis is that it facilitates the placing of cardiac target autoantigens in a location accessible to previously generated maternal autoantibodies. Tissue damage might be a consequence of being in the right place at the wrong time.

To investigate the hypothesis that apoptosis indeed facilitates accessibility of SSA/Ro and SSB/La in the heart, cultured human fetal cardiac myocytes were incubated with staurosporine or 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) (66). By phase contrast microscopy, morphologic signs of early apoptosis were observed in 40% of the cardiocytes after approximately 4 hours and increased to 95% after 7 hours. The cellular topology of SSA/Ro and SSB/La was evaluated with confocal microscopy and determined in non-apoptotic and apoptotic cardiocytes by indirect immunofluorescence using two previously characterized antisera, one “monospecific” anti-SSB/La, and the other recognizing both 52kD and 60kD SSA/Ro with goat anti-human IgG-FITC as secondary antibody. In nonapoptotic cardiocytes, SSA/Ro was predominantly nuclear with minor cytoplasmic staining, and SSB/La was confined to the nucleus. In early apoptotic cardiocytes, condensation of the SSA/Ro- or SSB/La-stained nucleus was observed accompanied in some cells by a “ring” of bright green fluorescence around the periphery. In the later stages of apoptosis, the nuclear SSA/Ro and SSB/La staining became weaker. Blebs could now be seen emerging from the cell surface, stained with both SSA/Ro and SSB/La. Scanning electron microscopy unambiguously confirmed the surface expression of SSA/Ro and SSB/La on cultured human fetal apoptotic cardiocytes.

Recent studies from our laboratory have focused on the structure/function of extrinsic and intrinsic apoptosis pathways in human fetal and adult heart, and surface accessibility of SSA/Ro-SSB/La antigens to maternal antibodies (67). High levels of Fas-associated death domain protein (FADD) and TNF-α receptor type 1-associated death domain protein (TRADD), key components in the apoptotic machinery, were observed in CHB but not normal cardiac tissues. Fetal cardiocytes readily underwent apoptosis following stimulation with either anti-Fas or TNF-α when plated on pHEMA (a nonadherent condition); however, these same stimuli did not induce apoptosis in adherent cells. Thus, in fetal cardiocytes adhesion to substrate was pivotal to escaping extrinsic pathway activation, whereas adult cardiocytes did not undergo apoptosis via the extrinsic pathway even in the absence of anchorage. However, adult cardiocytes treated with staurosporine underwent apoptosis, suggesting that these cells do have the machinery to execute apoptosis via the intrinsic pathway. Utilizing monoclonal antibodies generated from a chicken phage display library, it was demonstrated that Ro52, Ro60, and La48 are surface accessible on fetal cardiocytes regardless of the method used to induce apoptosis. Accessibility appeared to be restricted to select domains, because not all antibodies that stained permeabilized cells were reactive with intact apoptotic cells. In sum, this study identifies extrinsic activation of apoptosis, differentially operative in fetal compared to adult human cardiocytes, as a mechanism linking autoantibody to subsequent injury.

In vivo studies have confirmed the observations made in vitro. Tran et al. have demonstrated the translocation of SSB/La in apoptotic cardiocytes in the conduction system of the unmanipulated mouse fetal heart (68). Clustering of SSB/La near the surface of apoptotic bodies occurs in vivo under physiologic conditions. To assess proof of concept and examine whether SSB/La and/or SSA/Ro epitopes on apoptotic cells are accessible for binding by antibodies in vivo, these same investigators have exploited a murine passive transfer model in which the fate of human autoantibodies actively transported across the placenta could be traced in fetal tissues known to have high rates of apoptosis (69). Specifically, BALB/c pregnant mice were injected with human anti-SSA/Ro-SSB/La serum, monospecific anti-Ro60 serum, affinity-purified anti-SSB/La, anti-dsDNA, or normal human serum. Apoptotic cells identified in the fetal conducting tissue (present under normal physiologic conditions of remodelling), showed redistribution of SSB/La from the nucleus to the surface of apoptotic bodies. Fetuses from anti-SSA/Ro-SSB/La antibody-injected mothers showed a striking co-localization of human IgG with apoptotic cells in the atrium, AV node, liver, skin (with particulate epidermal deposition), and newly forming bone. The IgG-apoptotic cell complexes were organ-specific and not detected in thymus, lung, or gut. No IgG deposits were identified in fetuses from mothers injected with anti-dsDNA, anti-Ro60, or normal sera. Experiments with affinity-purified anti-SSB/La and anti-SSA/Ro-SSB/La antibodies absorbed with SSB/La confirmed the specificity of deposited IgG as anti-SSB/La.

The demonstration (in vitro and in vivo) of antigen-antibody binding at the cell surface (66 ,68 ,69 ,70) supports the hypothesis that maternal antibodies to components of the SSA/Ro-SSB/La complex are not simply markers of disease, but play an active part in the pathogenesis of CHB. A molecular explanation for subsequent damage to the specialized cells of the conduction system and working myocardium remains speculative. In this context, a mechanism is envisioned whereby unexpected circumstances convert the physiologic process of apoptosis into one in which an inflammatory component is evoked. Perhaps the unexpected event is opsonization. These findings suggest that circulating maternal autoantibodies opsonize cells undergoing physiological apoptosis, which then changes otherwise innocent degradation products into proinflammatory stimuli. This cascade could result in damage and ultimately permanent scarring in those tissues with low regenerative capacity.

To address the functional consequences of opsonization of apoptotic cardiocytes, we designed coincubation experiments with human macrophages (34 ,71). The Th1 cytokine, TNF-α, was chosen as a readout of inflammation. Basal production of TNF-α by the macrophages was 9.7 ± 0.9 SEM pg/mL and decreased to 3.3 ± 0.3 SEM pg/mL after coincubation with apoptotic cells, which was not observed in initial experiments using cardiocytes rendered necrotic after hypotonic lysis. Apoptotic cardiocytes preincubated with normal human IgG acted functionally as nontreated apoptotic cells; TNF-α production by the macrophages was 5.7 ± 0.9 SEM pg/mL. In contrast, when macrophages were cocultured with apoptotic cardiocytes incubated with affinity-purified antibodies to each of the components of the Ro/La complex, TNF-α production was increased by three- to five-fold over basal levels and 10- to 14-fold over that secreted after culture with apoptotic cells alone. Nonapoptotic cardiocytes incubated with medium alone or with serum containing antibodies reactive with 48kD SSB/La, 52kD SSA/Ro, and 60kD Ro did not modify the basal production of TNF-α by the macrophages. In subsequent studies, we have demonstrated that macrophage- derived factors induce phenotypic changes in cardiac fibroblasts supportive of scarring (72).

A potential role for TGF-α in fibrosis of the AV node was supported by additional experiments. Human fetal cardiac fibroblasts exposed to supernatants obtained from macrophages incubated with opsonized apoptotic cardiocytes markedly increased expression of the myofibroblast marker, smooth muscle actin (SMAc), associated with scarring. This effect was blocked by anti-TGF-ß antibodies (72).

In Vitro CHB Model

Immunohistology of the heart from a term male infant (diagnosed with AV block at 24 weeks of gestation and dying shortly after birth) supported macrophage crosstalk despite the 2-month lag time from detection to death (72). The ventricular tissue revealed microcalcification in which a predominant SMAc-positive infiltrate could be readily observed. Macrophages were also seen in areas of scar tissue. Notably, the fibrosis was not bland, but involved an infiltrate of activated myofibroblasts months after the initial insult. Recently, this heart and two others from fetuses with CHB (20- and 22-week fetal deaths) were examined for myofibroblasts, which were found in all specimens, supporting the persistence of the myofibroblast phenotype, because this collection encompassed a spectrum of disease severity and timing of death relative to clinical detection (34).

To evaluate the extent of fibrosis, cardiac sections were stained with picrosirius for detection of collagen. In both the 20- and 22-week CHB hearts, there was extensive fibrosis in the inferior portion of the atrial wall where the AV node is likely to reside. Collagen deposition was absent in the septal tissue of a normal (fetal-age-matched) control heart. In the 20- and 22-week CHB hearts, TGF-ß immunoreactivity was seen in the conduction tissue. In several sections, intense TGF-ß staining was present in the extracellular fibrous matrix between SMAc-positive myofibroblasts concentrated in the adjacent subendocardium and infiltrating CD68-positive macrophages. Double-labeling revealed colocalization of TGF-ß in the cytoplasm of macrophages, including multinucleate giant cells. No fibrosis or TGF-ß immunostaining was seen in conduction tissue or ventricles of control hearts from 22-, 23-week abortuses (34).

Murine Model of CHB

Although clinical data leave little doubt regarding the association of anti-SSA/Ro and/or SSB/La antibodies with the development of CHB, and experimental data are beginning to suggest pathogenicity, efforts to establish an animal model have been limited. Kalush et al. reported that offspring of BALB/c mice immunized with the monoclonal anti-DNA idiotype 16/6 had conduction abnormalities (73). Of 31 pups born to mothers with experimental SLE, eight had first-degree heart block, two had second-degree heart block, two had complete block, ten had bradycardia, and eight demonstrated widening of the QRS complex. None of these disorders could be detected in the 20 offspring of healthy control mice. One of the difficulties in interpreting these findings is that the immunized mothers synthesized a variety of autoantibodies, including antibodies reactive with 16/6 Id, ss/dsDNA, Sm, RNP, cardiolipin, SSA/Ro, and SSB/La. Accordingly, it was not possible to segregate out which specific antibody might be responsible for the arrhythmias detected in these pups. The electrocardiographic data are provocative; however, no histologic data were provided to assess the status of the SA or AV node, or the presence of myocarditis.

To further establish an antibody-specific murine model to correlate arrhythmogenic effects of maternal autoantibodies with the in vivo genesis of CHB, we have immunized female BALB/c mice with 100 µg of one of the following 6 × His human recombinant proteins purified by Ni^{2+} affinity chromatography: 48kD SSB/La, 60kD SSA/Ro, 52kD SSA/Ro (52α full-length), and 52ß (74). Control animals were given the same injections with a Ni^{2+} affinity-purified polypeptide encoded by pET-28 alone. Following primary immunization in complete Freund adjuvant and two boosters (50 µg) in incomplete Freund adjuvant, high titer immune responses to the respective antigens were established by ELISA and immunoblot of recombinant antigens, and immunoprecipitation of [^{35}S]-methionine-labeled in vitro translation products. Sera from mice immunized with either 52α or 52ß immunoprecipitated radiolabeled murine 52kD SSA/Ro, confirming that these mice were specifically reactive with the murine homologue. Moreover, immunoblot of a newborn murine heart demonstrated the presence of 52kD SSA/Ro. Mice were mated and boosters continued every 3 weeks to ensure continued high titer antibody responses. EKGs were performed on all pups using standard limb leads at birth or within 2 days postpartum. Maternal antibodies to the primary immunogens were detected by ELISA in the pups.

Of 54 pups born to six fertile mice immunized with 60kD SSA/Ro, none had CHB; of 27 pups born to three fertile mice immunized with 48kD SSB/La, none had CHB. In contrast, of 78 pups born to five fertile mice immunized with 52α and 86 pups born to five fertile mice immunized with 52β, one and five pups, respectively, had complete AV block. Accordingly, this antibody-specific animal model provides strong preliminary evidence for a pathogenic role of antibodies reactive with 52kD SSA/Ro, particularly the 52β form, in the development of CHB. Moreover, analogous to the frequency of 1% to 5% given for women with SLE who have anti-SSA/Ro and/or SSB/La antibodies (4 ,9), this model suggests that additional factors promote disease expression.

Candidate Antigen-Antibody Systems in NLS

The Target Autoantigens of the SSA/Ro-SSB/La System

Antibodies against SSA/Ro and SSB/La have been discussed in detail by Drs. Reichlin and Harley in Chapter 25 .

Eftekhari et al. recently reported that antibodies reactive with the serotoninergic 5-hydroxytryptamine (5-HT)$_{4A}$ receptor, cloned from human adult atrium, also bind 52kD SSA/Ro (75). Moreover, affinity-purified 5-HT$_4$ antibodies antagonized the serotonin-induced L-type Ca channel activation in human atrial cells. Two peptides in the C terminus of 52kD SSA/Ro, aa365–382 and aa380–396, were identified that shared some similarity with the 5-HT$_4$ receptor. The former was recognized by sera from mothers of children with NLS, and was reported to be cross-reactive with peptide aa165–185, derived from the second extracellular loop of the 5-HT$_4$ receptor. These findings are of particular importance, since more than 75% of sera from mothers whose children have CHB contain antibodies to 52kD SSA/Ro as detected by ELISA, immunoblot and immunoprecipitation (76 ,77).

Given the intriguing possibility that antibodies to the 5-HT$_4$ receptor might represent the hitherto elusive reactivity directly contributing to AV block, we examined whether the 5-HT$_4$ receptor is a target of the immune response in these mothers (78). Initial experiments demonstrated mRNA expression of the 5-HT$_4$ receptor in the human fetal atrium. Electrophysiologic studies established that human fetal atrial cells express functional 5-HT$_4$ receptors. Sera from 116 mothers enrolled in the RRNL, whose children have CHB, were evaluated: 99 (85%) contained antibodies to SSA/Ro, 84% of which were reactive with the 52kD SSA/Ro component by immunoblot. In sum, none of the 116 sera were reactive with the peptide spanning aa165–185 of the serotoninergic receptor. Rabbit antisera that recognized this peptide did not react with 52kD SSA/Ro. Accordingly, although 5-HT$_4$ receptors are present and functional in the human fetal heart, maternal antibodies to the 5-HT$_4$ receptor are not necessary for the development of CHB.

Most recently, Eftekhari's group and ours jointly assessed the role of anti-5-HT$_4$ antibodies (79). Sera from 128 patients (101 anti-SSA/Ro52 positive mothers, of whom 74 had children with CHB; 20 anti-SSA/Ro52 negative patients, of whom one had a child with CHB, six had children with structural HB, five had children who developed HB after birth; 18 healthy donors) were assessed in a single blind test using an ELISA coated with a 5-HT$_4$ receptor-derived peptide. Discrepancies between previous observations in our two groups could be ascribed to small differences in the set-up of the assay. Of the 74 sera from Ro52$^+$ mothers of children with CHB, 11 were reactive with the 5-HT$_4$ peptide. Sera from the Ro52$^-$ mother of a child with CHB, one of six Ro52$^-$ mothers of children with structural HB, three of 35 mothers of unaffected children, and two of 18 controls were also 5-HT$_4$-positive. Although 5-HT$_4$ receptor autoantibodies do not have the predictive value of anti-Ro52 autoantibodies, the presence of these antibodies in a minor subset of mothers whose children have CHB suggests an additional risk factor that may contribute to the pathogenesis of disease.

Fine Specificities of the Maternal SSA/Ro-SSB/La Autoantibody Response

The sensitivity and specificity of anti-52kD Ro, anti-60kD Ro, and anti-48kD La in predicting risk of CHB in an offspring were recently reassessed in sera from 125 mothers of children with NLS, using a commercial line immunoassay that employs natural 60kD Ro protein (Inno-Lia ANA Update, Innogenetics NV, Gent, Belgium) (80). By this method, 96% of the sera had antibodies to 60Ro, 86% to 52Ro, and 78% to 48La. Immunoblot of 65 CHB-mothers showed significantly fewer positive results for anti-60Ro (P <0.001) and anti-52Ro (P <0.05). Sensitivity of the three antibodies was assessed in 78 symptomatic CHB-mothers and 65 disease-matched controls with unaffected children using Inno-Lia ANA Update. The sensitivity of each antibody was compared by multiple logistic regression to adjust for maternal disease. There was no significant difference between the groups for 60Ro or 52Ro antibody. However, there was a significant difference for the anti-La antibody (P = 0.001), with an odds ratio of 3.59. This translates to an increase in risk from a published 2% for CHB in an anti-Ro-positive mother to 3.1% if the woman is also anti-La antibody-positive, and to a decrease in risk to 0.9% if anti-La-negative.

Novel Antigen/Antibody Systems

Although it is not known how maternal antibodies influence the development of cardiac versus cutaneous manifestations of NLS, to date, antibodies to U1RNP in the absence of reactivity to anti-SSA/Ro and/or SSB/La have never been reported in children with CHB. Sheth et al. have added two cases to eight previously reported in which anti-U1RNP antibodies but not anti-SSA/Ro-SSB/La antibodies were present in infants with cutaneous disease alone (81). Furthermore, Solomon et al. describe anti-U1RNP-positive, anti-SSA/Ro-SSB/La-negative dizygotic twins discordant for cutaneous

manifestations of NLS (neither twin had cardiac disease) (82). The segregation of anti-U1RNP antibodies with cutaneous disease may be a useful maternal marker and should guide research efforts in sorting out cardiac versus cutaneous susceptibility to antibody-mediated injury.

Complement Regulatory Proteins

With regard to the mechanism of tissue injury, consideration should be given to the possibility that protective molecules may be diminished on the surface of fetal cells. In adulthood, the organism's cells are protected from complement-mediated damage by membrane-bound proteins such as decay accelerating factor (DAF, CD55), protectin (CD59), and membrane cofactor protein (MCP, CD46) (83 ,84 ,85). DAF regulates C3/C5 convertases and CD59 regulates assembly of the terminal components of the membrane attack complex. MCP patrols cell membranes, inactivating the C4b and C3b that is inadvertently bound by acting as a cofactor for their factor I-mediated cleavage (86). MCP is expressed on a wide variety of cells including those of the epithelial, fibroblast, and endothelial cell lineage (87).

To examine fetal characteristics that might influence autoantibody-mediated diseases acquired in utero, such as heart block in NLS, the tissue expression of MCP was studied (88). Immunoblots of organs from six fetuses (aged 19 to 24 weeks) probed with rabbit anti-MCP antibodies revealed a band at 60kD in addition to the known 65kD and 55kD isoforms that comprise the codominant allelic system of MCP. Five fetuses expressed the most common MCP polymorphism (predominance of the 65kD isoform, upper band α phenotype) in the kidney, spleen, liver, and lung. In contrast, all hearts from these five fetuses demonstrated a different pattern in which there was a marked decrease in the intensity of the 65kD band and accentuation of the lower molecular weight bands. In a sixth fetus, which expressed the second most common polymorphism (equal expression of the 65kD and 55kD MCP isoforms, αß phenotype), the heart was similar to the other tissues. Preferential expression of the MCP ß isoform in five of six fetal hearts, irrespective of the phenotype of other organs, suggests tissue-specific RNA splicing or posttranslational modification. The clinical significance of this tissue-specific phenotype is unknown at present, but may provide an important clue to the susceptibility of the fetal heart to antibody-mediated damage.

Genetic Considerations in the Proposed Pathogenesis of NLS

Fibrosis-Promoting Genes

It is clear that maternal antibodies are not sufficient to directly cause cardiac scarring and that additional maternal and fetal factors, such as genetics, are likely to convert predisposition to clinical expression. At present, no model of inheritance for CHB is known, making this a complex genetic problem. As noted above, CHB occurs in 19% of siblings born subsequent to a CHB-affected infant (11), a rate 3,000 times higher than the population prevalence (1/20,000), implying a strong genetic effect. Driven by the proposed pathologic cascade supported by in vitro and in vivo data, we have begun an initial analysis of TGF-ß and TNF-α polymorphisms.

The human gene encoding TGF-ß is on chromosome 19q13 and is highly polymorphic. Awad et al. (89) have identified five polymorphisms in the TGF-ß gene: two in the promoter region at positions -800 and -509, one at position +72 in a nontranslated region, and two in the signal sequence at positions +869 and +915. The polymorphisms at positions +869 and +915, which change codon 10 (T→C, leucine→proline) and codon 25 →G→C, arginine(proline), are associated with interindividual variation in the levels of TGF-ß production. This has clinical relevance, because several animal and human studies have shown that high TGF-ß producers develop significantly more lung fibrosis in response to a number of inflammatory triggers, such as radiation (90), chemotherapy (91) and lung transplantation (92). The Pro^{25} allele is associated with lower TGF-ß synthesis in vitro and in vivo, whereas the Arg^{25} allele is associated with allograft fibrosis in transbronchial biopsies when compared with controls and with nonallograft fibrosis (93). It has been reported that lung allograft recipients with the Leu^{10} allele produced the highest amounts of TGF-ß (89), and chronic rejection after lung transplant is linked with high levels of TGF-ß (93). In parallel with our hypothesis that high levels of TGF-ß permit the development of CHB as a result of enhancement of extracellular matrix and increased fibrosis, patients with cystic fibrosis who develop rapid deterioration in lung function have an increased frequency of the Leu^{10} homozygosity (94).

Codons 10 and 25 of the TGF-ß gene were evaluated in 88 children (40 CHB, 17 rash, 31 unaffected siblings) and 74 mothers from the RRNL (95). The TGF-ß polymorphism Leu^{10} (associated with increased fibrosis) was significantly higher in CHB-children (genotypic frequency 60%, allelic frequency 78%) than unaffected offspring (genotypic frequency 29%, $P = 0.016$; allelic frequency 56%, $P = 0.011$) and controls, whereas there were no significant differences between controls and other NLS groups. For the TGF-ß polymorphism Arg^{25} there were no significant differences between NLS groups and controls.

The rash of neonatal lupus resembles subacute cutaneous systemic lupus erythematosus (SCLE) and is often photosensitive. There is evidence that the release of TNF-α by UV light-exposed keratinocytes contributes to the lesions of SCLE in individuals having the haplotype –308A TNF-α/DRB1*03 (96). The gene encoding TNF-α is highly polymorphic, and a substitution of G→A at position –308 (TNF2) in the promoter region has been associated with increased production of this cytokine (97). The common (wild-type) allele, –308G (TNF1), has a frequency of approximately 80% in Caucasians and 92% in African Americans (97). The link between anti-SSA/Ro antibodies, photosensitivity, and TNF-α promoter polymorphisms extends to HLA class II molecules. In Caucasians, there is a strong linkage disequilibrium

between the –308A allele and HLA-DRB1*03 (98). The presence of DRB1*03 is also common in individuals who synthesize anti-SSA/Ro and SSB/La antibodies.

DNA was isolated from the same RRNL cohort for genotyping of the TNF-α –308 promoter region and HLA-DRB1. There was a significantly higher –308A carrier frequency in children with NLS rash compared to healthy controls (64% vs. 23%; *P* = 0.002) (99). The DRB1 distribution for DRB1*03 was significantly higher in children with rash compared to controls (64% vs. 17%; *P* = 0.014). Interestingly, the carrier frequency of DRB1*03 was also significantly higher than controls for children with CHB (54%) and for mothers (73%) but not unaffected children (39%). Although the carrier frequency was higher in children with rash compared to children without rash, the difference did not reach statistical significance.

In the children with rash, the prevalence of the –308A allele paralleled the prevalence of DRB1*03. When individual subjects were tabulated by the presence or absence of –308A and DRB1*03, the two alleles together were significantly greater in the children with rash, children with CHB, and mothers, compared to controls. In contrast, unaffected children were not significantly different from controls. For children with rash, the association between the –308A allele and DRB1*03 occurred in all but one case (7%) (*P* = 0.012 vs. control). However, for children with CHB and unaffected children the presence of –308A in the absence of DRB1*03 was 25% and 38%, respectively, which did not differ significantly from controls.

Immunogenetics of the Mother and Host

The majority of studies to date have documented the near-universal presence of DR3 alleles in the mothers of affected offspring, frequently associated with the extended haplotype A1, B8 (100 ,101 ,102 ,103 ,104 ,105). In addition to the reports of others, 10 of 11 affected mothers studied at our institution were HLA DR3. The one exception was DR7. For comparison, of 25 women with SLE or SS who gave birth to healthy offspring, 48% were DR3, odds ratio = 16, *P* <0.003. Several investigators have noted an increased frequency of HLA-DR2 in anti-SSA/Ro positive mothers of normal infants (100 ,104). This is not surprising, because sera from the majority of mothers whose offspring have CHB contain both anti-SSA/Ro and -SSB/La antibodies and not anti-SSA/Ro alone (45 ,76 ,106). It is the former subgroup that is more strongly associated with the linked HLA alleles B8,DR3,DRw52,DQw2 and the latter subgroup with DR2,DQw1 (105).

As has been suggested for other HLA-linked diseases, it is possible that several genes within a particular haplotype may contribute to enhanced susceptibility. Arnaiz-Villena et al. found that Class III antigens BS and C4QOB1 are increased together with the A1,B8,DR3 haplotype in SSA/Ro positive mothers whose offspring have CHB (107). Moreover, these class II genes were not significantly increased in a group of SSA/Ro positive mothers whose offspring did not have CHB. Of particular interest was the observation that the most common DR3-bearing haplotype found in the Spanish population for adult SLE (A30,B18,DR3,BfF1,C2C,C4ABQ) was not increased among the mothers of offspring who had CHB. This study did not find an increased frequency of DR2 in the anti-SSA/Ro positive mothers of healthy offspring, i.e., $DR2^+$ = 3/15 (20%) versus 23% in their normal population.

Brucato et al. reviewed the literature and reported that of 28 mothers whose children have CHB, 50% were A1, 62% B8, and 96% DR3 (105). In their own cohort of 15 Italian mothers of children with CHB, Brucato found a significantly increased prevalence of DR3 and DQ2 and B8/DR3, DR3/DQ2, and A1/Cw7/B8/DR3/DQ2 haplotypes (105). In a study of 31 Finnish mothers (all anti-SSA/Ro and/or SSB/La positive) of children with CHB, HLA B8 and DR3 were present in 71% and 74% of the affected mothers, respectively (101). Thus, the genetic background of CHB-mothers is similar in Anglo-Saxon populations and populations from southern and northern Europe (101). The same Finnish study demonstrated that as a group, CHB-mothers were genetically more closely related to primary SS than to SLE (101).

At the present time a role for fetal genetic differences in the major histocompatibility complex influence susceptibility is not firmly established. In five earlier reports of affected neonates, only two were DR3 (100 ,102 ,103 ,104). These data emphasize the passive nature of NLS and the difference between acquiring manifestations of lupus and actually developing the disease. However, continued investigations of larger numbers of affected mothers add complexities to the picture. In the study of Arnaiz-Villena et al., four of five offspring with CHB were DR3-positive compared to two of five unaffected siblings (107).

Perhaps fetal HLA, not as an isolated risk factor, but as it relates to maternal HLA, is a fetal factor contributing to injury. A plausible hypothesis might be that tissue damage occurs either when the HLA relationship between the fetus and mother is bidirectionally compatible or unidirectionally compatible from the child's perspective. Relevant to the hypothesis that macrophage phagocytosis of apoptotic cardiocytes opsonized by autoantibodies initiates and inflammatory cascade leading to CHB, HLA class II compatible maternal Th1 cells would be able to provide help to fetal macrophages. In support of this notion, Miyagawa et al. reported on a limited study on 13 Japanese families in which children with CHB shared both HLA class II alleles with their mothers significantly more often than children without CHB (4/9 CHB vs. 0/12 healthy siblings, *P* <0.02) (108). Furthermore, Stevens et al. (109) have demonstrated maternal cells in the AV node, myocardium, and liver of two infants with NLS who died shortly after birth, possibly representing alloreactive hematopoietic cells that trigger inflammation leading to tissue destruction.

Given two limited studies implicating fetal/maternal class II sharing as contributory to the pathogenesis of CHB, the RRNL was utilized to further expand or refute this notion. DNA was isolated from 65 children (29 CHB, 12 rash, 24 unaffected siblings) and their 62 mothers (anti-SSA/Ro^+, Caucasian) enrolled in the RRNL. HLA-DQB1, DRB1, and

Cw7 alleles were determined by a DNA-based technique, with PCR amplification using sequence specific oligonucleotide primers. Specific HLA alleles were significantly enriched in the mothers: DRB1*03 (79%), DQB1*02 (84%), and Cw7 (92%). No class II allele was enriched in the children with CHB compared to healthy anti-SSA/Ro exposed children or those with rash. Maternofetal compatibility was noted for mother/rash dyads, but no disproportional sharing of HLA molecules was observed between mothers and children with CHB. This is further emphasized by the inclusion of a child with CHB (product of ovodonation) whose HLA molecules were unrelated to the anti-SSA/Ro^+ surrogate mother. In sum, although HLA associations are crucial for cellular immunity leading to maternal autoantibody production, these associations are not likely contributory as fetal factors in the development of CHB but only rash.

Lessons from Twins and Triplets

The study of twin gestations further emphasizes the complexity of NLS. These informative in vivo experiments of nature provide clues to the true relevance of the maternal antibody and host factors. Discordance of disease expression in monozygotic twins would be particularly intriguing, given that the placenta is shared and the fetal genetics are identical. Twins born to mothers with anti-SSA/Ro-SSB/La antibodies provide a unique opportunity to gauge the effect of a specific antibody profile on disease phenotype. Regardless of whether twins share a common placenta or not, it is likely that even if a mother had three types of antibodies (Type A causing CHB, Type B causing skin rashes, and Type C being nonpathogenic) the proportion of these antibodies should be similar in each fetal circulation. Types A and B by definition must be of the correct subclass and isotype (i.e., IgG, subclasses 1–4) to be transported across the trophoblast, whereas Type C might not be transported at all. One might envision quantitative differences in each twin, but it is difficult to conceive of a mechanism to explain why one placenta would transport antibodies more selectively than another unless Fc receptors were differentially expressed.

Of 24 twin pairs and two triplet sets published (1 ,11 ,82 ,102 ,110 ,111 ,112 ,113 ,114 ,115 ,116 ,117 ,118 ,119 ,120 ,121 ,122 ,123 ,124 ,125), only six are entirely concordant for disease expression (Table 53-1). Gawkrodger and Beveridge describe disparate disease expression in male twins who were in the same amniotic sac and shared a common placenta (110). The mother had anti-SSA/Ro and anti-SSB/La antibodies. One infant presented at 8 weeks of age with Stokes Adams attack and second-degree heart block, a facial rash and evidence of hemolytic anemia, whereas his twin brother had merely a “peeling” facial rash at 15 weeks of age. Cooley et al. reported two sets of monozygotic twins discordant for CHB (111). Watson et al. performed HLA studies and C4 phenotypes on dizygotic twins discordant for isolated CHB (117). Both infants had identical HLA (DR4, DR7) and C4 phenotypes (C4A3.QOB1.1). Serum from the mother and both cord bloods demonstrated anti-SSA/Ro and SSB/La antibodies with no significant difference in titer noted in the cord blood of either twin when compared with maternal titers. Brucato et al. reported a second pair of HLA identical twins discordant for CHB (118), and a third such pair was reported by Kaaja et al. (119). In our RRNL there were three sets of twins (11). Two sets were dizygotic, with only one twin in each set having CHB. Histologic and genetic analysis of the third set revealed a twin placenta (diamniotic, dichorionic) and monozygosity. One twin had CHB and the other was completely healthy.

Certainly these observations support a fetal contribution to the development of neonatal lupus, but perhaps even more intriguing is that in two sets of twins (110 ,116) and two sets of triplets (120 ,125), all children were affected but with discordant manifestations. Accordingly, even if antibody profile is an important predictor of risk for one manifestation of neonatal lupus versus another, the fetus (perhaps density of calcium channels, impaired healing, exuberant fibrosis) and/or its immediate environment (low oxygen) provide additional factor(s) required to convert susceptibility to overt disease. The logical inference from these reports is that developmental events in utero strongly contribute to the susceptibility of a particular fetus.

The Transient NLS Rash

In striking contrast to the typical late second trimester onset of CHB, the skin lesions generally become manifest several weeks into postnatal life. Less commonly the rash is present at birth. Ultraviolet exposure may be an initiating factor and can exacerbate an existing rash (126). Thornton et al. report that telangiectasia may be a presenting feature and can occur in sun-protected sites independent of “lupus dermatitis” (127). Cutaneous activity, inclusive of erythema and the continued appearance of new lesions, is generally present for several weeks with resolution by 6 to 8 months of age coincident with the clearance of maternal autoantibodies from the baby's circulation. However, hypopigmentation may persist into the second year of life. Lee has observed two children with persistent telangiectasias and one child with a small but persistent patch of hyperpigmentation (126); 11 other children, including several with extensive skin disease, have had complete resolution of the rash.

The rash frequently involves the face and scalp with a characteristic predilection for the upper eyelids. In some instances the rash is present in other locations and can cover virtually the entire body. A review of the corporeal distribution of 57 infants (20 males, 37 females) diagnosed with cutaneous NLS (absent heart disease) enrolled in the RRNL was recently reported (128). All had facial involvement (periorbital region most common) followed by the scalp, trunk, extremities, neck, intertriginous areas, and rarely the palms and soles. In most cases the infant's rash was temporally related to UV exposure; mean age of detection was 6 weeks and duration 22 weeks. The lesions are described as superficial inflammatory plaques resembling subacute cutaneous lupus erythematosus of the adult (129). They are typically annular or elliptical with erythema and scaling.

Hypopigmentation was frequent and may be a prominent feature. The more characteristic lesions of adult discoid lupus such as follicular plugging, dermal atrophy, and scarring were generally not observed in the neonatal skin rash. The lesional histology supports the clinical descriptions of subacute cutaneous lupus with basal cell damage in the epidermis and a superficial mononuclear cell infiltrate in the upper dermis (130 ,131). As observed in subacute cutaneous lupus, immunofluorescence is positive with the finding of a particulate pattern of IgG in the epidermis (126).

Table 53-1: Neonatal Lupus in Twins and Triplets

Study	Maternal Serology	Zygosity	Disease Manifestations		
			Sib 1	Sib 2	[Sib 3]
Gawkrodger (110)	SSA/Ro, SSB/La	monozygotic	CHB/rash/anemia	rash	
Cooley (111)	SSA/Ro	monozygotic	CHB	healthy	
Cooley (111)	SSA/Ro	monozygotic	CHB	healthy	
Buyon (11)	SSA/Ro, SSB/La	monozygotic, dichorionic	CHB	healthy	
Siren (112)	SSA/Ro, SSB/La	monozygotic, HLA Cw3	CHB	CHB	
Shimosegawa (113)	SSA/Ro, SSB/La	monozygotic, HLA Cw3	rash	rash	
Machado (114)	SSA/Ro	not reported	CHB	CHB	
McCue (115)*	+ RF, - ANA,	not reported	CHB-death*	CHB*	
Siren (112)	SSA/Ro, SSB/La	dizygotic, HLA Cw3	CHB	CHB	
Silverman (116)	SSA/Ro, SSB/La	dizygotic	CHB	rash	
Scott (1)	SSA/Ro	dizygotic	CHB	healthy	
Harley (102)	SSA/Ro	dizygotic	CHB	healthy	
Watson (117)	SSA/Ro, SSB/La	dizygotic, HLA identical	CHB	healthy	
Brucato (118)	SSA/Ro	dizygotic, HLA identical	CHB	healthy	
Kaaja (119)	SSA/Ro	dizygotic, HLA identical	CHB	healthy	
Buyon (11)	SSA/Ro	dizygotic	CHB	healthy	
Buyon (11)	SSA/Ro	dizygotic	CHB	healthy	
Stevens (120)	SSA/Ro, SSB/La	dizygotic	CHB	healthy	
Eronen (121)	not reported	dizygotic	CHB	healthy	
Lockshin (122)	SSA/Ro	dizygotic	CHB	healthy	
Lockshin (122)	SSA/Ro	dizygotic	rash	healthy	
Solomon (82)	U1RNP	dizygotic	rash	healthy	
Callen (123)	SSA/Ro	dizygotic	rash	healthy	
Lawrence (124)	SSA/Ro, SSB/La	dizygotic	rash	rash	
Stevens (120)	SSA/Ro, SSB/La	trizygotic	CHB (3°)	CHB (1°/2°)	liver
Yazici (125)	SSA/Ro	trizygotic	rash	rash	rash, thrombocytopenia, liver

1, 2, and 3 arbitrarily refer to the twin or triplet siblings but do not imply sequence of birth.
*Both twins had CHB recognized at birth; twin 2 had associated fibroelastosis and was asymptomatic at 1 year of age, twin 1 had L-transposition, ventricular septal defect, coarctation, and hypoplastic right ventricle, and died at 15 days of age.

Less Commonly Encountered Manifestations of NLS

The clinical spectrum of NLS includes hepatic involvement, a manifestation that could well be underestimated since routine neonatal evaluation does not include a liver profile. Permanent sequelae can occur in the liver, but unlike the heart may be clinically insignificant. Laxer et al. have described three living infants and one perinatal death with NLS associated with significant hepatic involvement (13). The living infants presented with neonatal cholestasis as a major component of their clinical picture. Pathologic changes included giant cell transformation, ductal obstruction and extramedullary hematopoiesis. The authors speculated that an inflammatory hepatitis proceeding to hepatic fibrosis may ensue, analogous to the mechanism hypothesized to occur in cardiac tissue. Rosh et al. reported an infant born with CHB in whom severe neonatal cholestasis developed, requiring surgical exploration to exclude extra hepatic biliary atresia (132). The clinical picture included an elevation of the serum glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, and gamma-GTP. A percutaneous liver biopsy revealed mild fibrosis, bile ductular proliferation, and a mixed inflammatory infiltrate in the portal tracts. Lee et al. described three infants with hepatic dysfunction; all had laboratory and histologic evidence of significant cholestasis (133). One occurred in the setting of intractable congestive heart failure. At autopsy, immunofluorescence revealed widespread deposits of IgG. A second infant had thrombocytopenia and hepatospleno-megaly at birth, followed at 3 weeks of age by a cutaneous eruption characteristic of NLS. Liver biopsy revealed hepatocellular cholestasis, lobular disarray, and mild pseudoacinar formation. A third neonate developed a typical rash at 2 weeks and transaminitis with jaundice by 8 weeks. Liver biopsy revealed canalicular and hepatocellular cholestasis.

Lee et al. (134) recently investigated the incidence of hepatobiliary manifestations among 219 NLS patients in the RRNL. Nineteen (9%) had probable or possible hepatobiliary disease, appearing as the sole manifestation of NLS in three cases, and in association with cardiac or cutaneous manifestations in 16 cases. Six (including one previously reported by Schoenlebe et al. (135)) of the 19 infants died, either during gestation or within the first few weeks of life. Three clinical variants were observed: (a) severe liver failure present during gestation or in the neonatal period, often with the phenotype of neonatal iron storage disease; (b) conjugated hyperbilirubinemia with mild or no elevations of aminotransferases, occurring in the first few weeks of life; and (c) mild elevations of aminotransferases occurring at approximately 2 to 3 months of life. The prognosis for the children in the last two categories was excellent.

These reports and others (136) suggest that the diagnosis of NLS-related liver disease should be considered in situations in which the liver enzymes and bilirubin levels are most consistent with cholestasis in the absence of a major structural abnormality of the biliary tree. Reassuringly, in babies that survive cardiac manifestations, the general observation is that hepatic disease resolves.

Although the nervous system has not been regarded as an organ characteristically affected in NLS, clinical detection may be a limiting factor. There have been several reports of neurologic sequelae in NLS. Specifically, aseptic meningitis occurred in an infant with CHB and circulating maternal anti-SSA/Ro and anti-SSB/La antibodies (137). There has been one case report of NLS and transient hypocalcemia with seizures (138). Wong et al. observed sonographic evidence of infantile lenticulostriate vasculopathy (LSV) in a case of NLS (139). The authors suggest that sonographic LSV is a nonspecific marker of a previous insult to the developing brain, the clinical significance of which is uncertain. Bourke et al. describe an infant with thrombocytopenia and a generalized annular rash with scattered telangiectases at birth in the setting of antibodies to SSA/Ro and SSB/La (140). At the age of 1 year, the child was found to have an abnormal gait and examination revealed mild spastic diplegia of the lower limbs. The authors appropriately point out that the central nervous system (CNS) abnormalities might have been due to an intracerebral hemorrhage in the neonatal period. This curious observation of late onset lower limb spasticity has been reported in one other infant with antibodies to SSA/Ro and butterfly rash at birth (141). In sum, the mechanism of these neurologic sequelae is entirely elusive and awaits further observation in other neonates born to mothers with anti-SSA/Ro-SSB/La antibodies.

Hematologic abnormalities have been described as a manifestation of NLS. Thrombocytopenia has been observed together with other manifestations of NLS (14). Some of these infants have a petechial or purpuric eruption as the initial feature. Thrombocytopenia was present in 10% of the neonates referred to Lee et al. (129). Gastrointestinal bleeding occurred in one of these infants. In contrast to the cardiac manifestations (which do not parallel disease in the mother) and the cutaneous manifestations (which occasionally occur in the mother but often not in synchrony with her affected offspring), the hematologic manifestations may more closely parallel maternal disease. However, Watson has described thrombocytopenia in offspring of anti-SSA/Ro positive mothers with no apparent history of thrombocytopenia (14). Despite this, the presence of thrombocytopenia raises some questions as to whether antiplatelet antibodies rather than antibodies to SSA/Ro-SSB/La are targeting the surface of fetal cells. These manifestations, while secondary to passively acquired autoimmunity (and therefore part of NLS), may be

more akin to the neonatal thrombocytopenia of idiopathic thrombocytopenic purpura (ITP). The disparate fetal and adult vulnerability appears more pronounced in NLS than in ITP.

Kanagasegar et al. (142) reported an infant with neutropenia and mildly abnormal liver functions, but no cardiac or cutaneous manifestations of NLS, born to a mother with anti-SSA/Ro-SSB/La antibodies. The child's neutropenia improved as maternal antibody was metabolized. Sera from this child and mother, as well as sera from two RRNL mothers who had given birth to infants with CHB and neutropenia, were shown to bind the cell surface of intact neutrophils (142). Binding to neutrophils was then inhibited (>80%) by incubating the sera with 60kD Ro antigen, suggesting that anti-60kD SSA/Ro is directly involved in the pathogenesis of neutropenia.

Wolach et al. extend the hematologic spectrum of NLS (143). They describe a 5-month-old infant with anti-SSA/Ro antibodies and typical cutaneous involvement in the setting of complete marrow aplasia, who recovered at 8 months with the disappearance of anti-SSA/Ro antibodies. However, before this complication is added to the “official list” of manifestations, it is curious that the mother herself was said to have “tested negative” for anti-SSA/Ro antibodies. The child died at 16 months from gram-negative sepsis.

Maternal Disease at Identification of Neonatal Lupus and Progression

In 1987, McCune et al. assessed the health status of 21 mothers and their children with NLS (144). This study suggested that all mothers eventually developed symptoms of a rheumatic disease but larger series have not reached similar conclusions (77 ,145 ,146 ,147).

To date we have evaluated 110 mothers of children with CHB enrolled in the RRNL; at the time CHB was identified, 39 mothers were asymptomatic, 17 had an undifferentiated autoimmune syndrome (UAS), 20 had SLE, 24 had SS, five had SLE/SS, and for five the diagnosis is unknown. At a mean follow-up of 8.4 years, 14 (36%) of the 39 initially asymptomatic mothers had developed symptoms of a rheumatic disease: one developed UAS, five SLE, two SS, and one SLE/SS. Twenty-five (64%) remained asymptomatic. Of the 17 mothers initially diagnosed with UAS, one developed SLE and four SS. Three mothers with SS at the birth of the affected child progressed to SLE/SS at follow-up.

Similar results were reported by Julkunen et al. (77). Fifteen (48%) of 31 mothers whose children had CHB were asymptomatic before the index delivery, seven of whom remained asymptomatic after a mean follow-up of 8 years. Two mothers had SLE, one mother had definite primary SS, one had probable SS, one had autoimmune hypothyroidism, and one had Grave disease. Six (19%) gave a self-reported diagnosis of a chronic autoimmune disease antedating the index delivery. Of two mothers who died, one subsequently developed SLE and died of a fatal cardiac arrhythmia 5 years after the birth of the affected child. The other died of alcoholic liver disease and had developed SLE after 11 years. No patient with SLE had nephritis. Importantly these authors noted that as a group, mothers of CHB children had clinical and immunologic characteristics more closely related to primary SS than SLE.

In a study of 64 CHB-mothers from Toronto, 42 (66%) were asymptomatic, two (3%) had SLE, two (3%) had linear scleroderma, two (3%) had RA, three (5%) had a history of rheumatic fever, one (2%) had SS, and 12 (2%) had UAS (145). Three of the 12 mothers with UAS progressed to SLE and two developed SS. Thirty-six of the 42 initially healthy mothers remained well, one developed SLE, one hyperthyroidism, one ankylosing spondylitis, and three UAS. Unlike the other larger studies, not all mothers included in this cohort were documented to have anti-SSA/Ro-SSB/La antibodies.

Taken together, it can be concluded that asymptomatic mothers do not invariably become ill (at least over 8 years or more of follow-up) and if an asymptomatic mother does develop SLE, it is not likely to be life-threatening. Although more formal epitope analysis of the autoantigens has not been done with regard to clinical outcome, the fine specificity of anti-SSA/Ro-SSB/La antibodies as assessed by immunoblot is highly stable for years, independent of the maternal clinical status (145).

Maternal health status may also be of interest with regard to the discordant clinical manifestations of neonatal lupus. A link between the health status of the mother and child might aid in defining risk for a given mother, an enormous benefit to the practitioner faced with family counseling, and in identifying a pathogenetic mechanism of injury. Maternal disease might reflect the fine specificity of an antibody response, which in turn could account for the preferential vulnerability of one particular organ versus another. Lawrence et al. compared a cohort of 24 women with anti-SSA/Ro-SSB/La antibodies whose children have only cutaneous manifestations of neonatal lupus (146) to their previously published cohort of 32 mothers with similar antibodies whose children have only cardiac manifestations (145). Maternal health status was considered as either symptomatic (inclusive of varied rheumatic diseases such as systemic lupus erythematosus, Sjögren syndrome, and undifferentiated autoimmune diseases) or completely asymptomatic. A significantly greater number of mothers whose children had CHB were asymptomatic (75%) compared to mothers of children with NLS rash alone (42%). At follow-up, mothers of children with CHB were more likely to remain asymptomatic (59%) than were mothers of children with NLS rash (25%).

This same question was also addressed by review of data in the RRNL; included were 105 mothers whose children had only CHB and 47 whose children had only NLS rash (148). All mothers had documented antibodies to SSA/Ro and/or SSB/La. Initially, 37% of the “CHB-mothers” and 28% of the “rash-mothers” were asymptomatic and, after at least 5 years of follow-up, 24% and 13%, respectively, remained symptom-free. There was a trend to more symptomatic disease in the mothers whose children had cutaneous manifestations, but the differences between rash-mothers and CHB-mothers in the RRNL did not reach statistical significance. These data are not completely in accord with those of Lawrence et al.

(147), the difference being in the distribution of maternal disease in the CHB groups. There was a significantly greater proportion of symptomatic CHB-mothers in the RRNL than in Toronto. Interestingly, when Fisher's exact test was used to compare the rash-mothers, the proportion of symptomatic individuals did not differ significantly between the Toronto and RRNL databases. The referral patterns of the two studies could plausibly explain their divergent findings with regard to the health of CHB-mothers. All subjects in the Toronto database are from within a single city, not from outside sources; the RRNL, in contrast, includes the entire United States. Although the referral source for the Toronto patients is not included in the report by Lawrence et al., in the RRNL 42% of CHB and 54% of rash cases were referred by rheumatologists (*P* = NS, CHB vs. rash). Data from two separate studies suggest that mothers who have children with cutaneous disease are more likely to be symptomatic than asymptomatic. However, this could be explained by the fact that recognizing a neonatal rash as NLS is more likely in a mother with rheumatic disease. Whether maternal health status (i.e., absence of clinical disease) is a marker for heart disease remains to be established.

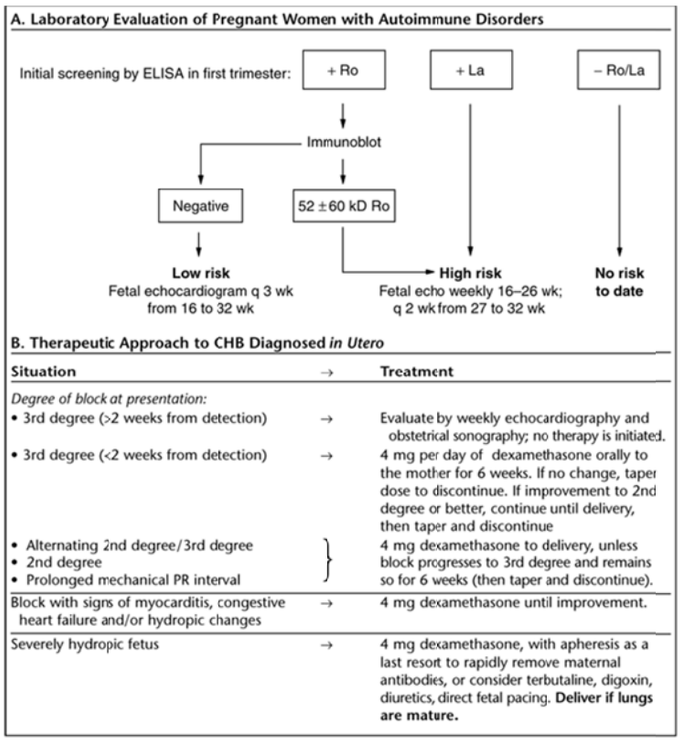

Table 53-2: Current Laboratory Evaluation and Clinical Management of Neonatal Lupus

Clinical Management of NLS

Identification and Management of the High-Risk Pregnancy

Table 53-2 presents an overall schema for management. These recommendations are considered experimental,

based on the author's experience, and have not been tested in a controlled trial.

Prenatal Considerations

Ideally, since CHB is most often identified from 18 to 24 weeks of gestation, intrauterine therapy should eventually be possible. The clinical approach to cardiac manifestations of NLS includes obstetric and rheumatologic management of (a) the fetus identified with CHB and (b) the fetus with a normal heart-beat but at high risk of developing CHB.

To address the treatment of identified CHB one needs to know if the presence of bradycardia represents an irreversible fibrotic process and if continued autoimmune tissue injury will cause progressive damage. McCue has reported a neonate described with first-degree heart block at birth, which resolved at 6 months (115). In contrast, Geggel et al. report an infant born with second-degree heart block, which progressed to third-degree block by 9 weeks of age (149). Data from the RRNL also emphasize that incomplete AV block is not always fixed and the degree of block is variable (11 ,33). There is a spectrum of conduction abnormalities even at the time of initial detection and in utero injury can have continued sequelae in some cases despite clearance of maternal antibodies from the neonatal circulation.

The rationale for treatment of identified heart block and prevention of potential heart block is to diminish a generalized inflammatory insult and reduce or eliminate maternal autoantibodies. Accordingly, several intrauterine therapeutic regimens have been tried including dexamethasone, which is not metabolized by the placenta and is available to the fetus in an active form, and plasmapheresis. Although there is not yet documentation in the literature regarding the reversal of third-degree heart block (complete fibrosis of the AV node would not be reversible) by maternal treatment with dexamethasone alone, the potential for diminishing an inflammatory fetal response attacking the myocardium is plausible. This would be an effect independent of a decrease in antibody titer. Precedent for this therapeutic rationale is the resolution of associated pleuropericardial effusions and ascites reported in separate investigations (17 ,46 ,150). In the largest retrospective study published to date it was observed that fluorinated glucocorticoids ameliorated incomplete atrioventricular block and hydropic changes in autoimmune-associated congenital heart block but did not reverse established third-degree block (151). Specifically, the data included 47 mothers whose sera contain anti-SSA/Ro-SSB/La antibodies, and their 50 children diagnosed with isolated CHB in utero and in whom at least four echocardiograms were performed thereafter. In 28 pregnancies, the mothers were treated with dexamethasone 4 to 9 mg/day for 3 to 19 weeks or betamethasone 12 to 24 mg/wk for more than 6 weeks (group A). In 22 pregnancies, fluorinated steroids were not used (group B). Third-degree block was present in 21 fetuses in group A and 18 fetuses in group B; none were reversible despite steroids. Three fetuses in group A and two in group B progressed from alternating second-degree/third-degree block to permanent third-degree block at birth and postnatally. Notably, of four fetuses in group A with second-degree block at presentation, all reverted to first-degree by birth. Of two fetuses in group B with second-degree block at presentation, both progressed to permanent third-degree block postnatally. Initial echocardiographic evaluation revealed pericardial effusions in 13 group A versus four group B (P = NS), pleural effusions in two group A versus none in group B (P = NS), ascites in eight group A versus none in Group B (P <0.007), hydrops in eight group A versus none in Group B (P <0.007), and intrauterine growth restriction (IUGR) in one group A versus one group B (P = NS). Pericardial effusions resolved and reappeared in both groups. Steroid therapy was most effective in the sustained resolution of pleural effusions (2/2), ascites (6/8), and hydrops (5/8). Oligohydramnios ensued in nine group A and two group B (P = NS). Although fetuses in group A had more associated complications at initial presentation than those in group B, there were no significant differences between the groups in the duration of pregnancy (35.7 vs. 37.0 wk), number of deaths (4 vs. 1), final degree of block, or requirement for permanent pacing (14 vs. 11).

In our experience and that of others, apheresis in addition to dexamethasone has not reversed third-degree heart block (17 ,152 ,153), although titers of maternal anti-SSA/Ro-SSB/La have been profoundly decreased (152). Maternal risks of dexamethasone are similar to any glucocorticoid and include infection, osteoporosis, osteonecrosis, diabetes, hypertension, and preeclampsia. Fetal risks include oligohydramnios, intrauterine growth retardation, and adrenal suppression. Intervention with glucocorticoids might decrease acute inflammation, but not necessarily prevent subsequent fibrosis.

Available data support serial cardiac monitoring of all fetuses with any bradyarrhythmias detected in utero and of neonates with incomplete blocks at birth whose mothers are previously known or currently identified to have anti-SSA/Ro-SSB/La antibodies. Fetal echocardiogram is essential to diagnose and follow the course of disease, and may suggest the presence of an associated myocarditis by the finding of decreased contractility in addition to the secondary changes associated with myocarditis such as an increase of cardiac size, pericardial effusions, and tricuspid regurgitation. The obstetric management should be guided by the degree of cardiac failure noted on the ultrasound images. The in utero environment is preferred as long as possible because of the low resistance circulatory pathways, thereby affording minimal work to maintain cardiac output.

The initiation of dexamethasone or plasmapheresis as a preventative measure has been considered. With regard to prophylactic therapy of the high-risk mother (documentation of high titer anti-SSA/Ro and SSB/La antibodies, anti-48kD SSB/La and 52kD SSA/Ro on immunoblot, and a previous child with NLS), administration of prednisone, dexamethasone, or plasmapheresis is not justified at the present time. Maternal prednisone (at least in low and moderate doses) early in pregnancy does not prevent the development of CHB (154). This might be anticipated, because prednisone given to the mother is not active in the fetus (155) and levels of anti-SSA/Ro and anti-SSB/La antibodies remain relatively constant

during steroid therapy. However, Shinohara et al. recently published uncontrolled data suggesting that prenatal use of maternal glucocorticoids has prophylactic merit (156). This recommendation was based on the finding that 15 of 61 infants born to 40 mothers with anti-SSA/Ro antibodies who did not receive glucocorticoids had CHB. The unexpectedly high prevalence may be explained by the retrospective nature of the study and potential referral bias. Conversely, CHB did not occur in any of 26 fetuses whose mothers were given steroids prior to the 16th week of gestation. If one accepts the rate of 2% then at least 40 mothers with anti-SSA/Ro antibodies may need to be followed to find one infant with complete block.

With regard to plasmapheresis as a prophylactic therapy, Barclay et al. initiated plasmapheresis during the late second trimester in a woman with anti-SSA/Ro antibodies and a history of four unsuccessful pregnancies, including a 32-week stillbirth with unexplained antenatal bradycardia. The pregnancy resulted in a healthy birth, and the titer of anti-SSA/Ro antibodies was decreased by 75%, although detectable antibodies were present in the cord blood (157). We similarly utilized “prophylactic” plasmapheresis in a pregnant woman with SS and a previous child with CHB (152). In this case, the fetus, despite having circulating levels of these antibodies detectable at birth, was exposed to only 10% of the potential maternal antibody load during the second and third trimesters. The child, now 8 years of age, never had any manifestations of NLS. In the absence of controlled studies, which may never be feasible given the rarity of CHB, plasmapheresis should be considered highly experimental and only reserved for those cases where the fetus is in a life-threatening situation with hydrops and deteriorating cardiac function.

The best approach for a mother with a previous offspring with CHB is unknown. We suggest that all mothers with anti-SSB/La antibodies or antibodies to components of SSA/Ro (particularly the 52kD) on immunoblot should have serial fetal echocardiography done by an experienced pediatric cardiologist weekly from 16 to 26 weeks and every other week until about 34 weeks. Thereafter, auscultation should be sufficient. In the last few years there have been major advances in fetal echocardiography. Until recently, the in utero detection of first-degree block was not technically feasible. However, the EKG equivalent of the PR interval can now be measured by echocardiography (158). A prospective NIH-supported multicenter study, PR Interval and Dexamethasone Evaluation (PRIDE) in CHB is underway to examine the mechanical PR interval weekly in pregnant woman with anti-SSA/Ro and/or anti-SSB/La antibodies. One of the goals of this trial is to identify the prevalence of first-degree block and to determine whether it is a marker for more advanced destruction of the conducting system. Such information will provide the optimal opportunity for reversibility. It is strongly recommended that all neonates born to mothers with anti-SSA/Ro-SSB/La antibodies have an EKG at birth to detect first-degree block.

Breastfeeding

Mothers often ask about the risks of breastfeeding. Askanase et al. (159) have addressed whether human breast milk contains antibodies to components of the SSA/Ro-SSB/La complex and, if so, whether breastfeeding might be associated with the postnatal manifestations of neonatal lupus. To accomplish these goals, breast milk from nine mothers with serologic evidence of anti-SSA/Ro and/or SSB/La antibodies was examined by ELISA and immunoblot for the presence of these same antibodies. Five of these breastfed infants were healthy without any manifestations of neonatal lupus, one had an isolated cardiomyopathy and died, two had cutaneous manifestations of neonatal lupus, and one had both CHB and rash. IgA and IgG antibodies to all components of the SSA/Ro-SSB/La complex were present in breast milk. Not unexpectedly, the antibody profiles of the breast milk paralleled those observed in the serum.

Of 237 mothers enrolled in the RRNL as of September 2000, 129 mothers answered a questionnaire regarding breastfeeding of their 266 children. Neonatal lupus was present in 149 of the children (55 with NLS rash alone, 72 with CHB alone, and 22 with both manifestations) and 117 were unaffected. The frequency of breastfeeding in the mothers enrolled in the RRNL was slightly lower (51%) than the national average (60%) of mothers breastfeeding upon leaving the hospital (160). This might be explained by the fact that mothers with anti-SSA/Ro-SSB/La antibodies are discouraged from breastfeeding by their physicians or in some cases might be too ill or on other medications. Overall, a total of 136 children (51%) were breastfed. Of 55 children with NLS rash, 33 were breastfed. Of 22 with both rash and CHB, 12 were breastfed. For the unaffected siblings, 60 of 117 were breastfed. The Fisher exact test revealed no significant differences between the breastfed and nonbreastfed children with isolated NLS rash, or those with associated CHB, compared to the unaffected children. Although there was a trend for the children who were breastfed to have the cutaneous manifestation of neonatal lupus appear at a later age than those who had rashes and were formula-fed, the difference in mean age of presentation of the rash did not reach statistical significance: 9.69 weeks vs. 7.55 weeks (*P* = NS). The duration of the rash was not influenced by breastfeeding: 14.7 weeks in the breastfed group versus 19 weeks in those not breastfed (*P* = NS). Not unexpectedly, of 72 children with isolated CHB, 31 were breastfed, which did not differ significantly from the unaffected children. Of seven infants in whom cardiomyopathy was detected after birth, four were breastfed.

Accordingly, the available data do not suggest that breastfeeding has pathologic consequences. Specifically, children with skin rashes were not breastfed more frequently than those who remained healthy. Furthermore, prematurity was not a factor contributing to the development of NLS rash in breastfed infants. There was a trend toward later presentation of the rash in the children who were breastfed, but there was no increase in duration compared to children who received formula. Because maternal antibodies transferred to the fetus during gestation would still be present for several months postpartum, the additional contribution of antibodies from breast milk may be inconsequential even if intestinal transport is effective. Mothers should be advised that autoantibodies are present in their breast milk but reassured that, at least within

the limits of published literature, breastfeeding is not associated with neonatal lupus. Given the potential for intestinal transport of the maternal antibodies, in the unusual circumstance of a worsening rash or developing cardiomyopathy consideration should be given to discontinuation of breastfeeding.

Treatment of Cutaneous Manifestations

Infants with CHB should be protected from excessive sun exposure since they are at risk for developing skin lesions until 8 to 12 months of age. In the absence of precise information regarding specific pathogenicity of the maternal antibody response, it is reasonable to consider all offspring of mothers with antibodies to components of the SSA/Ro-SSB/La ribonucleoproteins at risk for cutaneous disease in the first few months of postnatal life. Topical steroids, preferably those that are nonfluorinated, have been recommended for babies who develop lesions (126). High potency topical steroids to the skin of an infant can result in systemic effects. Because the lesions are transient and generally benign, systemic therapies such as antimalarials, which have a low toxic-to-therapeutic ratio in young children, have not been recommended (126).

Neiman et al. (128) reported on therapy and outcome of 57 infants (20 males, 37 females) diagnosed with cutaneous NLS (absent heart disease) between 1981 and 1997. Thirty-four (60%) were treated. Thirty-one were given only low to medium potency topical corticosteroid preparations. Three children were initially given topical antifungal agents and then subsequently treated with topical steroid preparations. None received systemic glucocorticoid therapy. The active rash resolved in all children regardless of treatment. In 51 children for whom reliable follow-up data were available, 37 rashes completely resolved without sequelae, of which 21 were treated and 16 received no therapy except avoidance of sun exposure. However, in 14 there were residual skin abnormalities: ten had telangiectasias, two had hyperpigmentation of the affected areas, ten had what was described as pitting, scarring or atrophy after at least 2 years of follow-up. Of these 14 children, ten were treated and four untreated. Although there was no significant difference in outcome between treated and untreated children by the Fisher exact test, firm conclusions are limited by the small number of cases.

Recurrence Rates

In the RRNL as of December 2005, 100 mothers have had pregnancies (lasting longer than 6 months) subsequent to the birth of child with CHB. In 73 (73%) of these pregnancies there were no AV conduction abnormalities or reported cutaneous manifestations of NLS in the children. Eighteen (18%) next pregnancies resulted in a second child with CHB, three in association with a rash. In seven (7%) next pregnancies, the offspring had cutaneous manifestations alone. Thus, the probability of having a second affected child with any manifestation of NLS was 25%. In one (1%) subsequent pregnancy, fetal demise occurred at 30 weeks, but with no apparent conduction abnormalities. Another subsequent pregnancy (1%) resulted in the birth of a boy who died at age 2 months. Although there were no documented conduction abnormalities, severe aortic stenosis and cardiomyopathy were present, which on biopsy was found to be associated with immunoglobulin deposition (24).

It is also important for the clinician to consider the outcomes of pregnancies subsequent to the birth of a child with NLS rash. Among RRNL mothers, as of December 2005, there have been 46 pregnancies (lasting longer than 6 months) following the birth of a child with NLS rash. In 17 (37%) of the offspring, there were no cardiac or cutaneous manifestations of NLS; 15 (33%) had NLS rash alone. It is striking that 13 (28%) next pregnancies resulted in a child with CHB, five in which there was also an associated rash. Although these recurrence rates from rash to CHB are disturbing, one caveat to consider is that many of these women entered the RRNL because of a child with CHB, but request for photos of younger siblings revealed characteristic NLS rashes.

Fetal Outcome

Data published from the RRNL reveal that 22 (19%) of the 113 offspring with CHB whose mothers were documented to have anti-SSA/Ro and/or SSB/La antibodies have died (12 boys, 10 girls). Six of these deaths occurred in utero. Ten neonates died in the first 3 months of life. Six children died between 3 months and 3 years of age. None of the 67 children between 3 and 10 years old remaining in the cohort have died. Twenty-two children are older than 10 years. However, the mortality is markedly reduced in those children born at later gestational ages. Specifically, only 8 (9%) of 86 children born at or after 34 weeks have died (11). Those infants who survive the neonatal period have an excellent prognosis. The cumulative probability of survival at 3 years is 79%.

With regard to the morbidity of CHB, 67 (63%) of the 107 children born alive have required pacemakers, 35 within the first 9 days of life. Fifteen additional children have been paced in the first year, and 17 after 1 year. One infant has had a cardiac transplant at 8 months of age, because of intractable cardiomyopathy. Moak et al. (8) recently underscored the need to recognize late-onset cardiomyopathy as a sequela of CHB with their report of 16 cases that developed despite adequate pacing.

In a study of 15 CHB patients followed by Silverman et al., there were three neonatal deaths, two late deaths due to pacemaker failure, and six who have required pacemaker therapy (106). Similarly, McCune reported a follow-up of 14 neonates with CHB, of whom five required pacemakers (144).

Long Term Follow-Up of Children with Varied Manifestations of NLS

Given the rarity of the disease, little information is available on the health outcome of children with neonatal lupus and their unaffected siblings. This is of interest from several perspectives. There is a genetic susceptibility for the

development of SLE (161 ,162 ,163), and relatives of SLE patients can have autoantibodies in the absence of clinical disease (164). Perhaps in addition to autoantibodies, the expression of SLE in the mother increases the risk of autoimmune disease in her offspring independent of whether the child has neonatal lupus or not. This generates the hypothesis that children with neonatal lupus, as well as their unaffected siblings, whose mothers have SLE might be at greater risk for the development of subsequent disease than children whose mothers are asymptomatic. Perhaps vulnerability to neonatal lupus is a marker for susceptibility to, or protection from, the development of actively acquired autoimmunity.

Martin et al. (165) report on the health of children 8 years of age or older who had manifestations of neonatal lupus (affected group) and their unaffected siblings (unaffected group). Questionnaires were sent to mothers (with anti-SSA/Ro-SSB/La antibodies) enrolled in the RRNL, and a control group composed of children of healthy mothers referred by the RRNL enrollees. Responses to the questionnaires were confirmed and expanded by review of medical records. Fifty-five mothers enrolled in the RRNL returned questionnaires on 49 children with neonatal lupus and their 45 unaffected siblings. Six children were identified with definite rheumatologic/autoimmune diseases: two with juvenile rheumatoid arthritis, one with Hashimoto thyroiditis, one with psoriasis and iritis, one with diabetes mellitus and psoriasis, and one with congenital hypothyroidism and nephrotic syndrome. All had manifestations of neonatal lupus and their mothers have manifestations of autoimmune diseases: four Sjögren syndrome, one systemic lupus erythematosus/Sjögren syndrome, and one undifferentiated autoimmune disease. Four of 55 sera tested were positive for ANA (two of 33 affected children and two of 22 unaffected children). No serum contained antibodies reactive with SSA/Ro or SSB/La antigens. These data suggest that children with neonatal lupus require continued follow-up, especially prior to adolescence and if the mother herself has an autoimmune disease. Although there was no apparent increased risk of SLE, the development of some form of autoimmune disease (systemic or organ-specific) in early childhood may be of concern. During adolescence and young adulthood, individuals with neonatal lupus and their unaffected siblings do not appear to have an increased risk of developing systemic rheumatic diseases.

In one small series, Brucato et al. (105) found that, at a mean follow-up of 18 years, none of 13 children with CHB developed clinical symptoms or serologic abnormalities suggesting immune disease.

Despite these encouraging results, other authors have reported seven cases in which an autoimmune disease developed in a child with manifestations of neonatal lupus. All the children were female, and in each instance except one the mother had SLE. Specifically, Esscher and Scott (166) reported a female with CHB who developed SLE at age 15 years. Jackson and Gulliver (167) reported an infant with cutaneous neonatal lupus who developed SLE at age 13, and Fox et al. (168) described a similar patient who developed SLE at age 19 years. In a patient described by Waterworth (169), CHB was identified at age 6 years and SLE diagnosed at age 13 years; no information was provided on the mother. Lanham et al. (170) reported two children with CHB; one subsequently developed primary SS at age 23 years, and the other arthritis, positive ANA and antibodies to dsDNA at age 19 years. Hübscher et al. (171) reported the development of a scleroderma of the face, puffy hands, and Raynaud phenomenon in a 13-year-old girl with CHB. At age 15 she was found to be seropositive for anti-SSA/Ro and U1RNP. It should be emphasized that maternal anti-SSA/Ro-SSB/La antibodies were not actually documented for any of these mothers.

Conclusions

The presence of antibodies reactive with several components of the SSA/Ro-SSB/La ribonucleoprotein particle, notably the 48kD SSB/La and the 52kD SSA/Ro antigens, is to date a near-universal characteristic of all mothers giving birth to offspring with NLS. Although NLS is rare, its discussion is an integral part of all pregnancy counseling of women with SLE, SS, and UAS. Overall studies suggest that there is an unacceptable morbidity and mortality. A major clue to defining the pathogenesis of antibody-mediated damage is the selective vulnerability of the fetal heart. Vulnerability could relate to a direct or indirect antigen target differentially expressed in the developing human heart. Alternatively, but not mutually exclusively, biologic events operative during fetal life, such as apoptosis, could facilitate accessibility of intracellular antigens to the extracellular environment. Opsonization of apoptotic cardiocytes might alter the normal events of cell removal by macrophages. Inadvertent activation of the macrophage and subsequent dysregulation of the fibroblast may lead to permanent injury. Perturbation of L-type calcium channels, which propagate the action potential in the AV node, provides a clue to a definable pathogenetic effect of anti-SSA/Ro antibodies. How apoptosis and calcium channel dysfunction relate, if at all, is not intuitive; there may be more than one mechanism involved in pathogenesis. A reproducible murine model of CHB would be a critical tool to define antibody pathogenicity at the histologic and molecular level with subsequent application for testing prophylactic and therapeutic interventions. The availability of a research registry devoted to neonatal lupus should continue provide an invaluable resource for basic and clinical research. The reporting of recurrence rates, mortality and morbidity, and maternal outcomes in a large number of patients has greatly facilitated family counseling. We eagerly await the results of the PRIDE study in the hope that with more detailed echocardiographic data, risk can be better defined and reversibility of early injury, a reality.

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Chapter 54

Reproductive Issues in Women with Autoimmune Disease

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Introduction

The human physiologic process that protects pregnancy from interruption by the maternal immune system remains a mystery. In recent years, investigators have theorized that breeches in this system may result in either infertility or recurrent pregnancy loss (RPL). Numerous immunologic mechanisms for so-called “reproductive failure” have been proposed and several immunomodulating treatments have been suggested as infertility therapy. This chapter will examine the evidence pertaining to proposed mechanisms and treatment regimens relating to immunologic reproductive failure.

Embryologic Development of the Immune System

The development of the immune system begins at conception and continues throughout pregnancy and into the newborn period. During weeks two and three of gestation, pluripotential yolk sac stem cells form the precursors for all the blood cell series. The thymus develops in the human embryo at week six of gestation, and lymphocyte differentiation proceeds in the absence of foreign antigens. Small lymphocytes appear in the peripheral blood at week seven and around lymphocyte plexuses by week eight. As early as 13 weeks of gestation, T cells that can respond to mitogens and recognize histoincompatible cells begin to appear. By 20 weeks of gestation, the human fetus has the ability to respond to congenital infections by producing plasma cells and antibodies.

The presence of an intact trophoblastic cellular barrier prevents the movement of large numbers of immunocompetent cells into or out of the fetus during pregnancy. In contrast, maternal IgG, by virtue of its Fc fragment, is specifically selected for placental transfer. Fetal IgG concentrations are about 10% of adult levels by the middle of the first trimester. Adequate humoral immunity in the neonatal period depends on the circulating immune globulins that have crossed the placenta, and fetal blood levels of IgG reflect maternal levels. The specific antibodies depend on the mother's own antigenic experience and function primarily to protect the neonate from infection. Additionally, maternal autoimmune disorders characterized by production of IgG antibodies may result in the transplacental passage of potentially antibodies to the fetus and newborn.

Maternal–Fetal Immunology

The normal relationship between mother and fetus appears to be growth promoting rather than the usual allogeneic model of destruction (1). Although incompletely understood, several immunologic mechanisms have been suggested. Gleicher (2) noted a generally higher prevalence of autoantibodies in women than men, speculating that selectively regulated autoantibodies have evolved in women as a compensatory mechanism to combat the excessive autoantigen load imposed by pregnancy (3 ,4). The implication is that autoantibodies may be considered normal and perhaps essential for normal reproduction.

Circulating blocking factors have been theorized to attenuate maternal immunologic reaction. These suppressor or blocking antibodies are measured using maternal-paternal mixed lymphocyte reactions (MLR) or flow cytometric cross-match (5). One progesterone-induced blocking factor suppresses production of lymphocytes and pro-inflammatory cytokines. Additionally, so-called blocking antibodies may prevent lymphocytic destruction by binding to receptors on fetoplacental tissues. Alternatively, blocking antibodies may be directed against antigen-specific combining sites (idiotypes) on maternally produced antibodies that prevent them from assisting lymphocytes in targeting cells on the conceptus. The concept of protection by blocking antibodies is indeed intriguing. However, their relevance remains unsettled. They are not universally present in the sera of women with normal pregnancies (6), but are often present in patients with RPL (7). Second, there is no evidence they are the cause of inhibition in the mixed lymphocyte response which is nonspecific and may be a result of factors other than immunoglobulins (8). Not only do

agammaglobulinemic women without antibody production have normal pregnancies (9), antibodies do not frequently appear until late in the first or second trimester of the first pregnancy.

Immunotolerance during pregnancy is probably a local phenomenon at the maternal-fetal interface rather than from generalized immunosuppressive state. Before conception, endometrial stromal cells transform into decidual cells that contain T cell subtypes with immunosuppressive activity. One T helper cell subset secretes cytokines that are beneficial or neutral to the presence of the fetus. Another is thought to prevent colonization with microbial pathogens. Additional evidence suggests that some subsets of the decidual T cells promote growth of the placenta through the secretion of cytokines that suppress inflammation. Hormones, enzymes, growth factors, and endometrial proteins within maternal decidual tissue also have potent immunomodulatory properties that promote a favorable interaction between the conceptus and the mother.

The presence of large granular lymphocytes in luteal phase and early to midpregnancy deciduus has generated considerable interest among reproductive immunologists (10). Under normal circumstances, these nonspecific innate immune effector cells, similar to natural killer (NK) cells, kill standard NK cell-targets with the notable exception of trophoblastic cells (11). Their absence or alteration has been associated with pregnancy loss.

The placenta also plays an active role in protection of the fetus from maternal immune responses. Villous cytotrophoblasts and syncytiotrophoblasts escape destruction because both express nonclassic MHC antigens that prevent trophoblast destruction through inhibition of lysis by activated NK cells, limitation of leukocyte cytotoxic activity, suppression of proinflammatory cytokine production, and induction of T cell death. Nonclassical MHC antigens also promote trophoblast proliferation and invasion. Altered expression of nonclassical MHC antigens has been linked to recurrent miscarriage and preeclampsia.

Placental expression of a protein known as the Fas ligand may also play a role in pregnancy success through selective deletion of antifetal T cell clones. In animal studies, binding to the Fas ligand causes death and removal of autoreactive T cells. The placenta may also inhibit T cell proliferation by sequestering nutrients. Placental indoleamine 2,3 dioxidase (IDO) inactivates the amino acid tryptophan that is essential for the proliferation of T cells. The role of IDO in recurrent pregnancy loss in humans has not yet been widely investigated.

Recurrent Pregnancy Loss

Background

Most pregnancy losses are sporadic, nonconsecutive spontaneous abortions that occur as an isolated event in the reproductive career of a woman with other successful pregnancies. Approximately 10% to 20% of pregnant women experience sporadic loss of a clinically recognized pregnancy (12). By comparison, only 2% of pregnant women experience two consecutive pregnancy losses. The diagnosis of recurrent pregnancy loss (RPL) is traditionally reserved for women with three or more consecutive losses and is estimated to occur in 0.5% to 1% of couples (13). Etiological categories include genetic, uterine pathology, endocrine, immunologic, thrombophilia, and environmental factors. Unfortunately, a cause for RPL is identified in only about 50% of affected couples (14).

Pregnancy loss prior to 20 weeks' gestation (menstrual dates) has traditionally been termed miscarriage or spontaneous abortion with fetal death thereafter considered a stillbirth. This oversimplified classification scheme ignores precepts of developmental biology as well as the clinical realities of pregnancy loss. In reality, the development of the conceptus from fertilized egg to live born infant involves numerous complex, inter-related steps. The pre-embryonic period lasts from conception through the fourth week from the first day of the last menstrual period (3 weeks after fertilization). During this period, the early trophoblast differentiates from the tissue destined to become the embryo (the inner cell mass) and accomplishes implantation into the maternal endometrium (days 6 to 7 after fertilization). The pre-embryo develops into a bilaminar and then trilaminar disk of cells and microscopically observable alterations of the cell disk define the cranial end central neural axis of the pre-embryo. Oxygen and nutrient needs are met by diffusion across maternal tissues.

The embryonic period is thought to begin around the fifth week of gestation, lasting through the ninth week of gestation during which time the trilaminar disk folds to become cylindrical, the head and tail regions become recognizable as cranial and caudal folds, definite segmentation is seen, and all organs form (organogenesis). The fetal period begins at the tenth week of gestation and extends through pregnancy until delivery. This period is characterized by substantial growth and differentiation of previously formed structures with relatively little organogenesis.

There are also distinct phases in the development of the placenta and the maternal-fetal circulation. Careful histologic examination (15) and evidence from Doppler ultrasound (16) indicates that normal pregnancies are characterized by obstruction of uteroplacental arteries by invading trophoblastic cells, which severely limits maternal blood flow into the intervillous tissue during the first 10 to 12 weeks. During this time, the intervillous space is relatively hypoxic (17) and is filled with an acellular fluid (15 ,18). Trophoblastic regression and dislocation of arteriolar trophoblastic plugs are thought to begin around 10 weeks' gestation allowing the initiation of true intervillous blood flow and an increase in intervillous oxygen tension. Until then, oxygenation of the embryonic tissues is presumed to occur largely through diffusion across adjacent tissues, rather than an organized circulatory delivery.

Accurate dating of pregnancy loss is often difficult because of the nature of its clinical presentation. The

symptoms of early loss, typically uterine bleeding and cramping, usually occur several days after the demise of the conceptus so that a pre-embryonic or embryonic loss may not become clinically apparent until 10 to 12 weeks' gestation. In other cases, fetal death may not occur until shortly before or even after clinical symptoms appear, as in the case of cervical insufficiency.

Epidemiology of Recurrent Pregnancy Loss

Approximately 50% of conceptions are thought to end in failure (19); the vast majority going unrecognized because they occur prior to or with the expected next menses (20). In one study of 232 early pregnancies of which 13% ended in failure, 90% occurred prior to 12 to 14 weeks' gestation (from last menses) and only 2% between 14 and 20 weeks' gestation (21). Miscarriage was infrequent between 8.5 to 14 weeks' gestation with no embryos alive at 8.5 weeks' gestation dying before 14 weeks' gestation, suggesting that pregnancy loss is somewhat biphasic in its distribution. Including both fetal deaths and early neonatal deaths, approximately 5% of all pregnancies end in pregnancy loss from 14 weeks' gestation through term (22 ,23 ,24). It is not clear whether failure of so-called “chemical pregnancies,” diagnosed solely by sensitive human chorionic gonadotropin assays, should be considered pregnancy losses.

Most cases of RPL take place in the pre-embryonic/embryonic period. Primary RPL refers to consecutive losses without any live births, whereas secondary RPL refers to losses following a live birth (25 ,26). The prognosis for a successful pregnancy is better after secondary RPL (Paukku, 1999). Women with three successive pre-embryonic or embryonic losses have a recurrence risk of approximately 25% to 30% (13 ,27 ,28 ,29). The recurrence risk is thought to increase with the number of successive losses. Other risk factors include tobacco and moderate alcohol use (30 ,31). Advanced maternal age also increases the likelihood of both sporadic and RPL. Even so, 70% of untreated couples with a history of RPL loss will have a live birth in a subsequent pregnancy. The risk of recurrent fetal death is less well understood. When one fetal death has occurred, the risk increases substantially, the magnitude depending on the gestational age at which it occurred (32 ,33). The risk increases 20-fold if fetal death occurs between 16 to 27 weeks but only fivefold if it occurs after 28 weeks' gestation (32).

Immunologic Recurrent Pregnancy Loss

The immunologic nature of otherwise unexplained RPL has generated considerable controversy in recent years. Both antibody-mediated and cellular-mediated mechanisms have been proposed. An association between systemic lupus erythematosus (SLE) and RPL is uncertain. In a recent retrospective study, women with SLE were about twice as likely to have spontaneous miscarriage or choose elective termination as controls (34). However, the authors noted that race and cultural background were more predictive of family size than the presence of SLE or even the number of miscarriages. To date, the only scientifically validated humoral cause for RPL remains antiphospholipid antibodies (APA), particularly the lupus anticoagulant (LA) and anticardiolipin antibodies (aCL).

An association between RPL and APA was first noticed in the latter third of the last century. The term antiphospholipid syndrome (APS) was introduced in 1986 (35) and formalized the association between RPL and APA as well as the association between APA and thrombotic events. The pathogenesis of pregnancy loss in women with APS has been ascribed to abnormal placental function, probably resulting from maldevelopment of the uteroplacental circulation. Extensive infarction, necrosis, and thrombosis have been identified in placentas from failed pregnancies in women with APA (36 ,37 ,38 ,39). A spiral arterial vasculopathy in decidual vessels also has been linked to APA-related fetal loss (40). It must be said, however, that the histologic abnormalities seen in APS cases are nonspecific.

Antiphospholipid Antibody Related Pregnancy Loss

The first investigators assumed that the hypercoagulability of APS was in some way having a negative impact on uteroplacental circulation, and numerous mechanisms have been subsequently considered. It is likely that vascular injury and/or endothelial cell activation (“second hit”) immediately precede the occurrence of thrombosis in patients with APS. The role of a second hit or cell activation step in APA-associated pregnancy loss or morbidity is uncertain. One proposed mechanism begins with activation or apoptosis of platelets and endothelial cells during which negatively charged phosphatidylserine migrates from the inner to the normally electrically neutral outer cell membrane. In the placenta, the migration of phosphatidylserine may occur during trophoblast syncytium formation. Circulating $ß_2$-glyoprotein I ($ß_2$GPI) then binds to phosphatidylserine. Antiphospholipid antibodies bind to the $ß_2$GPI dimer (41), activating complement and initiating a signaling cascade that induces cell surface tissue factor expression and adhesion molecules, causing platelets to aggregate and initiate thrombosis (42). An alternative, thrombotic hypothesis holds that APA competes in the placenta for phosphatidylserine with the natural anticoagulant placental anticoagulant protein I (annexin V) (43 ,44), possibly interrupting a shield that is thought to protect the fetus from maternal prothrombotic mechanisms (45 ,46). Others suggest that APA may operate through the signaling cascade. They inhibit production of placental prolactin, insulin growth factor $ß_1$, and the signal transducer and activator of transcription 5 (Stat5) (47), and they adversely affect formation of a trophoblast syncytium, placental apoptosis, and trophoblast invasion, all processes required for normal establishment of placental function.

In vitro, pathogenic APA induce adhesion molecule and tissue factor expression in endothelial cells and expression of GPIIb/IIIa on platelets (48) resulting in enhanced adherence of leukocytes to endothelium (49). In experimental animal models, APA cause fetal resorption (50 ,51) and the size and duration of trauma-induced venous and arterial thrombi (52 ,53). Inhibiting complement activation by a variety of mechanisms prevents experimental APA-induced fetal death (51); C5 knock-out mice carry pregnancies normally despite APA (54).

Pregnancy Loss Specific to Antiphospholipid Antibodies

Antiphospholipid antibodies are not frequently associated with sporadic pregnancy loss (55). Early pregnancy losses are more commonly due to chromosomal and other etiologies. The original description of APS included only women with fetal death, rather than earlier pregnancy loss. Subsequent series reported that positive tests for LA or IgG or IgM aCL are found in up to 20% of women with RPL (56 ,57 ,58 ,59 ,60 ,61); the majority of prospective treatment trials (62 ,63 ,64 ,65 ,66 ,67) have been composed mainly of healthy women with recurrent pre-embryonic and embryonic losses. The median rates of fetal death, preeclampsia, and preterm birth in these trials were relatively low, 4.5% (range, 0% to 15%), 10.5% (range, 0% to 15%), and 10.5% (range, 5% to 40%), respectively.

It should be noted that some investigators believe that women with APA identified in the setting of recurrent pre-embryonic and embryonic loss, without history of other clinical manifestations of APS, represent a different population from those identified because of thromboembolic disease, SLE, or adverse second or third trimester obstetric outcomes. Women with LA or medium-to-high positive IgG aCL have been shown to have losses more specific to the fetal period (68 ,69). In one study of 366 women with two or more consecutive pregnancy losses, investigators found that women with moderate-to-high levels of APA had significantly different pregnancy loss histories compared to women with low or absent levels (68). Although the overall rates of prior loss were similar in both groups (84%), 50% of the prior losses in women with moderate-to-high levels of APA were fetal deaths, compared to less than 15% in women without low or absent APA. In other prospective, large case series that included women with SLE, prior thrombosis, and other medical conditions, women with APS experienced high rates of preterm birth secondary to gestational hypertension-preeclampsia and uteroplacental insufficiency as manifested by fetal growth restriction, oligohydramnios, and nonreassuring fetal surveillance (70 ,71 ,72).

The etiology of APA related adverse pre-embryonic and embryonic outcomes (presenting as RPL) and later APA-related fetal complications are thought to be the same by some experts (73). The implication is that APA-mediated inflammation operates along the continuum of gestation to cause either pre-embryonic and embryonic or fetal damage. Other investigators question this theory, suggesting that women with recurrent pre-embryonic and embryonic losses and APA represent a largely different patient population than those who present with fetal death and other late pregnancy complications (74 ,75). The International Congress on Antiphospholipid Antibodies included both pre-embryonic-embryonic losses and the fetal-neonatal complications in their 1999 criteria, dividing them into three categories:

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
- One or more premature births of a morphologically normal neonate at or before the 34th week of gestation
- Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation

Clinicians and investigators were strongly encouraged to stratify patients according to these clinical and laboratory criteria.

Treatment of Antiphospholipid Syndrome during Pregnancy

Treatment strategies for women with APA in pregnancy have been designed to suppress the immune system (prednisone and intravenous immunoglobulin [IVIG]), prevent thrombosis (heparin and aspirin), and improve placental blood flow by decreasing the thromboxane-to-prostacyclin ratio (aspirin). In addition to decreasing the risk of thrombosis, the ideal treatment would also improve maternal and fetal-neonatal outcome by preventing pregnancy loss, preeclampsia, placental insufficiency, and preterm birth. High-dose prednisone (at least 40 mg per day) in combination with low-dose aspirin (80 mg per day) was initially used to treat APS in pregnancy, resulting in successful pregnancies in 60% to 70% of cases (37 ,38 ,76). However, heparin became the treatment of choice after publication of a small, randomized trial found that maternally administered heparin was as effective as prednisone in reducing the risk of pregnancy loss (62). In most case series and trials, daily low-dose aspirin is included in the treatment regimen (62 ,63 ,64 ,65 ,66 ,77 ,78). In a recent meta-analysis of treatment trials (78), the live birth rate was improved by 54%. Other pregnancy complications associated with APS occur in spite of appropriate treatment (71 ,79). In a recent observational study of 107 pregnancies complicated by APS, preeclampsia occurred in 20%, preterm birth in 24%, and growth restriction in 15% of treated women (79).

The safe and effective dose of heparin for pregnant women with APS is debated, but should probably depend on individual patient history (74). Most authorities recommend full, adjusted-dose heparin for women with a history of a thrombotic event (80 ,81). Table 54-1 presents suggested regimens. Patients in most published series also received low-dose aspirin, although the benefit of adding aspirin is unknown. The goal of adjusted dose anticoagulation therapy with unfractionated heparin is to maintain an activated partial thromboplastin time (aPTT) 1.5–2.5 times the

normal value. Antifactor Xa levels are followed in patients positive for LA and should fall between 0.4 to 0.7 U/mL. Low molecular weight heparin (LMWH) has been also been used safely in women with APS during pregnancy (82 ,83). Full anticoagulation with enoxaparin is usually achieved by administering 1 mg/kg twice daily, in two equal doses 12 hours apart. However, because of pregnancy induced alterations in the pharmakinetics of LMWH, intermittent monitoring of antifactor Xa levels are recommended to ensure adequate dosing. The target antifactor Xa level for full anticoagulation using LMWH is 0.5 to 1.1 U/mL. In most cases, heparin should not be used in combination with high-dose prednisone because of an increased risk of osteopenic fractures and lack of improvement in fetal outcome with combination therapy (71).

Table 54-1: Subcutaneous Heparin Regimens Used in the Treatment of Antiphospholipid Syndrome during Pregnancy

Prophylactic Regimens	
Indications	Recommended in women with no history of thrombotic events. Diagnosis of antiphospholipid syndrome based on recurrent pre-embryonic and embryonic loss or prior fetal death or early delivery because of severe preeclampsia or severe placental insufficiency
Unfractionated Heparin	7,500–10,000 U every 12 hours in the first trimester, 10,000 U every 12 hours in the second and third trimesters
Low Molecular Weight Heparin	(1) Enoxaparin 40 mg once daily or dalteparin 5,000 U once daily, OR (2) Enoxaparin 30 mg every 12 hours or dalteparin 5,000 U every 12 hours
Adjusted-Dose Anticoagulation Regimens	
Indications	Recommended in women with antiphospholipid syndrome with a history of thrombotic event(s).
Unfractionated Heparin	>7,500 U every 8–12 hours adjusted to maintain the mid-interval heparin levels* in the therapeutic range
Low Molecular Weight Heparin	(1) Enoxaparin 1 mg/kg every 12 hours or dalteparin 200 U/kg every 12 hours (2) Intermediate dose (e.g., enoxaparin 40 mg once daily or dalteparin 5,000 U once daily until 16 weeks' gestation and every 12 hours from 16 weeks' gestation onwards

*Heparin levels = antifactor Xa levels. Women without a lupus anticoagulant in whom the activated partial thromboplastin time is normal can be followed using the activated partial thromboplastin time.

Heparin is also considered the treatment of choice during pregnancy for women with APS and no history of a thrombotic event. Treatment is usually initiated in the early first trimester after ultrasonographic demonstration of a live embryo. There is no evidence to support the initiation of heparin prior to conception. The dose of heparin required for safe and effective treatment is debated and both low-dose prophylaxis and adjusted-dose anticoagulation regimens have been reported (78). Live rates have exceeded 70% using either strategy (62 ,64). Women with a history of fetal death alone may be at higher risk for thromboembolism during pregnancy (84) and should probably receive higher doses of heparin prophylaxis. It is our practice to treat such women with generous thromboprophylaxis (e.g., 15,000 to 20,000 units of standard heparin or 60 mg of enoxaparin in divided doses daily) (74).

Warfarin is a known teratogen and should be discontinued either prior to conception or by the fifth week of gestation (menstrual). Even so, some experts recommend the judicious use of warfarin anticoagulation in pregnancy for women with particularly egregious thrombotic histories, such those with recurrent thrombotic events or cerebral thrombotic events and those who have had a recurrent thrombosis while on heparin (74). Likewise, clopidogrel and newer antithrombotic agents are not cleared for use in pregnancy and should be considered only patients unable to use heparin in consultation with a maternal-fetal medicine specialist.

Pregnancy losses continue to occur in 20% to 30% of cases even when heparin prophylaxis is given (71 ,78 ,85). Several alternative therapies have been tried in so-called refractory cases. Glucocorticoids, often in high doses, have sometimes been added to regimens of heparin and low-dose aspirin. Although there are anecdotal reports of success, this practice has never been studied in appropriately designed trials and the combination of glucocorticoids and heparin may increase the risk of preeclampsia and osteoporotic fracture (62). IVIG is also sometimes been given during pregnancy to women with APS who have continued to have poor obstetric outcomes despite treatment with heparin (86). However, two randomized, controlled trials found no benefit to this expensive therapy compared to heparin and low-dose aspirin (87 ,88). Hydroxychloroquine has been shown to diminish the thrombogenic properties of APA in a murine thrombosis model (89). Past concerns about ocular damage or defects with in utero exposure have been put to rest by recent reports of hydroxychloroquine use in pregnant women with lupus (90). Nevertheless, there are few case reports and no controlled trials of APS patients being treated during pregnancy with hydroxychloroquine.

Healthy women with recurrent embryonic and pre-embyronic loss and low titers of aPL probably do not require treatment (91). The controlled trial of Pattison et al. included a majority of such women and found no difference in live birth rates using either low-dose aspirin or placebo (66).

Alloimmune Theories of Recurrent Pregnancy Loss

Perhaps no subject in the area of immunologic reproductive failure has generated more controversy than the idea that many cases are a result of a maternal alloimmune response to her fetus, semiallogenic, because of maternally and paternally inherited gene products and tissue-specific antigens. Several potential allogeneic factors have been suggested and extensively investigated. Early reports proposed that human leukocyte antigens (HLA) compatibility of couples, the absence of maternal leukocytotoxic antibodies, or the absence of maternal blocking antibodies were related to RPL. Defects in molecular immunosuppressive factors (cytokines and growth factors) at the local decidual/trophoblast level were initially implicated (92 ,93). Elevated levels of maternal systemic NK cells have also been associated with RPL (94 ,95 ,96). Recent evidence suggests that pregnancy survival depends on inhibition of local inflammatory mediators (97 ,98 ,99 ,100).

Though no clear alloimmune mechanism for RPL has to date been identified, several immunologic treatments are given in some centers to women with RPL. Most commonly this includes transfusion of paternal leukocytes (lymphocytes) prior to conception and/or passive immunization with IVIG during pregnancy. Paternal leukocyte immunization treatment is based on the salutary effect that third party leukocytes have on allograft rejection in transplant patients. There is also some evidence that it results in a decreased number of NK cells in RPL patients (101). The use of IVIG is based on the possibility that it may cause down regulation of systemic NK cells (101 ,102) and abrogation of their activity at the implantation site resulting in a lower likelihood of miscarriage (95 ,103). Both treatments have been extensively studied in randomized controlled trials, most of which found no benefit to either treatment (104 ,105 ,106 ,107 ,108). Only two of seven trials of leukocyte immunization have found a beneficial effect on live births in women with a history of RPL (109 ,110). Both were heavily criticized because of an unusually low live birth rate in women who received placebo. One trial of IVIG, which included women with only two prior losses, reported an improvement in live births in women with RPL (111). Several others, which limited inclusion to women with three or more prior losses, found no statistically significant difference in live birth rates in women who received IVIG compared to those who were given placebo (112 ,113 ,114 ,115 ,116). Although there continues to be considerable debate regarding the efficacy of both leukocyte immunization and IVIG for the treatment of RPL, their use should currently be considered experimental and limited to research protocols.

Infertility

Background

Infertility is traditionally defined as 1 year of unprotected coitus without conception. Approximately 10% to 15% of reproductive age couples are affected (117). Causal factors are traditionally broken down into several categories including tubal and pelvic pathology, male factor, and ovulatory dysfunction. Infertility remains unexplained in 10% to 20% of affected couples who undergo evaluation (118).

Advancing maternal age probably contributes to unexplained infertility. Based on studies in a stable population of Hutterite men and women not using contraception, Tietze found that 33% of women were infertile at age 40, and 87% were infertile at age 45 (119). This may play a role in the observed rise in the rate of infertility in the United States where a steady increase in births among women aged 35 to 44 has been observed over the last several years (120 ,121).

Treatment of Infertility

Assisted reproductive technologies (ART) describe several treatments given to infertile couples attempting to conceive. Ovulation induction (OI) or stimulation begins with administration of gonadotropin-releasing hormone (GnRH) analogues to bring about desensitization of the pituitary gonadotropes to endogenous GnRH. Human menopausal gonadotropin is given to stimulate ovarian follicle maturation, followed by human chorionic gonadotropin (hcG), which is given to induce ovulation. The luteal phase is maintained by low doses of hCG and/or intramuscular injections of progesterone. The most promising oocytes, usually five, are retrieved using transvaginal ultrasound guidance. Ovulation induction is not without risks. Estrogen production must be monitored carefully as overproduction can lead to hyperstimulation syndrome, characterized by pain, markedly enlarged ovaries, capillary leak syndrome, fluid retention, and cytokine release syndrome. Hypertension and renal failure may occur in the most severe cases.

In vitro fertilization-embryo transfer (IVF-ET) refers to a number of techniques in which sperm and oocytes are manipulated to achieve fertilization, with the intent to place a growing zygote within the uterine cavity to establish a pregnancy. Table 54-2 describes other less common ART.

Immunologic Mechanisms of Female Infertility

Whether or not autoimmunity is responsible for primary infertility or an increased risk of failed ART is a matter of great debate. Antisperm antibodies, killer T cells, and a variety of more narrowly specified lymphocyte subsets, antileukocyte antibodies, antiphospholipid antibodies, and other immunologic aberrations have all been suggested as the cause of primary infertility and failure of IVF-ET. Aberrations in the immune system could theoretically lead

to premature ovarian failure (POF) and subsequent infertility. Because the ovary is not an immunologically privileged site, the immune system is free to attack its stromal, epithelial, and germ cell components. However, evaluations of an immune mechanism relating to infertility are severely hampered by the lack of adequately validated antiovarian antibody assays. Attempts to diagnose female immune infertility have relied on assays for nonspecific autoantibodies and other markers of immune system activation or dysregulation. In the end, autoimmunity probably plays only minor role in POF (~20%). Very rare autoimmune polyglandular syndromes, also characterized by idiopathic Addison disease, are found in about 3% of women with POF.

Table 54-2: Assisted Reproductive Technologies (ART)

Technology	Description
In vitro fertilization (IVF)	Laboratory culture of aspirated oocytes and spermatozoa followed by transcervical embryo transfer (ET)
Gamete intrafallopian transfer (GIFT)	Direct placement of aspirated oocytes and spermatozoa into fallopian tubes
Controlled ovarian hyperstimulation (COH)	Ovulation induction with monitoring in normal ovulatory women; intent is to induce multiple ovarian follicles
Intrauterine insemination (IUI) intracervical insemination (ICI) intratubal insemination (ITI)	Separation of spermatozoa from seminal fluid with suspension in buffer or culture media and insemination into the uterus, cervix, or fallopian tube
Zygote intrafallopian transfer (ZIFT)	Laboratory culture of aspirated oocytes with spermatozoa followed by direct placement of fertilized zygotes or embryos into fallopian tubes
Oocyte donation (OD)	Laboratory culture of aspirated oocytes from a donor woman followed by sperm/oocytes culture and transfer
Intracytoplasmic spermatozoa injection (ICSI)	Micromanipulation technique in which a single sperm is injected into an oocyte. It is used in cases of abnormal spermatozoal function or very low sperm counts

It has been hypothesized that the same immunologic mechanisms responsible for some cases of RPL could also affect earlier, unrecognized pregnancies as well, leading to unexplained infertility. Antibodies directed toward gametes or other critical components could theoretically inhibit fertilization, impair early embryo development, or hinder implantation or postimplantation/placental development. However, no disease-specific autoantibodies that adversely affect fertility have been convincingly identified.

SLE and Infertility

Fertility in patients with SLE (SLE) is thought to be equivalent to that of the general population (122). Likewise, disease activity does not appear to affect fertility (123 ,124). Some immunosuppressive treatments appear to have an adverse effect on fertility. Cyclophosphamide pulse therapy has been associated with POF in women undergoing treatment for lupus nephritis (125 ,126 ,127). Ranging from 12% to 62%, the incidence appears to be related to route of delivery, dosage and patient age at the time of therapy (128 ,129 ,130 ,131). Long-term nonsteroidal anti-inflammatory drugs (NSAIDs) have also been shown to interfere with ovulation (132 ,133). Successful pregnancy has been reported after withdrawal of NSAID therapy (134).

The increased serum estrogen concentrations that result from ovarian stimulation in preparation for ART have been associated with increased SLE disease activity (135 ,136), as well as with the onset of SLE in women who were previously healthy (137 ,138). In one retrospective series of ART in rheumatic disease pregnancies (139), 6/19 women suffered complications, regardless of whether or not ART was successful. Three patients experienced worsening of the primary rheumatic disease and three others suffered complications of the procedure itself.

The pregnancies that result from ART are in and of themselves complicated, in large part because many are multiple gestation pregnancies.

ART in autoimmune disease patients are not trivial. Infertile women with any type of rheumatic disease should be informed of risks prior to undertaking any form of ART. If competently done in carefully selected patients, ART do not appear to add substantial risk, nor are major disease specific complications apparent. However, multiple gestation pregnancies risk additional harm to the mother. No ART procedure should be performed in women with active or unstable disease.

Antiphospholipid Syndrome and Infertility

Antiphospholipid antibodies have been studied extensively in women with otherwise unexplained infertility. Proposed mechanisms by which these antibodies might affect fertility or IVF success include abnormal implantation, placentation, and embryonic vascular compromise. Laboratories across the globe disagree whether APAs (as well as other immunologic anomalies) contribute to infertility (140). Even so, it is not uncommon for reproductive failure clinics to perform so-called APA panels on women with

unexplained infertility and those contemplating IVF (141 ,142). In addition to LA and aCL, large APA panels typically include several other APA types, including antiphosphatidylserine, antiphosphatidyl choline, antiphosphatidylglycerol, antiphosphatidylethanolamine, and antiphos-phatidyl inositol. Based on the results of retrospective studies showing an increased prevalence of aCL in women with unexplained infertility (143 ,144 ,145), immunomodulating treatments and anticoagulation have been given to women who test positive for one or more types of antibodies in the APA panels. However, a meta-analysis of data from seven studies and more than 2,000 subjects undergoing IVF-ET found no statistically significant associations between the presence of APA and the clinical outcomes of IVF-ET, including clinical pregnancies and live births (146).

Some centers offer immunosuppressive and anticoagulation treatment to all APA-positive women undergoing IVF-ET (147 ,148). However, an association between APA status and infertility in well-designed prospective trials has not been confirmed (149 ,150 ,151). In a randomized, double-blinded, controlled trial, Stem et al. reported no difference in implantation rates between women attempting IVF-ET whether they were treated with aspirin plus heparin (8.9%) or placebo (9%) (149). In the most recent randomized double-blinded, placebo-controlled trial of heparin and aspirin for women undergoing IVF-ET with a history of prior IVF-ET failure, Stern et al. (150) reported no differences in pregnancy or implantation rates in 300 embryo transfers in 143 women, regardless of whether or not they received heparin and aspirin or placebo. Although other investigators have reported improved outcomes with heparin treatment in APA-positive women undergoing IVF-ET, none of their trials were randomized and only a few were controlled (Table 54-3).

Table 54-3: Empiric Treatment for Presumed Antibody-mediated Infertility

Trial	APA positive subjects treated	Treatment	Treatment Criteria	Outcome
Birkenfield et al., 1994 (153)	15	Aspirin/prednisone IVF	≥1 of 4 antibodies positive Prior IVF-ET failure	7/15 (46.6%) pregnancies, no differences from prior untreated cycles for # of ova or embryos
Sher et al., 1994 (147)	169	Heparin/aspirin IVF	≥1 of 18 APA positive organic pelvic pathology	82/169 (49%) clinical pregnancies
Schenk et al., 1996 (154)	35	Heparin/aspirin IVF	≥2 of 12 APA assays positive; unselected IVF-ET candidates	10/35 (51.4%) clinical pregnancies in seropositive/treated vs. 12/40 (30%) clinical pregnancies in seronegative/untreated [NS]. Implantation rates 28/140 (20%) in seropositive/treated vs. 9.7% in seronegative/untreated (p = 0.014)
Kutteh et al., 1997 (151)	19	Heparin/aspirin IVF	≥1 of 3 assays for ACL isotypes positive, no history of RPL	10/19 (52.6%) clinical pregnancies seropositive/treated 8/19 (42.1%) clinical pregnancies seropositive
Sher et al., 1998 (152)	52	Heparin/aspirin IVF	≥4 IVF failures	42% APA positive and 19% APA negative delivered (p = 0.02)
Geva et al., 2000 (155)	52	Aspirin/prednisone IVF	≥1 of 4 autoantibody or rheumatoid factor positive and ≥1 IVF-ET failure	17/52 (32.7%) clinical pregnancy rate, uncontrolled
Stern et al., 2003 (150)	143	Heparin/aspirin IVF	≥1 APA, ANA, and ≥10 prior IVF-ET failure	Implantation rates 20/296 (6.8%) for treated cycles and 22/259 (8.5%) for placebo cycles

Based on the available evidence, there is little on which to base the performance of APA panel testing in couples with otherwise unexplained infertility. There is even less justification to prescribe potentially dangerous immunomodulating

and anticoagulation treatment regimens to infertile women who do not meet the criteria for bona fide APS. Indeed, a pregnancy-related death associated with heparin and aspirin treatment for infertility was reported by the Centers for Disease Control (CDC) in 1996.

Subclinical Autoimmunity and Reproductive Failure

A role for autoimmunity in otherwise unexplained, primary infertility remains far from certain (156). Not withstanding, so-called reproductive immunology clinics offer a vast array of therapies said to improve the likelihood of IVF-ET success. Couples desperate to have children are drawn to these centers where they are subjected to a staggering number of assays for autoantibodies (157), the results of which are used to prescribe an equally impressive number of expensive and sometimes dangerous immunomodulating treatments. In truth, no adequately controlled trials of these therapies have been conducted in couples attempting ART. The use of desensitization, immunosuppression, anticoagulation, intravenous immunoglobulin, anti-TNF medications and other forms of immunomodulation is justified by only by reports of successful implantation in small case series and anecdotal reports.

Interventions of the past underline the need for caution in the use of unproven therapies during IVF-ET and in early pregnancy. One needs only to point to the tragedy of diethylstilboestrol (DES) in the last century. Based on the findings of one uncontrolled trial (158), DES was routinely prescribed to pregnant women thought to be at risk for miscarriage. Reports of an increased risk of cancer in laboratory animals (159) and the findings of a placebo-controlled trial showing no preventive effect on miscarriage and other pregnancy complications did little to dissuade physicians and patients from using DES (160). It was not until 1971 that a red flag was raised after Herbst et al. published the first case-control study showing that maternal use of DES could result in cancer and reproductive abnormalities in their female offspring 20 years after exposure (161).

Corticosteroid therapy is known to reduce the percentage of peripheral blood NK cells (162) and has been reported to improve pregnancy rates in women undergoing IVF-ET (153 ,155 ,163). Other investigators of have found no beneficial effect of corticosteroid therapy on implantation or pregnancy rates in women undergoing IVF-ET (164 ,165 ,166). A meta-analysis of treatments in APA positive women found no beneficial effect but reported a higher risk of pregnancy complications compared to women receiving placebo (78). Although necessary to control disease activity in women with unstable autoimmune disease during pregnancy, the use of corticosteroids for healthy women undergoing IVF-ET seems unnecessarily dangerous.

There is little to no evidence to support the use of other immunomodulation treatments in healthy women undergoing IVF-ET.

After a review of the available evidence, expert committees have found no evidence that autoimmunity is a cause of primary infertility (167 ,168). Moreover, unbiased authorities recommend that that the use immunomodulation therapies be restricted to research protocols under carefully controlled conditions.

Summary

- Immunotolerance during pregnancy is a poorly understood local phenomenon at the maternal-fetal interface.
- Most pregnancy loss is a sporadic, isolated event in the reproductive life of women with other successful pregnancies.
- Recurrent pregnancy loss is estimated to occur in 0.5% to 1% of couples.
- The etiology of recurrent pregnancy loss is found in 50% of couples undergoing evaluation.
- Most pregnancy loss occurs during the pre-embryonic-embryonic period (<10 weeks of gestation).
- Recurrent pregnancy loss does not appear to be more common among women with systemic lupus erythematosus.
- Antiphospholipid antibodies, particularly the lupus anticoagulant and IgG anticardiolipin antibodies, remain the only scientifically validated cause of recurrent pregnancy loss.
- Patients with antiphospholipid antibodies identified because of recurrent early pregnancy loss probably represent a different population than those identified because of fetal death or thrombosis.
- Heparin (usually with low-dose aspirin) is the treatment of choice for antiphospholipid syndrome during pregnancy.
- No clear mechanism for alloimmune recurrent pregnancy loss has been identified.
- There is no evidence to support the use of any immunomodulating treatments in women with so-called alloimmune recurrent pregnancy loss.
- Infertility is defined as 1 year of unprotected coitus without conception.
- Ten percent to 15% of couples attempting pregnancy are infertile.
- Assisted reproductive technologies (ART) describe several treatment regimens given to infertile couples attempting to conceive.
- Fertility in patients with systemic lupus erythematosus appears to be equivalent to that of the general population.
- Increased serum estrogen levels during ovulation induction may result in lupus flare.
- ART should not be performed in women with active or unstable systemic lupus erythematosus.
- Antiphospholipid antibodies are probably not associated with infertility or failure of IVF-ET.
- There is no evidence to support the use of immunomodulating treatments in healthy women undergoing IVF-ET.

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Chapter 55

Lupus Nephritis: Pathology and Pathogenesis

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Introduction

Lupus nephritis (LN) contributes significantly to the morbidity and mortality of SLE patients (1 ,2 ,3). This is in part because of the renal disease itself, and in part because of the side effects of therapies directed to the renal manifestations. Recent studies have clarified the major histologic patterns of renal involvement as well as differences in their clinical presentations and outcomes. The use of the World Health Organization (WHO) classification of LN, supplemented by activity and chronicity indices, has become the "gold standard" to gauge prognosis and determine appropriate therapy. A revised classification system for lupus nephritis, the ISN/RPS "WHO Classification Revisited" published in 2004, has replaced older classifications (4 ,5). With appropriate validation and increasing acceptance, this classification should provide a more reproducible system for investigators from different centers to standardize pathologic interpretation and to determine optimal therapy and study outcomes.

Histopathologic Classifications of Lupus Nephritis (LN)

LN is extremely pleomorphic. All four renal compartments, the glomeruli, tubules, interstitium, and blood vessels, may be affected. Adjacent glomeruli from a single biopsy may show variable involvement, as may the biopsies from patients with similar clinical manifestations. Over time glomerular lesions may transform from one pattern to another. Through the years, investigators have sought to define and quantify the many morphologic lesions of lupus nephritis in a comprehensive, systematic fashion. The earliest classifications of renal involvement in SLE patients divided glomerular changes only into mild forms (lupus glomerulitis), severe proliferative forms (active lupus glomerulonephritis) and membranous glomerulopathy (6 ,7).

Three major classification systems have been proposed over the last three decades. The original WHO classification was formulated in 1974 and recognized five major classes of lupus nephritis (Table 55-1) (8 ,9). In 1982, a modified WHO classification was promulgated by the International Study of Kidney Disease in Children (ISKDC), with further revisions in 1995 (10 ,11) (Table 55-2). It defines six major classes of lupus nephritis and a large number of subclasses with emphasis on distribution, activity, and chronicity of the lesions. Although it is much more detailed and precise than the original WHO classification, it has not been as widely accepted because of its greater complexity with excessive reliance on subclasses. Moreover, its treatment of mixed or overlapping classes of lupus nephritis has been controversial. A third classification proposed in 2004 by a consensus conference organized jointly by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) retains the simplicity of the original WHO classification, while incorporating some of the refinements introduced by the modified WHO classification (4 ,5) (Table 55-3). The ISN/RPS classification has the advantage of standardizing pathologic criteria and defining more precisely the distinctions between the classes.

All three classifications are based entirely on evaluation of glomerular alterations. Although tubular, interstitial, and vascular lesions are common in lupus nephritis and may contribute significantly to overall disease severity, activity, and chronicity, they are not factored into these classifications. Accurate classification requires careful assessment of the glomerular alterations by light microscopy, followed by integration of the immunofluorescence and electron microscopic findings. The first step is to determine whether there is glomerular hypercellularity in the mesangial, endocapillary, or extracapillary zones. The distribution of hypercellularity (focal: <50% of glomeruli; diffuse: ≥50% of glomeruli affected) is assessed. Attention is given to the presence of infiltrating leukocytes, necrotizing lesions, and glomerular basement membrane thickening. These light microscopic findings are then interpreted in the context of the distribution of the glomerular immune deposits in mesangial, subendothelial, and subepithelial locations as detected by light microscopy, immunofluorescence and electron microscopy (Table 55-4). Immunofluorescence and electron microscopy are sensitive techniques for the detection of immune deposits. Since some lesions are focal, a proper classification depends on the adequacy of the glomerular sampling by the three modalities.

Table 55-1: Original WHO Classification of Lupus Nephritis (1974)

Class I	Normal glomeruli (by LM, IF, EM)
Class II	Purely mesangial disease a. Normocellular mesangium by LM but mesangial deposits by IF and/or EM b. Mesangial hypercellularity with mesangial deposits by IF and/or EM
Class III	Focal segmental proliferative glomerulonephritis (<50%)
Class IV	Diffuse proliferative glomerulonephritis (≥50%)
Class V	Membranous glomerulonephritis

Several recent studies have evaluated the reproducibility and validity of the new ISN/RPS classification versus the older WHO classification of lupus nephritis. A Japanese study reviewed the renal biopsies of 60 LN patients with a mean follow-up of 187 months (12). When compared with the modified WHO classification, the ISN/RPS classification gave higher consensus on pathologic interpretation (98% vs. 83%) and proved valuable in predicting prognosis. Likewise, a recent analysis of 420 Chinese patients found the ISN/RPS classification to be more reproducible than the modified WHO classification (96% vs. 87%) (13). Additionally, recent studies have already tested the validity of the subdivision of ISN class IV into diffuse segmental and diffuse global subtypes (14 ,15).

Table 55-2: Modified WHO Classification of Lupus Nephritis (1982)

Class I	a. Normal glomeruli (by LM, IF, EM) b. Normal glomeruli by LM but deposits seen by IF and/or EM
Class II	Purely mesangial alterations (Mesangiopathy) a. Mesangial widening and/or mild hypercellularity (+) b. Moderate hypercellularity (++)
Class III	Focal segmental glomerulonephritis (associated with mild or moderate mesangial alterations) a. With active necrotizing lesions b. With active and sclerosing lesions c. With sclerosing lesions
Class IV	Diffuse glomerulonephritis (severe mesangial, endocapillary or mesangiocapillary proliferation, and/or extensive subendothelial deposits). Mesangial deposits are present invariably and subepithelial deposits often, and may be numerous. a. Without segmental lesions b. With active necrotizing lesions c. With active and sclerosing lesions d. With sclerosing lesions
Class V	Membranous glomerulonephritis a. Pure membranous glomerulonephritis b. Associated with lesions of category II (a or b) c. *Associated with lesions of category III (a, b, or c) d. *Associated with lesions of category IV (a,b,c, or d)
Class VI	Advanced sclerosing glomerulonephritis

*Deleted from the 1995 Modified WHO Classification

Pathologic Features of Lupus Nephritis According to the ISN/RPS Classification

Class I (Minimal Mesangial Lupus Nephritis)

Class I denotes normal glomeruli by light microscopy with mesangial immune deposits detected by immunofluorescence and/or electron microscopy. The original WHO class I, defined as an entirely normal renal biopsy, was rarely if ever encountered because such patients typically have no clinical renal abnormalities and are not subjected to renal biopsy. Therefore, this “normal” category was eliminated from the ISN/RPS classification. By light microscopy the glomeruli are normocellular (Fig. 55-1). By immunofluorescence, immune deposits are limited to the mesangium (Fig. 55-2). The mesangial deposits tend to be small and vary from segmental to global in distribution. By electron microscopy corresponding electron dense deposits are present in the mesangium, without involvement of the peripheral glomerular capillary walls.

Class II (Mesangial Proliferative Lupus Nephritis)

Class II is defined as pure mesangial hypercellularity of any degree and/or mesangial matrix expansion by LM with mesangial immune deposits. Mesangial hypercellularity is defined as more than or equal to 3 mesangial cells in mesangial areas away from the vascular pole, assessed in 3 micron thick histologic sections. The mesangial proliferation is usually mild to moderate and does not compromise the glomerular capillary lumens (Fig. 55-3). By immunofluorescence, there are granular mesangial immune deposits. The pattern by immunofluorescence outlines the axial framework of the glomerulus, corresponding to the mesangial stalk (Fig. 55-4). By electron microscopy there are electron dense deposits within the mesangial matrix (Fig. 55-5). Strictly speaking, pure class II lupus nephritis should have no detectable subendothelial or subepithelial deposits. However, in practice, some cases of purely mesangial proliferative lupus nephritis will manifest rare small subendothelial electron dense deposits, particularly extending out from the adjacent mesangium. Cases of lupus nephritis with severe, but purely mesangial hypercellularity, without obliteration of the capillary lumina, may pose difficulties in classification. If the immune deposits are limited to the mesangium by immunofluorescence and electron microscopy, even cases of severe diffuse mesangial

proliferation should be classified as class II. If significant subendothelial deposits are present by immunofluorescence or by electron microscopy, or if they are visible by light microscopy, the case should be classified as focal proliferative (class III) or diffuse proliferative (class IV) depending on their distribution.

Table 55-3: ISN/RPS Classification of Lupus Nephritis (LN) (2004)

Class I	Minimal mesangial LN Normal glomeruli by LM, but mesangial immune deposits by IF
Class II	Mesangial proliferative LN Purely mesangial hypercellularity of any degree or mesangial matrix expansion by LM, with mesangial immune deposits. There may be a few isolated subepithelial or subendothelial deposits visible by IF or EM, but not by LM.
Class III	Focal LN* Active or inactive focal, segmental and/or global endo- and/or extracapillary GN involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations. III (A): Purely active lesions: focal proliferative LN III (A/C): Active and chronic lesions: focal proliferative and sclerosing LN III (C): Chronic inactive with glomerular scars: focal sclerosing LN
Class IV	Diffuse LN* Active or inactive diffuse, segmental and/or global endo- and/or extracapillary GN involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is divided into diffuse segmental (IV-S) when >50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) when >50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. IV-S (A) or IV-G (A): Purely active lesions: diffuse segmental or global proliferative LN IV-S (A/C) or IV-G (A/C): Active and chronic lesions: diffuse segmental or global proliferative and sclerosing LN IV-S (C) or IV-G (C): inactive with glomerular scars: diffuse segmental or global sclerosing LN
Class V	Membranous LN† Global or segmental subepithelial immune deposits or their morphologic sequelae by LM and by IF or EM, with or without mesangial alterations
Class VI	Advanced sclerosing LN ≥90% of glomeruli globally sclerosed without residual activity

Definitions of Pathologic Terms

Diffuse: a lesion involving most (≥50%) glomeruli.

Focal: a lesion involving <50% of glomeruli.

Global: a lesion involving more than half of the glomerular tuft.

Segmental: a lesion involving less than half of the glomerular tuft.

Mesangial hypercellularity: ≥3 mesangial cells per mesangial region in a 3 μ-thick section.

Endocapillary proliferation: endocapillary hypercellularity due to increased number of mesangial cells, endothelial cells and infiltrating monocytes, and causing narrowing of the glomerular capillary lumina.

Extracapillary proliferation or cellular crescent: extracapillary cell proliferation of more than two cell layers occupying one fourth or more of the glomerular capsular circumference.

Karyorrhexis: presence of apoptotic, pyknotic and fragmented nuclei.

Necrosis: a lesion characterized by fragmentation of nuclei or disruption of the basement membrane, often associated with the presence of fibrin-rich material.

Hyaline thrombi: intracapillary eosinophilic material of a homogeneous consistency which by immunofluorescence has been shown to consist of immune deposits.

Proportion of involved glomeruli indicates the percentage of total glomeruli affected by lupus nephritis, but excluding ischemic glomeruli with inadequate perfusion due to vascular pathology separate from LGN.

Combination of class III and class V requires membranous involvement of at least 50% of the glomerular capillary surface area of at least 50% of glomeruli by LM or IF.

Combination of class IV and class V requires membranous involvement of at least 50% of the glomerular capillary surface area of at least 50% of glomeruli by LM or IF.

In the report, active lesions have to be specified; the percentage of glomeruli with capillary wall disruption (necrosis) and crescents should be included in the diagnostic line.

*Indicate the proportion of glomeruli with active and with sclerotic lesions

Indicate the proportion of glomeruli with fibrinoid necrosis and with cellular crescents

Indicate and grade (mild, moderate, severe) tubular atrophy, interstitial inflammation and fibrosis, severity of arteriosclerosis or other vascular lesions.

†May occur in combination with III or IV in which case both will be diagnosed; may show advanced sclerosis

Table 55-4: Integration of LM, IF, and EM Findings by WHO Class

	Light Microscopy		Immunofluorescence		Electron Microscopy		
CLASS	MES	PCW	MES	PCW	MES	SENDO	SEPI
I	0	0	+	0	+	0	0
II	+	0	+	0	+	0	0
III	+	+	++	+	++	+	+/-
IV	++	++	++	++	++	++	+/-
V	+	++	+	++	+	+/-	++

EM, electron microscopic location of deposits; IF, immunofluorescence positivity; LM, light microscopic abnormalities; MES, mesangial; PCW, peripheral capillary wall; SENDO, subendothelial; SEP, subepithelial.

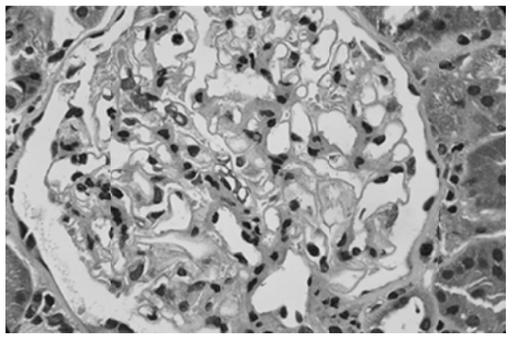

Figure 55-1. (See color plate.) Lupus Nephritis Class I. The glomerular tuft is normocellular with patent capillaries and glomerular basement membranes of normal thickness (Hematoxylin and eosin, × 400).

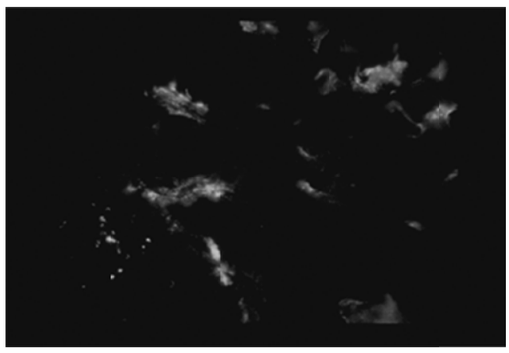

Figure 55-2. (See color plate.) Lupus Nephritis Class I. Immunofluorescence reveals delicate mesangial deposits of IgG. No deposits are identified involving the peripheral capillary walls. × 500).

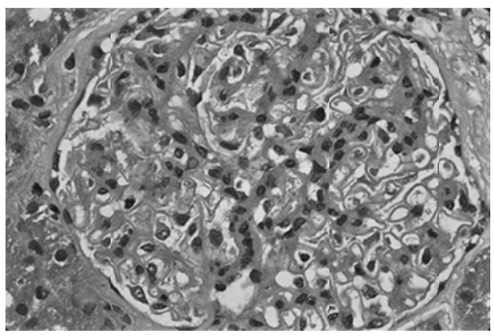

Figure 55-3. (See color plate.) Lupus Nephritis Class II. There is mild global mesangial hypercellularity. (H&E, × 400).

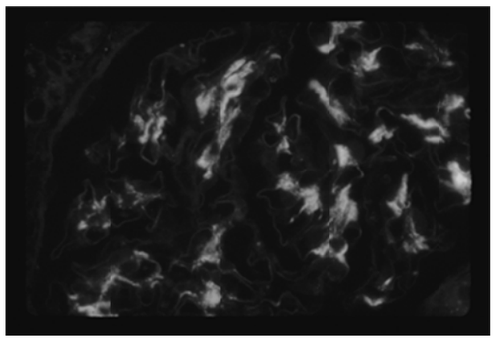

Figure 55-4. (See color plate.) Lupus Nephritis Class II. Immunofluorescence reveals global deposits of IgG outlining the mesangial stalk, with sparing of the peripheral capillary loops. (× 400).

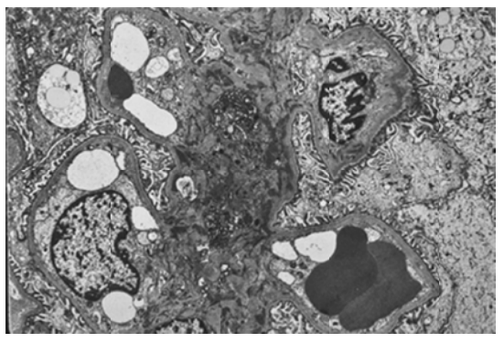

Figure 55-5. (See color plate.) Lupus Nephritis Class II. Electron microscopy shows electron dense deposits within the mesangial matrix, between adjacent capillaries. No deposits are detected involving the peripheral capillary walls (× 2,000).

Class III (Focal Lupus Nephritis) and Class IV (Diffuse Lupus Nephritis)

Most investigators consider class III and class IV lupus nephritis to be qualitatively similar glomerular lesions that differ only in severity and distribution. Therefore, these two related classes will be described together. Class III lupus nephritis is defined as focal segmental and/or global endocapillary and/or extracapillary glomerulonephritis affecting less than 50% of the total glomeruli sampled (Fig. 55-6). Class IV is defined as diffuse segmental and/or global endocapillary and/or extracapillary glomerulonephritis affecting more than or equal to 50% of glomeruli (Fig. 55-7). Both class III and class IV manifest subendothelial immune deposits (relatively focal in class III and diffuse in class IV), with or without mesangial alterations. The ISN/RPS Classification subdivides lupus nephritis class IV into those cases with diffuse segmental versus diffuse global proliferation (Table 55-3). The designation IV-S is used if more than 50% of affected glomeruli have segmental lesions (Fig. 55-8); the designation IV-G is used if more than 50% of affected glomeruli have global lesions. This subdivision was proposed to facilitate future studies addressing possible differences in outcome and pathogenesis between these subgroups.

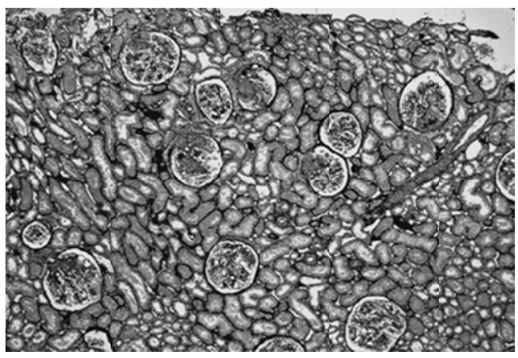

Figure 55-6. (See color plate.) Lupus Nephritis Class III. On low power examination, there is focal and segmental proliferation of the glomeruli. Overall, endocapillary or extracapillary proliferation affected <50% of the total glomeruli in this biopsy, qualifying as class III. (Jones methenamine silver, × 4).

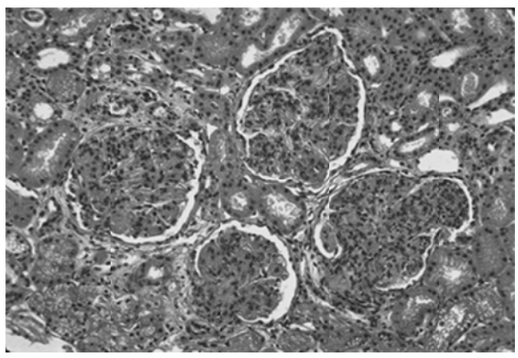

Figure 55-7. (See color plate.) Lupus Nephritis Class IV-G. A pattern of diffuse and global endocapillary proliferation is present. All four glomeruli illustrated show similar degree of glomerular involvement. (H&E, × 80).

Both class III and class IV may have active (proliferative) and/or inactive (sclerosing) lesions. In determining the percentage of total glomeruli affected by glomerulonephritis, both the proliferative and sclerosing lesions must be taken into account. Although most active glomerular lesions are endocapillary proliferative in nature,

both class III and class IV factor in glomerular lesions that are membranoproliferative, extracapillary proliferative, or consist of wire-loop deposits without associated proliferation. For these reasons, the ISN classification prefers the broader terms “focal lupus nephritis” and “diffuse lupus nephritis” over the more restrictive terms “focal proliferative lupus nephritis” and “diffuse proliferative lupus nephritis” used in the original WHO classification.

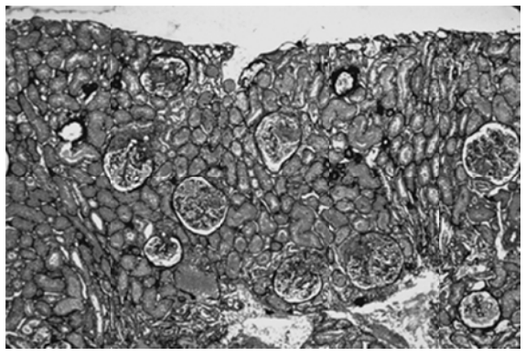

Figure 55-8. (See color plate.) Lupus Nephritis Class IV-S. Low power examination of the biopsy shows a pattern of diffuse glomerular proliferation involving >50% of glomeruli with a predominantly segmental distribution, involving a portion of each glomerular tuft (Jones methenamine silver, × 4).

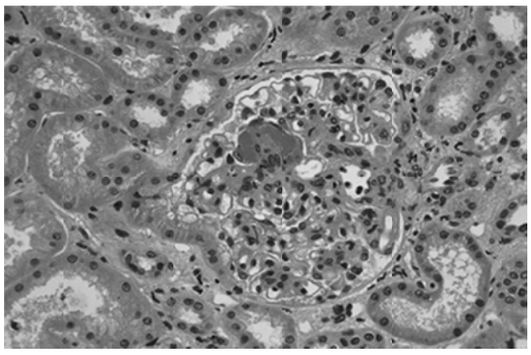

Figure 55-9. (See color plate.) Lupus Nephritis Class III. There is segmental glomerular necrosis with deposition of eosinophilic fibrinoid material, leukocyte infiltration and pyknosis (H&E, × 200).

The endocapillary proliferative lesions in class III tend to be relatively segmental (involving only a portion of the glomerular tuft), although some glomeruli may be affected globally (Fig. 55-9). In class IV, the endocapillary proliferation is typically more diffuse and global (Fig. 55-10). However, some examples of class IV have a diffuse and segmental distribution (designated IV-S in the ISN/RPS classification). The glomerular lesions in class III and IV are qualitatively similar. Common light microscopic features include wire-loop deposits, hyaline thrombi, leukocyte infiltration, necrosis, hematoxylin bodies, cellular crescents, and glomerular scarring, each of which is described below.

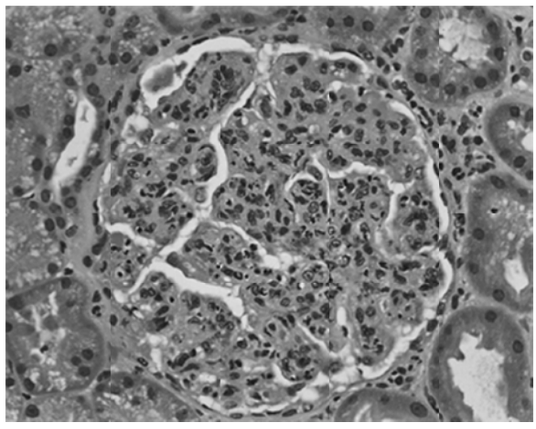

Figure 55-10. (See color plate.) Lupus Nephritis Class IV. A representative glomerulus displays global endocapillary proliferation including many infiltrating neutrophils, causing occlusion of the capillary lumina. (H&E, × 320)

In class III and class IV lupus nephritis, subendothelial immune deposits may be large enough to detect by light microscopy, forming “wire-loop” thickenings of the glomerular capillary walls (Fig. 55-11). Special stains reveal the deposits to be entirely or largely subendothelial, with preservation of an outer peripheral layer of glomerular basement membrane. In some cases, the subendothelial deposits are incorporated into the glomerular capillary wall by a subendothelial layer of neomembrane, producing a double contour. This may be accompanied by mesangial interposition giving a membranoproliferative appearance. Some cases of class III or class IV manifest large intracapillary deposits forming “hyaline thrombi” (Fig. 55-11). This term is actually a misnomer because these do not represent true fibrin thrombi but massive intracapillary immune deposits with the same composition by immunofluorescence as the neighboring subendothelial immune deposits.

In most cases of class III and class IV lupus nephritis, the endocapillary hypercellularity results from proliferation of glomerular endothelial and mesangial cells, as well as by leukocyte infiltration, including neutrophils, monocytes, and lymphocytes. However, several morphologic variants of class IV lack the typical picture of florid endocapillary proliferation with leukocyte infiltration. The first is the membranoproliferative variant. In this form, the endocapillary proliferation has a distinctly membranoproliferative aspect, with extensive mesangial interposition and duplication of glomerular basement membranes resembling membranoproliferative glomerulonephritis type 1. Other histologic variations including diffuse wire-loop deposits without glomerular hypercellularity or diffuse wire-loop deposits accompanied by mesangial proliferation. In each of these histologic variants, the sine qua non of active class IV is the presence of diffuse

subendothelial deposits, albeit with variable patterns of glomerular proliferation.

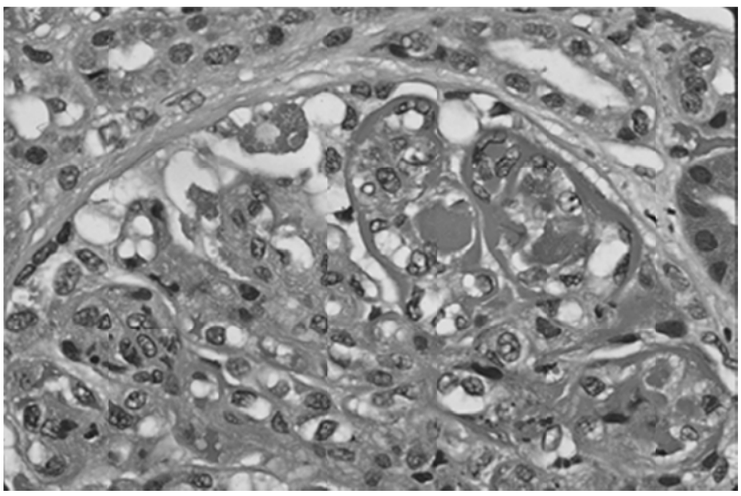

Figure 55-11. (See color plate.) Lupus Nephritis Class IV. A wire-loop thickening of the glomerular capillary wall is caused by a large subendothelial deposit. An adjacent glomerular capillary is occluded by an intraluminal immune deposit of similar composition, forming a "hyaline thrombus." (H&E, × 600).

Glomerular necrosis is a feature of active class III and class IV lupus nephritis and consists of foci of smudgy fibrinoid degeneration of the glomerular tuft. Necrosis may be accompanied by deposition of intracapillary fibrin, glomerular basement membrane rupture, and apoptosis of infiltrating neutrophils producing pyknotic or karyorrhectic nuclear debris ("nuclear dust"). Necrotizing lesions are typically segmental in distribution, but more than one glomerular lobule may be affected, particularly in diffuse proliferative lupus nephritis.

Hematoxylin bodies are the only truly pathognomonic lesion of lupus nephritis. However, they are extremely uncommon, affecting less than 2% of biopsy specimens of lupus nephritis (16). They consist of smudgy lilac-staining structures that may be smaller or larger than normal nuclei (17) (Fig. 55-12). They may be isolated or clustered and usually occur in glomeruli with very active proliferative and necrotizing lesions. Hematoxylin bodies are the tissue equivalent of the LE body and consist of naked nuclei whose chromatin has been altered following cell death with extrusion of the nucleus and binding to ambient circulating ANA.

Cellular crescents are a feature of active lupus nephritis that may be encountered frequently in both class III and class IV lupus nephritis (Fig. 55-13). They are common overlying necrotizing lesions. Glomerular scarring is a feature of chronic glomerular injury in class III and class IV lupus nephritis. In class III, the glomerular scarring is often initially focal and segmental. Associated fibrous crescents may form synechiae to the sclerotic segments. In chronic class IV lupus nephritis, the glomerular scarring is typically more global and diffuse.

By immunofluorescence in class III and IV lupus nephritis, there are subendothelial immune deposits that generally follow the distribution of the endocapillary proliferation. Thus subendothelial deposits tend to be relatively focal and segmental in class III (Fig. 55-14) and more diffuse and global in class IV lupus nephritis (Fig. 55-15). These subendothelial deposits are typically superimposed on generalized mesangial immune deposits. Hyaline thrombi form occlusive intracapillary globular deposits. Scattered subepithelial deposits are not uncommon in class III or IV and usually have a more finely granular quality. However, according to the ISN/RPS classification, the presence of regular subepithelial deposits involving over 50% of the glomerular capillary surface area of at least 50% of glomeruli exceeds what is acceptable in class III or IV alone and would warrant an additional diagnosis of membranous lupus nephritis class V. By electron microscopy, class III and IV lupus nephritis typically display subendothelial electron dense deposits that tend to be focal and segmental in class III and more diffuse and global in class IV, superimposed on a substratum of mesangial deposits (Fig. 55-16). The extent and distribution of deposits seen by electron microscopy

usually parallels that seen by immunofluorescence. Rare cases of class III of IV lupus nephritis have relatively sparse subendothelial deposits relative to the extent and severity of the active necrotizing lesions. Such cases resemble examples of “pauciimmune” focal segmental necrotizing glomerulonephritis (18), and some may be associated with antineutrophil cytoplasmic antibodies (ANCA) (19).

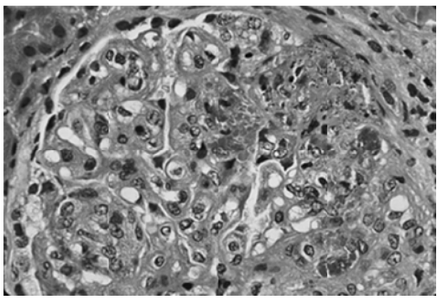

Figure 55-12. (See color plate.) Lupus Nephritis Class IV. Hematoxylin bodies appear as lilac-colored rounded bodies within a glomerulus with active proliferation. These structures consist of nuclei that have been extruded from damaged cells and have undergone clumping of their chromatin following interaction with ANA. (H&E, × 600).

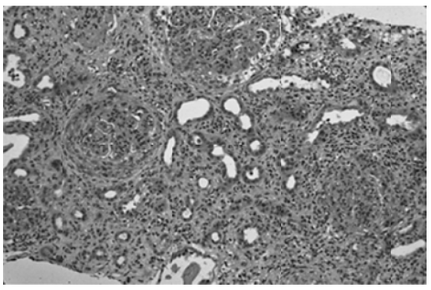

Figure 55-13. (See color plate.) Lupus Nephritis Class IV. A severe example shows glomerular crescents (extracapillary proliferation), as well as endocapillary proliferation. Severe interstitial inflammation and edema accompany the glomerular disease. (H&E, × 80).

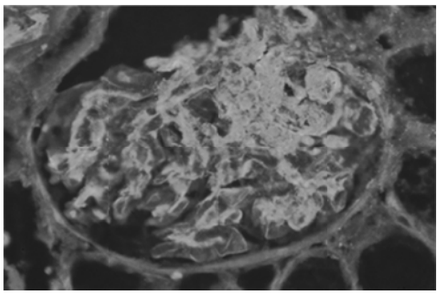

Figure 55-14. (See color plate.) Lupus Nephritis Class III. By immunofluorescence, there are segmental subendothelial deposits involving one segment of the glomerulus. Smaller mesangial deposits are noted in the adjacent segments (IgG, × 400).

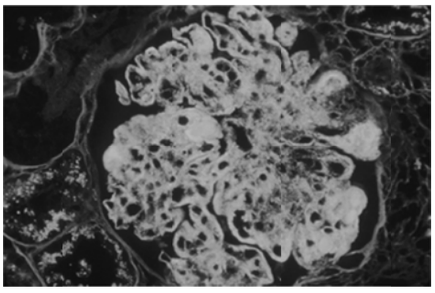

Figure 55-15. (See color plate.) Lupus Nephritis Class IV. By immunofluorescence, there are globally distributed subendothelial and mesangial immune deposits of IgG. Some glomerular capillaries are actually occluded by immune deposits, corresponding to “hyaline thrombi” (× 400)

Class V (Membranous Lupus Nephritis)

Class V designates membranous lupus nephritis, as defined by subepithelial immune deposits or their morphologic sequelae. The membranous alterations may be present alone or on a background of mesangial hypercellularity and mesangial immune deposits. There may be few small subendothelial immune deposits identified by immunofluorescence and/or electron microscopy, but not by light microscopy.

In the modified WHO classification, membranous lupus nephritis was subdivided into four subclasses, designated Va through Vd. It is important to be familiar with these categories because older outcome studies frequently employed these designations (20). Class Va denotes pure membranous lupus nephritis without associated mesangial deposits or mesangial proliferation. Class Vb included the typical peripheral capillary wall features of membranous glomerulopathy together with mesangial alterations, either mesangial deposits alone or with mesangial hypercellularity. The modified WHO classification also recognized class Vc (combined classes Va and III) in which there are typical features of focal and segmental endocapillary proliferative glomerulonephritis superimposed on the membranous pattern, and class Vd (combined classes Va and IV), in which there is superimposed diffuse endocapillary proliferative and membranous lupus nephritis. A major disadvantage of this system was to place undue emphasis on the membranous component rather than on the more serious proliferative component. According to the ISN classification, the designation mixed class III and class V replaces the Vc lesion, and the designation of mixed class IV and class V replaces the Vd lesion. In this schema, the additional designation of class V in the setting of class III or IV requires membranous involvement of at least 50% of glomerular capillary surface area of at least 50% of glomeruli by LM and/or IF. This approach is amply supported by clinical-pathologic studies demonstrating that class Vd has an extremely poor prognosis, even worse than pure diffuse proliferative class IV (21).

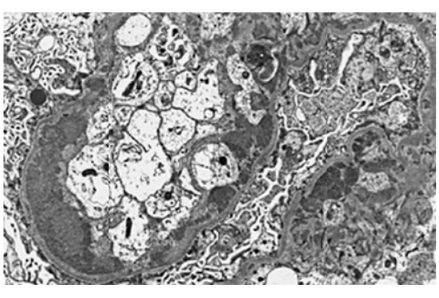

Figure 55-16. Lupus Nephritis Class IV. Electron microscopy shows a representative glomerular capillary with a large subendothelial electron dense deposit located between the endothelium and the overlying glomerular basement membrane. Smaller electron dense deposits are present in the adjacent mesangium at bottom (× 3,000).

By light microscopy, the peripheral glomerular capillary wall alterations display a spectrum and evolution similar to those of idiopathic membranous glomerulopathy. In early stages, the glomerular capillary walls may appear normal in thickness and texture by light microscopy, but subepithelial deposits are detected by immunofluorescence and electron microscopy. At this stage, the glomerular capillaries often have a rigid, ectatic appearance with visceral epithelial cell swelling. Well-established membranous lesions are typically characterized by uniform and diffuse thickening of the glomerular capillary walls (Fig. 55-17) with well-developed spikes of the GBM that are best demonstrated with

the silver stain (Fig. 55-18). As the lesions evolve, the deposits may become largely resorbed and overlaid by neomembrane formation producing a vacuolated glomerular basement membrane profile.

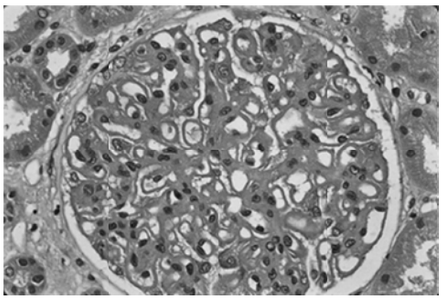

Figure 55-17. (See color plate.) Lupus Nephritis Class V. Membranous lupus nephritis displays global thickening of the glomerular capillary walls, which display a rigid aspect. Several mesangial areas also appear mildly hypercellular. (H&E, × 500).

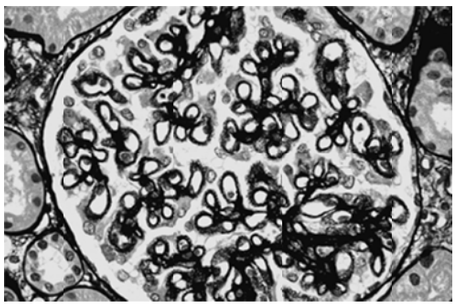

Figure 55-18. (See color plate.) Lupus Nephritis Class V. The Jones methenamine silver stain demonstrates that the thickening of glomerular capillary walls is due to basement membrane spikes. Spikes project at right angles from the glomerular basement membrane, like the bristles on a comb (× 500).

Patients with lupus nephritis class V are at risk for the development of renal vein thrombosis. Examination of the renal biopsy may provide clues to the occurrence of superimposed renal vein thrombosis. Suspicious findings include erythrocyte congestion and focal fibrin thrombosis of the glomerular capillaries as well as diffuse interstitial edema. In chronic renal vein thrombosis, there may be diffuse tubular atrophy and interstitial fibrosis out of proportion to the degree of glomerular sclerosis.

By immunofluorescence a diagnosis of class V is based on the presence of global or segmental continuous granular subepithelial immune deposits (Fig. 55-19). A background of mesangial immune deposits is commonly observed. By electron microscopy electron dense subepithelial deposits range from small to large, but involve the majority of capillaries. As the disease progresses, the same ultrastructural stages seen in idiopathic membranous glomerulopathy may evolve. Glomerular basement membrane spikes often separate the subepithelial deposits (Fig. 55-20). In more chronic cases, the deposits become overlaid by neomembrane and later become resorbed and relatively electron lucent. There is extensive foot process effacement in the distribution of the subepithelial deposits. Mesangial electron dense deposits are commonly observed. Sparse small subendothelial electron dense deposits also may be found, but are not accompanied by endocapillary proliferation.

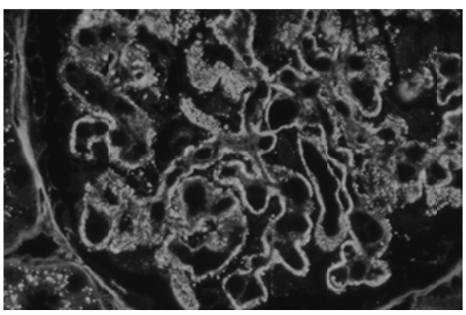

Figure 55-19. (See color plate.) Lupus Nephritis Class V. By immunofluorescence, there are finely granular deposits of IgG outlining the glomerular capillary walls in a subepithelial distribution (× 600).

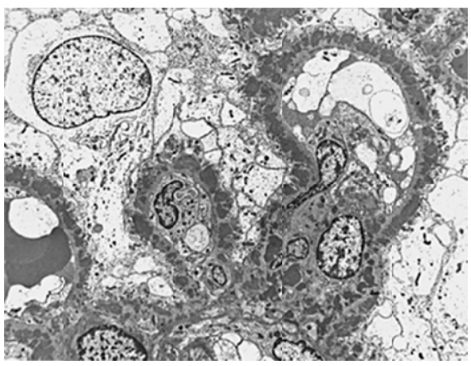

Figure 55-20. Lupus Nephritis Class V. Electron microscopy shows several glomerular capillaries with thickening of their walls owing to abundant subepithelial electron dense deposits. The deposits are located between the glomerular basement membrane and the visceral epithelial cells. Glomerular basement membrane spikes separate the deposits. Several electron dense deposits are also identified in the adjacent mesangial area (× 2,000).

Class VI (Advanced Sclerosing Lupus Nephritis)

Class VI is defined by global glomerular sclerosis affecting more than 90% of glomeruli without residual activity. Most such cases likely represent advanced class IV disease. However, patients with class V or repeated flares of class III also may progress to end-stage and manifest this pattern late in their course. By light microscopy there is extensive global glomerular sclerosis involving over 90% of glomeruli, without significant ongoing activity. Some glomeruli may be segmentally sclerotic. Glomeruli with less advanced sclerosis may display residual hypercellularity or thickenings of the glomerular basement membranes. Vestiges of old crescents may be discernible with the PAS stain as subcapsular fibrous proliferations with disruptions of Bowman's capsule. Severe tubular atrophy, interstitial fibrosis, inflammation, and arteriosclerosis usually accompany the glomerular sclerosis. In some cases, the process is so end-stage that a diagnosis of chronic lupus nephritis is difficult on morphologic grounds. By immunofluorescence there are usually some residual immune deposits in the few nonsclerotic glomeruli, as well as in the obsolescent glomeruli. Granular deposits may also be detected in the tubulointerstitial compartment or vessel walls. Despite the advanced

glomerular scarring, small electron dense granular deposits may still be detectable in the sclerosing tuft, as well as in the tubulointerstitial and vascular compartments.

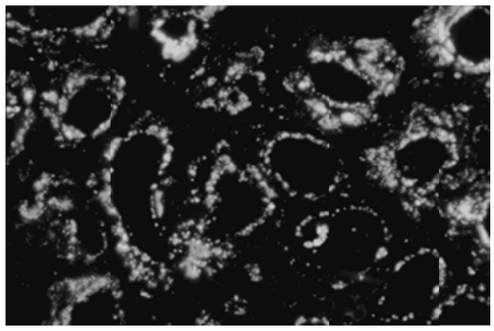

Figure 55-21. (See color plate.) Lupus Nephritis Class IV. Immunofluorescence reveals numerous granular deposits of C1q within the tubular basement membranes (× 100).

Immunofluorescence (General Features)

LN is one of the few renal diseases in which immune deposits can be found in all renal compartments, the glomeruli, tubules, interstitium, and blood vessels (Figs. 55-21 and 55-22) (8 ,22 ,23 ,24). More than one class of immunoglobulin is usually found. IgG is almost universally present, with codeposits of IgM and IgA in most specimens. IgG is usually dominant in intensity. Both C3 and C1q are commonly identified, and staining for C1q may be particularly intense (25). When all three immunoglobulin classes and both complement components are found, the designation “full house” staining is often used. Staining for fibrin-fibrinogen is common in the distribution of crescents and segmental necrotizing lesions. A frequently observed phenomenon in renal biopsies of lupus patients is the “tissue ANA” (26). This refers to nuclear staining of renal parenchymal cells in frozen sections stained with fluoresceinated antisera to human IgG. It results from the binding of patient's own ANA to nuclei that have been exposed in the course of cryostat sectioning. Tissue ANA may be found in all classes of LN and does not correlate with the activity of the LN.

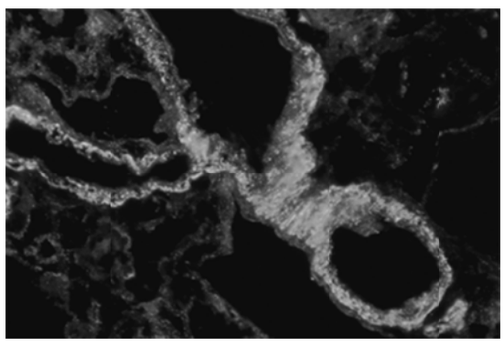

Figure 55-22. (See color plate.) Lupus Nephritis Class IV. A medium-sized artery contains finely granular deposits of IgG outlining its media (× 100).

Electron Microscopy (General Features)

The distribution of immune deposits seen by immunofluorescence corresponds to that observed by electron microscopy (8 ,22 ,23 ,24 ,27 ,28). Mesangial electron dense deposits are typically observed in all classes of lupus nephritis. Thus they may be considered the common substratum upon which the higher classes are built. Class III and class IV lupus nephritis display subendothelial as well as mesangial deposits. The distribution of the subendothelial deposits is more focal and segmental in class III and more diffuse and global in class IV, corresponding to the general distribution of the endocapillary proliferative lesions. An exception is the occasional case of class III or IV, in which there is a paucity of peripheral capillary wall deposits despite active necrotizing lesions, resembling the pattern in “pauci-immune” focal crescentic glomerulonephritis (18). Although scattered subepithelial deposits may also be detected in class III and class IV, regular subepithelial deposits are a distinguishing feature of class V.

In most cases, the texture of the glomerular electron dense deposits is granular. However, in a small percentage of cases, the deposits may exhibit an organized substructure known as “fingerprinting”(29). This corresponds to the presence of curvilinear microtubular or fibrillar structures composed of bands ranging from 10 to 15 nm in diameter. Fingerprint substructure may be more common in lupus patients with circulating cryoglobulins (30).

A common ultrastructural finding in lupus nephritis is “tubuloreticular inclusions” (TRI), 24-nm interanastamosing tubular structures located in the dilated cisternae of endoplasmic reticulum of renal endothelial cells (Fig. 55-23) (31 ,32 ,33 ,34).

These “interferon footprints” are readily identified in all classes of lupus nephritis, irrespective of disease activity.

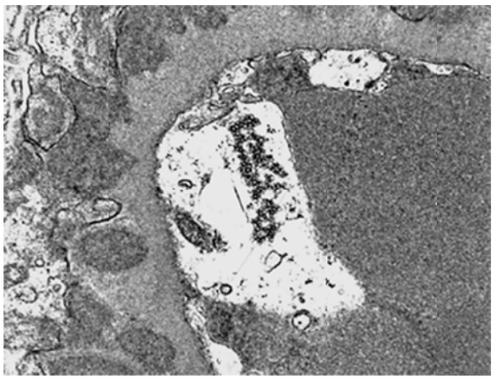

Figure 55-23. Lupus Nephritis Class V. A tubuloreticular inclusion (“interferon footprint”) is seen within the cytoplasm of a glomerular endothelial cell. It consists of an interanastamosing tubular structure located within cisternae of endoplasmic reticulum. Subepithelial electron dense deposits thicken the adjacent glomerular capillary wall, consistent with membranous features (× 15,000).

Pathogenesis of Lupus Nephritis

Although numerous immunologic abnormalities occur in SLE, it is unclear which factors relate directly to the pathogenesis of LN. SLE is a disease in which immune dysregulation leads to loss of self-tolerance and autoimmune responses. SLE has been associated with a decreased number of cytotoxic and suppressor T cells, increased helper ($CD4^+$) T cells, dysfunctional T-cell signaling, abnormal Th1 and Th2 cytokine production, polyclonal activation of B cells and defective B-cell tolerance (35 ,36 ,37 ,38). Activation and clonal expansion of $CD4^+$ T cells promote the activation of autoreactive B cells, leading to their proliferation and differentiation into cells that produce an excess of antibodies against a host of nuclear antigens, including DNA, Sm, RNA, Ro, and La (39). Immune complexes form, not only in kidney, but also at the dermal-epidermal junction, in the choroid plexus, pericardium, lung, and pleura.

Glomerular involvement in SLE has often been considered a human prototype of classic chronic immune complex induced glomerulonephritis as defined in experimental models. Three major mechanisms of immune deposition in the kidney have been identified: (1) binding of autoantibodies to nonglomerular autoantigens that have been planted in the glomerulus; (2) binding of autoantibodies to intrinsic glomerular antigens; (3) deposition of preformed circulating immune complexes. These mechanisms are not mutually exclusive and each may contribute in varying degrees to the pathogenesis of LN in a particular individual.

The size, charge, and avidity of the immune complexes, autoantibody specificity and cross-reactivity with glomerular constituents, and clearance of immune deposits through FcR interactions all influence localization within the glomerulus. Anti-dsDNA antibodies predominate in kidney eluates from patients with LN (40 ,41 ,42). Antibodies with specificity for nucleosomes, Sm, RNP, histone, SSA, SSB, and C1q have also been eluted from the kidney (40 ,41 ,42). Nucleosomes consisting of DNA bound to histone have affinity for anionic components of the glomerular basement membrane such as heparan sulfate (43 ,44). Nucleosomes liberated from apoptotic cells may bind to negatively charged cell surfaces or matrix components of the glomerulus in the course of filtration. Once planted in the glomerular filter through charge interactions, nucleosomal antigen may interact with circulating autoantibody, leading to the formation of ICs in situ (45).

There is also evidence that anti-DNA antibodies exhibit a broad range of cross-reactivities to normal glomerular constituents such as surface antigens on endothelial or mesangial cells (45 ,46 ,47), heparan sulfate proteoglycan (48), phospholipids (48 ,49 ,50), laminin (42 ,52 ,53), type IV collagen (52 ,54), podocyte antigens (52 ,54), and cytoskeletal proteins such as vimentin and α-actinin-4 (55 ,56 ,57). Such autoantibody cross-reactivities could promote in situ immune complex formation to glomerular structural proteins.

Passive deposition of preformed circulating immune complexes represents another possible mechanism of immune injury in LN (58). Drawing from models of chronic serum sickness, mesangial deposition is favored by relatively small amounts of intermediate-sized, high avidity complexes (59). When present in larger quantities, these may spill out into the subendothelial areas. Subepithelial deposits may be favored by smaller, low-avidity ICs formed in relative antigen excess (60). Such deposits may dissociate and reform in situ, allowing electrostatic interactions with the glomerular capillary wall's polyanionic constituents. The clearing ability of the mesangium and local hemodynamic factors also may play a role (61 ,62). In all these models, glomerular immune deposits may be amplified in situ by the binding of anti-C1q antibodies (63 ,64).

Once immune complexes are deposited, the complement cascade is activated leading to complement mediated damage, activation of procoagulant factors, monocyte and neutrophil infiltration, release of proteolytic enzymes, and elaboration of various cytokines regulating glomerular cellular proliferation and matrix synthesis (65). Renal influx of FcR-bearing cells as effector cells in lupus nephritis is critically important to the initiation of nephritis (66). Important chemoattractants implicated in murine or human LN include colony-stimulating factor, VCAM-1, ICAM-1, MCP-1, RANTES, and osteopontin (67 ,68 ,69 ,70 ,71 ,72). A number of inflammatory and fibrogenic cytokines are upregulated in active nephritis, including IL-1, IL-2, IL-6, TNF-α, IFN-γ, endothelin-1 and TGF-β (73 ,74 ,75 ,76 ,77 ,78 ,79). The dysregulated cytokine milieu promotes mesangial proliferation, crescent formation and progressive glomerulosclerosis. Glomerular and vascular damage may be potentiated by hypertension and coagulation abnormalities. The presence of antiphospholipid antibodies directed against a phospholipid-β_2-glycoprotein complex may promote endothelial and platelet dysfunction. These include procoagulant and antifibrinolytic effects through reduced production of prostacyclin, reduced prothrombinase activity, inhibition of protein C and S, and enhanced platelet aggregation (80 ,81).

Activity and Chronicity Indices

Most investigators have found it useful to supplement the WHO classification of LN with a semiquantitative grading of features of activity (potentially reversible lesions) and chronicity (irreversible lesions) (82 ,83). The NIH system of activity and chronicity indices is widely used. (Table 55-5). The activity index is calculated form the renal biopsy by grading each of six histologic features (including endocapillary proliferation, glomerular leukocyte infiltration, wire loop deposits, fibrinoid necrosis and karyorrhexis, cellular crescents and interstitial inflammation) on a scale of 0 to 3+.

Crescents and fibrinoid necrosis, the most severe lesions, are assigned double weight. The sum of the individual components yields a total histologic activity index score of from 0 to 24. Likewise, a chronicity index of 0 to 12 is derived from the sum of four features, each graded on a scale of 0 to 3+, including glomerulosclerosis, fibrous crescents, tubular atrophy and interstitial fibrosis. Early investigations found that either a high activity index (>12) or an elevated chronicity index (>4) predicted a reduced renal survival (82). However, subsequent studies not restricted to class IV disease but including all WHO classes failed to find a correlation between either the activity or chronicity index and long-term prognosis (84 ,85). More recent NIH studies on Class IV diffuse proliferative lupus nephritis patients have shown that the combination of an elevated activity index (>7) and chronicity index (>3) added prognostic information about long-term outcome (86). One clear benefit of these indices is to monitor changes in activity and chronicity over time in sequential biopsies from individual patients. This provides useful information about the efficacy of therapy and the relative degree of progressive histologic damage (87).

Table 55-5: Activity and Chronicity Indices

Index of Activity (0–24)	
Endocapillary hypercellularity	(0–3+)
Leukocyte infiltration	(0–3+)
Subendothelial hyaline deposits	(0–3+)
Fibrinoid necrosis/karyorrhexis	(0–3+) × 2
Cellular crescents	(0–3+) × 2
Interstitial inflammation	(0–3+)
Index of Chronicity (0–12)	
Glomerular sclerosis	(0–3+)
Fibrous crescents	(0–3+)
Tubular atrophy	(0–3+)
Interstitial fibrosis	(0–3+)

Tubulointerstitial Lesions

Tubulointerstitial disease commonly accompanies the glomerular lesions of LN (88 ,89 ,90). Whereas tubulointerstitial involvement may occur rarely as an isolated histologic finding in lupus biopsies, it is far more common for severe acute tubulointerstitial disease to accompany active proliferative lupus glomerulonephritis. Active lesions typically consist of interstitial inflammatory infiltrates including T lymphocytes (both CD4 and CD8 positive cells), monocytes, and plasma cells accompanied by interstitial edema (91 ,92). Immune deposits of immunoglobulins and/or complement components may be present in the tubular basement membranes, interstitial capillary walls or interstitial collagen (Fig. 55-21). Some studies have correlated the quantity of tubulointerstitial immune deposits with serologic activity (89 ,90). The degree of interstitial inflammation has also been correlated with a reduction in glomerular filtration rate and an elevated serum creatinine (90). In the more chronic stages of LN, interstitial fibrosis and/or tubular atrophy develop. Recently quantitative morphometry has been applied to the biopsies of patients with lupus nephritis (93). Using special stain picro-Sirius red and digitized light microscopic images, the cortex of renal biopsies from 48 patients with lupus nephritis was evaluated for the area occupied by nuclei, intratubular space, fibrillary collagen, and collagen matrix. Higher nuclear index correlated with parameters of clinical disease activity; higher collagen matrix index predicted relapse and progression to end stage renal disease; higher fibrillary collagen index and increased intratubular space correlated with an elevated serum creatinine (93).

Vascular Lesions

Vascular disease is common in lupus nephritis and may assume several morphologically distinct forms (94 ,95 ,96). Although vascular lesions contribute to disease severity and may influence prognosis, they are not factored into the WHO classification or the activity and chronicity indices.

Uncomplicated vascular immune deposits are a frequent and highly specific feature of lupus nephritis. They are most common in class III and IV lupus nephritis, but may be found in class II and V as well. They affect predominantly small arteries and arterioles, and to a lesser extent veins. By immunofluorescence and electron microscopy, granular immune deposits are detectable in the extracellular matrix of the media or the intimal basement membrane (Fig. 55-22). Usually these vascular deposits produce no obvious light microscopic changes. Rarely, thickening of the vascular basement membranes may be identified, but without significant compromise of the lumen. These lesions do not influence clinical course or prognosis.

Lupus vasculopathy is a term that refers to a noninflammatory necrotizing vascular lesion that primarily affects arterioles, most frequently in severe class IV lupus nephritis. The vascular wall is obscured by smudgy eosinophilic fibrinoid material that typically expands the intima and may occlude the lumen (97). There is frequent necrosis of medial myocytes and endothelial cells, but without inflammation of the vessel wall (Fig. 55-24). Thus, this lesion represents a vasculopathy rather than a true inflammatory vasculitis. By immunofluorescence, both immunoglobulins and fibrin-related antigens are detectable in the intima and media, indicating injury from combined immune deposition and intravascular coagulation. This lesion carries a poor prognosis with frequent associated hypertension and a rapid course to renal failure (95).

Thrombotic microangiopathy may affect small arteries, arterioles, and glomerular capillaries. It occurs in several distinct clinical settings including lupus anticoagulant syndrome, hemolytic uremic syndrome, thrombotic

thrombocytopenic purpura, and overlap with systemic sclerosis in some patients with mixed connective tissue disease (95 ,98 ,99 ,100 ,101 ,102). The renal thrombotic lesions may be limited to the kidney or may occur as part of a systemic thrombotic microangiopathy. Some patients with these lesions have documented anticardiolipin/antiphospholipid antibodies or manifest HUS-TTP like syndrome because of autoantibody to the Von willebrand factor cleaving protease (103). Thrombotic microangiopathy may supervene on any class of lupus nephritis. It should be suspected whenever there is intravascular coagulation that cannot be explained by the activity of the glomerular disease. Light microscopic features include fibrin thrombosis of glomerular capillaries and vessels, mesangiolysis, and arterial mucoid intimal edema with entrapment of fragmented erythrocytes (Fig. 55-25). Unlike lupus vasculopathy, these lesions contain predominantly fibrin-related antigens by immunofluorescence, without associated immune deposits.

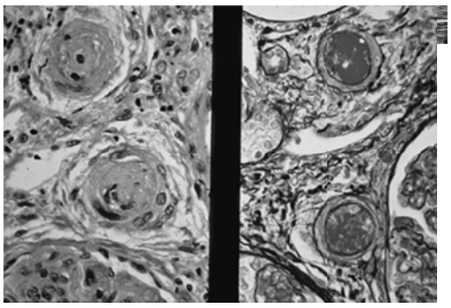

Figure 55-24. (See color plate.) Lupus vasculopathy. The left panel (H&E) shows luminal occlusion by fibrinoid eosinophilic material with smudgy necrosis of the arteriolar wall. The right panel (Lendrum stain for fibrin) shows that the intravascular eosinophilic material consists in part of fibrin (× 500).

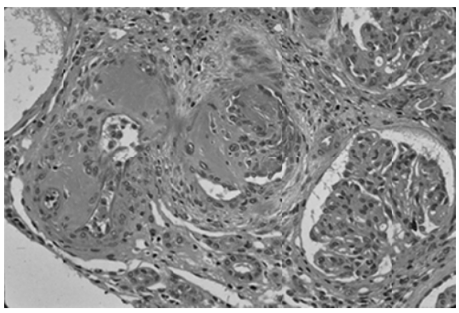

Figure 55-25. (See color plate.) Lupus anticoagulant syndrome with thrombotic microangiopathy. Several small arteries are severely narrowed by organizing thrombi. Fresh fibrin is deposited on a more chronic recanalized lesion, indicating acute and chronic thrombotic microangiopathy. The adjacent glomerulus displays ischemic retraction of the tuft and there is surrounding interstitial fibrosis. (H&E, × 80).

True necrotizing vasculitis is rarely identified in lupus nephritis (94 ,95). It may be renal-limited or associated with systemic vasculitis. It resembles microscopic polyangiitis with fibrinoid necrosis and inflammatory infiltration of the vessel wall. In most cases, no vascular immune deposits are detectable by immunofluorescence or electron microscopy. This lesion may occur in any class of lupus nephritis, regardless of the activity of the glomerular disease.

Silent Lupus Nephritis

“Silent lupus nephritis” defined as histologic renal involvement without clinically evident renal disease has been reported in a number of series of lupus patients (104 ,105 ,106 ,107). Some investigators require normal serology, inactive urinary sediment, absence of proteinuria, and a normal creatinine concentration to qualify as true “silent” disease, whereas others only require normal renal parameters. Although well described, both forms of “silent” LN appear to be rare (8 ,108). The majority of such patients will eventually manifest clinical renal findings at some time during the course of their nephritis.

Clinicopathologic Correlations and Outcomes According to Class

Patients with ISN/RPS class I, minimal mesangial lupus, have no evidence of clinical renal disease. Likewise, patients with class II, mesangial proliferative lupus nephritis, usually have no, or at most mild evidence of clinical renal disease (2 ,3 ,8 ,22 ,84 ,109). Although these patients may have an elevated anti-DNA antibody titer or low serum complement, urinary sediment is inactive, hypertension is infrequent, and the serum creatinine and GFR are usually normal. Proteinuria is rarely above a gram daily in patients with mesangial lesions. Class I and class II patients generally have an excellent prognosis unless they transform to another pattern.

An exception to this presentation is the concurrence of SLE and minimal change disease, known as lupus “podocytopathy” (110 ,111 ,112). These SLE patients present with sudden onset of the nephrotic syndrome and have renal biopsy findings of LN class I or II with extensive foot process effacement, correlating with the heavy albuminuria. The severe foot process effacement cannot be explained by the low immune complex load, which is limited to the mesangium, thereby supporting the presence of a primary podocyte injury. In individual patients, it is uncertain whether such cases represent a podocytopathy secondary to the altered cytokine milieu of SLE or a chance superimposition of two unrelated diseases. The nephrotic syndrome responds rapidly to steroid therapy akin to minimal change nephrotic syndrome.

ISN/RPS class III is often associated with markers of active serologic disease. However, the degree of serologic

activity does not necessarily correlate with the severity or extent of the histologic damage (109 ,113 ,114 ,115). Patients commonly have hypertension, proteinuria, and an active urinary sediment containing dysmorphic erythrocytes and erythrocyte casts. From one fourth to one third of patients present with the nephrotic syndrome, and as many as one fourth have an elevated serum creatinine at the time of renal biopsy. Some patients with class III disease have less extensive glomerular involvement and are more likely to be normotensive and have preserved renal function. Others may have more chronic scarred lesions with associated hypertension and reduced renal function, but without active urinary sediment. The course of patients with class III is extremely varied. Some with mild proliferation involving a small percentage of the glomeruli will respond well to therapy, with fewer than 5% progressing to renal failure over 5 years of follow-up (84 ,115 ,116 ,117 ,118). Others with more extensive glomerular involvement or with necrotizing features and crescent formation have a prognosis similar to class IV(A), active diffuse lupus nephritis.

Patients with class IV LN typically have the most active and severe clinical features. For this reason, most treatment studies of LN have included predominantly class IV patients. These patients have active serologic markers with have high anti-DNA antibody titers and low serum complement levels. Their urinary sediment is very active, containing erythrocytes and multiple urinary casts, hypertension is common, and proteinuria is universal with up to one half presenting with nephrotic range proteinuria at the time of renal biopsy (8 ,22 ,84 ,119 ,120). Although the serum creatinine may appear to be within the “normal” range, when measured, the glomerular filtration rate is usually reduced. Although the prognosis for patients with class IV diffuse proliferative disease has markedly improved in recent years, in most studies this group still has the worst renal survival. Survival is influenced greatly by such prognostic features as race, socioeconomic status, and renal features at presentation. A number of studies have confirmed a worse prognosis for patients with class IV compared to other classes.

A new and controversial feature of the ISN/RPS classification is the division of LN class IV into subcategories with diffuse segmental (IV-S) and diffuse global (IV-G) involvement. One study of 85 LN patients actually found a worse renal prognosis for “severe” focal proliferative class III patients (ISN/RPS class IV-S) than for patients with diffuse proliferative class IV lesions (ISN/RPS class IV-G) (121). This study emphasizes the different usages of the designation focal proliferative lupus nephritis among investigators using the older WHO classification and stresses the bad outcome for patients with segmental necrotizing lesions. A Japanese study of 60 lupus nephritis patients found that those patients with LN class IV-S had worse renal survival than those with IV-G (95-month survival vs. 214 months), although this did not reach statistical significance (12). By contrast, two studies from Boston and Paris have failed to find outcome differences between these subgroups (14 ,15). Further studies are needed to address the usefulness of this distinction.

ISN/RPS class V membranous LN differs importantly from the 1982 modified WHO class V. The older designation included patients with combined membranous and proliferative lesions under the rubric of class V. Hence a patient with focal proliferation and membranous features formerly was classified as WHO class Vc and one with diffuse proliferation and membranous features as WHO class Vd. These patients would now be designated ISN/RPS class V + III and ISN class V + IV, respectively. Patients with mixed membranous and proliferative patterns on biopsy have clinical features that reflect both components. Only those patients with pure membranous lupus nephritis or associated mild mesangial lesions are now included in ISN/RPS class V. Such patients typically present with proteinuria, and features of the nephrotic syndrome (20 ,84 ,109 ,122 ,123 ,124). However, up to 40% will have subnephrotic proteinuria, and up to 20% will have less than 1 gram of proteinuria daily at the time of renal biopsy. Patients with ISN/RPS class V typically have less serologic activity with normal serum complement levels and low anti-DNA antibody titers (84). Although some patients have active urinary sediment, hypertension, and renal dysfunction, these are far less common than in patients with proliferative lesions. Some ISN/RPS class V patients develop heavy proteinuria or what appears to be idiopathic nephrotic syndrome before the appearance of other clinical and laboratory manifestations of SLE (84). Patients with lupus membranous nephropathy are predisposed to thrombotic complications such as renal vein thrombosis and pulmonary emboli akin to patients with idiopathic membranous glomerulopathy (125).

Renal survival rates in class V patients depend upon whether patients have pure membranous lesions or superimposed (class III or IV) proliferative lesions (84 ,123 ,126). One U.S. study found the ten year survival rate was 72% for patients with pure membranous lesions but only 20% to 48% for those with superimposed proliferative lesions (126). In an analysis of 60 pure membranous lesions, we found a 75% 10-year renal survival with risk factors for renal progression by univariate analysis including Black race, elevated serum creatinine, higher degrees of proteinuria, hypertension, and transformation to another WHO pattern (unpublished observations). By multivariate analysis, only race (Black) was a significant predictor of progressive renal failure. Other U.S. studies in membranous SLE have had 10-year renal survival rates as high as 85% (117 ,124). In retrospective Italian studies largely composed of Caucasians, 10-year renal survival for class V was 93% (123). Outcomes were worse in those with superimposed proliferative lesions (WHO classes V + III or V + IV). Thus, the variability of prognosis in older studies of membranous LN can be explained in part by differences in racial composition of the study group, in biopsy findings and in therapy.

Advanced sclerosing lupus nephritis, ISN/RPS class VI, usually represents “burnt out” proliferative class III or IV

lupus nephritis. It may result from a single severe episode of resistant or overwhelming active disease, but more commonly is the result of years of lupus flares alternating with periods of inactivity. Nonimmunologic progression of renal disease with hyperfiltration of remaining glomeruli clearly contributes to this form of LN. Despite the lack of active or proliferative lesions on biopsy, and the lack of serologic activity, many class VI patients will have persistent microhematuria and some proteinuria. Hypertension and decreased glomerular filtration rate are common.

Other Histologic Prognostic Factors

Aside from histologic class, several other histologic parameters have been found to have prognostic value in patients with LN. These include: the degree of activity and chronicity on renal biopsy, the severity of tubulointerstitial involvement, the presence of crescents, and/or the presence of severe interstitial fibrosis. Additionally, particular histologic features on initial biopsy and their persistence on repeat renal biopsy have been shown to have prognostic value.

Utilizing biopsy features of reversible (active) or irreversible (chronic) damage, trials at the NIH documented that in patients with severe proliferative LN class IV, those with a higher activity index or chronicity index were more likely to progress to renal failure (82 ,83 ,86 ,116 ,127 ,128 ,129). In different patient populations, however, neither the reproducibility of these indices nor their prognostic value could be confirmed (84 ,85 ,130). Despite disagreement concerning the prognostic import of individual components of histologic damage, most studies agree that extensive glomerulosclerosis and interstitial fibrosis confer a worse long-term prognosis (131 ,132 ,133). In a large NIH study, patients with a combination of high activity (activity index >7) and high chronicity on biopsy (chronicity index >3) had worse outcomes (86). Similarly, those with the combination of cellular crescents and interstitial fibrosis on biopsy fared worse (86). In an independent study of 89 class IV patients, although no individual histologic variable predicted outcome, combined activity and chronicity on the biopsy did predict a poor outcome (134). In many studies, “flares” of renal disease have predicted a poor renal outcome (133 ,135). An Italian study of 91 patients with diffuse proliferative lupus nephritis found over 50% of patients to experience a renal flare over time, which correlated with higher activity index on biopsy (135).

Detailed histologic and clinicopathologic studies by a group of investigators assigned an important prognostic role to repeat renal biopsies performed 6 months after initiation of maximal therapy for LN (88 ,89 ,131 ,136). Certain histologic features found on repeat biopsy predicted 5-year progression to renal failure, including ongoing inflammation with cellular crescents, macrophages in the tubular lumens, and persistent subendothelial and mesangial deposits. The findings of both crescents and interstitial fibrosis on the repeat biopsy negatively impacted prognosis. By contrast, reversal of interstitial fibrosis and the presence of glomerular segmental scarring together with reduction of interstitial inflammation and immune deposition were favorable prognostic findings (136). Thus, not all chronic histologic changes are necessarily cumulative or immutable, and their stabilization or reversal despite repeated flares of disease activity may be critical to prevent ESRD.

Conclusions

The prognostic significance of the different patterns of renal involvement in lupus nephritis has been recognized since the seminal observations of Pollak and Pirani (7). The 2004 ISN/RPS classification was designed to standardize pathologic terminology, better define the threshold for diagnosis of each class and improve reproducibility between centers. Further validation of this classification in the coming years should confirm its benefits and facilitate multicenter studies exploring differences in optimal treatment and pathogenesis.

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Chapter 56
Clinical and Laboratory Features of Lupus Nephritis

Mary Anne Dooley

Introduction

Renal involvement in systemic lupus erythematosus (SLE) remains the strongest predictor of overall patient morbidity and mortality. Although survival in lupus has improved with more than 90% 10-year survival in many cohorts, the survival in patients with nephritis lags behind at 83% over 10 years (1). The proportion of patients receiving dialysis in the United States as a result of lupus nephritis is rising (2). Renal involvement remains more frequent and severe among children, ethnic groups such as African Americans, and male patients (3). Although mortality rates from SLE have been relatively stable among Caucasians since the 1970s, they have increased among African-American patients, particularly ages 45 to 64, during this time span (1).

Chronic glomerulonephritis was first described in SLE in 1922 (4). Baehr et al. (5) observed wire-loop lesions at autopsy in 13 of 23 patients with lupus in 1935. In the late 1950s, Dixon, Holman, Mellors, Kunkel, Muller-Eberhard, among others (6 ,7 ,8), noted that positive LE-cell preps often were found in patients who had immune deposits in renal tissue. Introduction of the LE-cell prep in 1948 (9) allowed investigators to evaluate the prevalence of SLE in patients with idiopathic nephritis (10 ,11). Prior to the development of corticosteroid therapy and nitrogen mustard in the late 1940s and hemodialysis in the 1960s, onset of lupus nephritis was associated with a significant risk of death within 2 years.

This chapter discusses the epidemiology, incidence, clinical features, and natural course, and it reviews the general management concepts of renal lupus. Chapter 50 covers the immunopathogenesis, pathology, and clinicopathologic correlates of lupus nephritis. Detailed discussions of specific treatment modalities for renal disease are covered separately. The reader is referred to Chapter 56 , Systemic Corticosteroid Therapy in Systemic Lupus Erythematosus (corticosteroids and pulse steroids); Chapter 57 , Immunosuppressive Drug Therapy (cyclophosphamide, nitrogen mustard, azathioprine, chlorambucil, mycophenolate mofetil, cyclosporin and methotrexate); Chapter 58 , Nonpharmacologic Therapeutic Methods (apheresis, total lymphoid irradiation, hemodialysis, and transplantation); and Chapter 59 , Occasional, Innovative, and Experimental Therapies (γ globulin, 2-CDA).

Definition, Epidemiology, and Prevalence

Active lupus nephritis can be defined clinically and histopathologically. Clinical evaluation for lupus nephritis includes dipstick and microscopic urinalysis, urinary protein and creatinine excretion, serum creatinine determinations, and serologic studies (anti-dsDNA antibody titers and serum complement components C3 and C4). Additionally, serum albumin and cholesterol can be used to define the renal disease as nephrotic.

The urinary sediment is useful to characterize disease activity as the presence of glomerular hematuria, leukocyturia, or casts are typical only during periods of disease activity. Interestingly, in one large series of 520 cases of SLE, red cell casts were only present in 39 cases or 7.5% (12). In descending order, the most common abnormal sediment findings are leukocyturia, hematuria, and granular casts. As mentioned, a rising anti-DNA titer and hypocomplementemia, especially with low C3, is a strong indicator or predictor of active lupus renal disease. Hypoalbuminemia and hypercholesterolemia accompanied by significant proteinuria are components of the nephrotic syndrome, which may accompany active lupus renal disease. Although much diagnostic import is placed on glomerular findings, there is increasing recognition of tubulointerstitial injury in lupus nephritis. Urowitz's group have described proliferative lupus nephritis presenting with sterile pyuria alone (13). Typically, the severity of interstitial inflammation parallels the degree of renal impairment. Tubular damage, fibrosis, and atrophy are linearly associated with long-term renal function and can be associated with hyperuricemia and renal tubular acidosis.

Lupus renal disease is also defined immunohistopathologically. Tissue obtained by renal biopsy should be evaluated by light microscopy, immunofluorescence, and electron microscopy. There is a correlation between the pathologic class of lupus nephritis and clinical features (14 ,15 ,16). Despite this association, there are patients with so-called silent lupus nephritis who have normal urinalyses, absence of proteinuria, and normal serum creatinine, but who, on renal biopsy, have anywhere from mesangial to proliferative nephritis (17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26). Fortunately, progressive loss of renal

function typically does not occur without changes in urinary sediment and protein excretion. Lupus glomerulonephritis is now defined by the ISN classification developed by nephropathologists in conjunction with rheumatologists and nephrologists (27). This classification, discussed in Chapter 50 , must be compared with the pre-existing WHO classification system and the NIH-developed activity and chronicity indices as prognosis and therapeutic guidelines have been based on the prior system. Few studies have validated the relationship of the ISN scoring system with clinical outcomes to date (28).

The prevalence of renal disease in eight large cohort studies consisting of 2,649 SLE patients varied from 31% to 65% (12 ,29 ,30 ,31 ,32 ,33 ,34 ,35). A recent study reported a 10% annual incidence of nephritis in 384 lupus patients followed at the Johns Hopkins Medical Center between 1992 and 1994 (36). Most patients with SLE diagnosed prior to the 1982 revised American College of Rheumatology (ACR) criteria for SLE were found to have histologic evidence of lupus nephritis on renal biopsy, even in the absence of urine sediment abnormalities, elevated protein measurements, abnormal serum creatinine, and depressed C3 complement levels, and anti–double-stranded DNA (anti-dsDNA) determinations (17 ,18 ,19 ,20 ,21 ,22 ,23 ,24 ,25 ,26). This has been termed silent lupus nephropathy and is typically nonprogressive. Although these instances generally represent minimal disease histologically, diffuse, proliferative lesions occasionally have been found.

In a series of patients from the Wallace/Dubois practice studied from 1950 to 1991, the following criteria for lupus nephritis were found to have a greater than 95% sensitivity (12 ,37 ,38). One of the following must be present: (1) a renal biopsy showing WHO class IIb mesangial, focal proliferative, diffuse proliferative, or membranous glomerulonephritis; (2) a 30% decrease in creatinine clearance over a 1-year period in a patient with active lupus; and (3) urine protein greater than 1 g in 24 hours. Alternatively, at least three of the following in a 12-month period allow us to make a diagnosis of nephritis in an SLE patient: (1) a serum albumin level greater than 3 g/dL; (2) sustained 2 to 4+ proteinuria; (3) oval fat bodies or granular, hyaline, or red cell casts in the urine; and (4) persistent hematuria of greater than five red cell casts per high-power field in the urine. For each of these criteria, an alternative cause must be excluded. These criteria differ from the less extensive criteria of the ACR (Chapter 2 , Definition, Classification, Activity, and Damage Indices). In the 1987 ACR criteria, renal disorders of SLE are defined as persistent proteinuria (>0.5 g/day or >-3+), or cellular casts of any kind. With these or similar criteria, the prevalence of renal involvement varied from 29% to 65% among the eight series described in Table 56-1 . Tertiary referral centers tended to have higher percentages of patients with renal disease, as did studies that were published before 1965 (when antinuclear antibodies [ANA] became widely available and identified more mild cases of SLE). The true prevalence probably is approximately 40% (39). Nephritis is present in most children (see Chapter 43 , Systemic Lupus Erythematosus in Childhood and Adolescence). Although once thought rare in the elderly (40 ,41), subsequent studies have shown that race confounded the relationship with age (42). When race is controlled, older-onset lupus patients do not have “milder” features and the outcome of lupus may be worse a result of the increased prevalence of comorbidities (43 ,44).

Table 56-1: Findings in Patients with Systemic Lupus Erythematosus and Nephritis (*n* = 128) Compared with Those without Nephritis (*n* = 336)[a]

More Frequent	Less Frequent
Family history of SLE	Other CNS sx
Anemia	Seizures
High sedimentation rate	Thrombocytopenia
High serum cholesterol	Fibromyalgia
High serum triglycerides	
Positive ANA	
High anti-dsDNA	
Low C3 complement	
Low C4 complement	

[a] $P < .01$.
ANA, antinuclear antibody; dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus

Several serologic and genetic risk factors are also associated with an increased risk of developing SLE nephritis. Patients with nephritis are more likely to have a family history of SLE, anemia, high anti-dsDNA antibody titers, and hypocomplementemia (37). The presence of anti-Sm autoantibodies is associated with more severe expressions of SLE, including lupus nephritis, and some studies suggest that anti-dsDNA and antihistone autoantibodies are associated with an increased risk of proliferative lupus nephritis (45).

The incidence and prevalence of SLE nephritis differs among patients of different racial/ethnic backgrounds. Despite much investigative attention, racial differences in lupus expression remain poorly understood. African Americans have a threefold increased incidence of SLE, develop the disorder at younger ages, and more frequently express anti-Sm and RNP autoantibodies (46 ,47 ,48 ,49 ,50 ,51). These patients develop nephritis more frequently than Caucasians and have onset of nephritis earlier in the course of their SLE. In an inception cohort of lupus patients in the southeastern United States, 31% of African-American patients versus 13% of Caucasian patients met ACR renal criteria within 18 months of diagnosis (52). Hispanic and Asian patients also have greater frequency and severity of nephritis compared with Caucasians. Once they have nephritis, African Americans and Hispanics are more likely to progress to end-stage renal disease (ESRD) than Caucasians (53 ,54 ,55 ,56 ,57 ,58 ,59 ,60). These findings have also been reported in studies based outside the United States (61 ,62). Despite the greater frequency of nephritis in Asians, generally good outcomes of cytotoxic therapy have been observed (63 ,64 ,65 ,66 ,67).

Important comorbidities such as diabetes, hypertension, patient compliance with medical regimens, socioeconomic, and psychosocial variables may also influence renal outcomes. In several studies, however, the worse outcome for African-American patients with lupus nephritis was independent of health care access, compliance with medications, and socioeconomic status (60 ,68). Others have shown a strong impact of poverty on renal outcomes (69 ,70). In some series, these patients may have more frequent or uncontrolled hypertension, hypocomplementemia, higher chronicity indices including interstitial fibrosis, and poorer compliance with therapy (56 ,57 ,58 ,59). However, other series have shown poorer response to cyclophosphamide despite similar presence and control of hypertension and activity or chronicity indices or a combination of these measures on biopsy (60 ,71). Lupus nephritis is only one of many kidney diseases in which African-American patients suffer more frequent adverse consequences. Ethnicity may also be associated with a nonlupus-related predisposition toward kidney failure following renal injury. African-American patients with hypertension, diabetes mellitus, HIV nephropathy, or focal segmental sclerosis develop renal failure significantly more often than Caucasian patients

The mean age at disease onset in patients with nephritis is younger than in those with SLE without nephritis (12 ,71 ,72). Most patients develop nephritis early in their disease (68 ,52 ,72); although, as some investigators have noted, the onset of renal disease can occur at any point in the patients disease course (36). The oldest patient with SLE and new-onset nephritis was 80 (B Hahn, pers. comm.). Smaller-scale surveys and the population-based Carolina Lupus study suggest that males have a relatively increased incidence or activity of renal disease (52 ,73 ,74).

Inheritance of the DR2 and B8 gene is associated with an increased risk of developing nephritis in some populations, and this risk is amplified if certain DQ beta genes also are present (see Chapter 6 , The Genetics of Human Lupus). Inheritance of the DR4 gene reduces the risk for lupus nephritis (75). Several studies have shown that allelic variants of the IgG Fcγ receptor RIIA and RIIIA, associated with poor binding and phagocytosis of IgG1 and IgG2 increase risk for lupus nephritis in several different ethnic populations (76). Such individuals may clear immune complexes less efficiently than persons who inherit other alleles for these receptors. However, the association has not been found in all populations, and a recent meta-analyses including data on over 3,000 patients with lupus, noted the population-attributable fractions of SLE cases because of the FcγRIIa-R131 allele were 13%, 40%, and 24% in subjects of European, African, and Asian descent, but was not a risk for lupus nephritis. Other researchers have noted familial antinuclear factors not associated with disease (77) and polymorphisms other pathways, such as the angiotensin-converting enzyme gene that may be associated with lupus (78). More recently, an interferon signature has been reported in patients with SLE, though not yet prospectively associated with disease activity or manifestations (79 ,80).

Clinical and Laboratory Presentation

Wallace et al. noted lupus patients with nephritis also expressed increased frequency of other severe lupus manifestations (12). Table 56-1 summarizes these findings. Piette's French group reported 180 of 436 patients with SLE nephritis. Renal patients had more malar rashes, psychosis, myocarditis, pericarditis, lymphadenopathy, hypertension, anti-DNA, and low C3 (81). Some investigators have proposed that rheumatoid-like arthritis is associated with a lower incidence of renal disease, especially if rheumatoid factor is present and the HLA-DR4 haplotype is present (82 ,83), although not all (12). Walker et al. (84) noted that arthritis and arthralgia were the most common symptoms in a group of 45 patients with lupus nephritis who were followed in New Zealand. Usually, elevated Westergren sedimentation rates, low C3 complement, elevated anti-dsDNA levels, and low serum albumin levels are associated with more active nephritis. Gallium scans demonstrate increased renal uptake with active disease (85 ,86) and roughly correlate with a biopsy activity index). Doppler ultrasounds are of value to exclude thrombosis, and elevated resistive indices, thinner cortex, and increased echogenicity correlate with greater chronicity index on biopsy (87 ,88).

Klippel (89) described five clinical types of lupus nephritis: occult, chronic active nephritis, rapidly progressive nephritis, nephrotic syndrome, and progressive renal insufficiency in patients with repeatedly normal urinalyses. Glomerulosclerosis, hypertension, diabetes, and occasionally, drugs (especially nonsteroidal anti-inflammatory drugs) probably cause renal insufficiency in most of the latter group. Patients in this group, along with those with occult disease and chronic active nephritis, often are asymptomatic. These clinical subtypes reflect the extraordinary clinical diversity of expression of nephritis in lupus. Mesangial lupus nephritis is often accompanied by normal diagnostic findings or a mild degree of proteinuria, but typically the absence of hypertension or abnormal urinary sediment. Focal and diffuse proliferative lupus glomerulonephritis are often associated with the worst prognosis for renal survival and can be accompanied by nephrotic syndrome, significant hypertension, and abnormal urine sediment. Membranous lupus nephritis often presents with moderate- to high-grade proteinuria, without hematuria or casts and often the absence of hypertension. The majority of patients have a good prognosis and relative preservation of renal function. However, in up to one third of patients, often those with persistent nephrotic range proteinuria, membranous lupus nephropathy can lead to decreased renal function and end stage renal disease (ESRD) (90).

Presentation with acute deterioration in renal function was observed in 36 (18.4%) of 196 SLE hospital admissions in the group followed by Yeung et al. (91 ,92). Infection and active central nervous system disease were frequent precipitating events, and recovery of renal function with aggressive management was reported in 76%. Others have confirmed these findings, even in patients requiring dialysis at presentation (49).

Nephrotic syndrome (defined as a serum albumin <2.8 g/dL with >3.5 g of urine protein per 24 hours) was observed in 13% to 26% of all patients with SLE in the eight well-detailed series shown in Table 56-1 . In contrast to mildly nephrotic subjects who may only have ankle edema on examination, frankly nephrotic states are associated with ascites, presacral edema, as well as pleural and pericardial effusions. Serositis as a result of lupus may be distinguished from uremia, because inflammatory effusions will have exudative rather than transudative features. Physical examinations often are deceptively normal except for hypertension in patients with isolated lupus nephritis. Indeed, as these patients are often young, blood pressure measurements may well be under 140/90 mm Hg, although hypertensive for the age of the patient. Patients with SLE have an increased incidence of renal tubular dysfunction, which is clinically characterized by a proximal or distal renal tubular acidosis (93). This is particularly evident in patients with Sjögren syndrome. (See Chapters 39 and 50 for a discussion of renal physiology abnormalities in SLE.).

Serologic features

Antinuclear Antibodies (ANA)

ANA are a sensitive screening test as more than 95% of lupus patients will be positive when the test is performed using a substrate containing human nuclei, such as HEP-2 cells. In 3% to 6% of cases, however, renal disease manifestations constitute the initial presentation of SLE (94 ,95 ,96 ,97). Cairns et al. (96) reported 11 ANA-negative patients whose onset of SLE began with clinical glomerulonephritis as the initial manifestation. All became ANA positive over a 6-year period. A similar group of 17 patients was described by Adu et al. (97), and three children by Gianuti et al. (98). ACR criteria may not be fulfilled at first even if the ANA is positive (95).

Complement

Complement is a protein whose levels are reduced with inflammation. Various tests of complement are available in the clinical laboratory that are relevant to lupus nephritis: C3, C4, total hemolytic complement, antibodies to C1q and C3d:C4d ratios. (Chapters 13 , Complement and Systemic Lupus Erythematosus, and 48 , Clinical Application of Serologic Abnormalities in Systemic Lupus Erythematosus, review the biology and clinical importance of complement). Low complement levels are associated with greater renal-disease activity (99 ,100 ,101 ,102 ,103 ,104 ,105 ,106 ,107 ,108 ,109 ,110 ,111 ,112 ,113 ,114 ,115). Falls in complement often predict disease exacerbation (100 ,101 ,102 ,103 ,104 ,107 ,108 ,109). The studies cited here suggest that the most specific test is C3, followed by total hemolytic complement and then C4. C3 correlates with activity indices on biopsy (112), and long-term normalization of complement is associated with a better prognosis (113). Conversely, low complement levels also may denote congenital or acquired deficiencies of various components, and a few patients have persistently low complement levels with no clinical evidence of disease activity. Gladman et al. (116) found 14 such patients in a group of 180 with SLE. Followed for a mean of 4.25 years, they had no symptoms and were on no medications, and none developed any evidence of lupus activity.

Anti-DSDNA

Anti-dsDNA is elevated in most patients with active nephritis, although its precipitous decline can presage a flare (100 ,102 ,103 ,104 ,107 ,113 ,117 ,118 ,119 ,120 ,121 ,122 ,123). Test methods include the enzyme immunoassays or the Farr assay to quantitate its presence. The *Crithidia luciliae* test also is available. Anti-DNA is found in 50% to 75% of patients with active nephritis; its levels often are normal in patients with pure membranous disease. Chapter 21 , Antibodies to DNA, and Chapter 48 , Clinical Application of Serologic Abnormalities in Systemic Lupus Erythematosus, review the biology and clinical importance of anti-DNA, which is not as reliable as C3 complement in assessing renal disease activity (101 ,124) and it may be elevated if extrarenal lupus activity is evident.

Other clinical correlates that might be useful in following renal disease activity have been sought. These include cryoglobulins (125 ,126), autoantibodies to poly(ADP)ribose (127), circulating immune complexes (103 ,128 ,129 ,130), IL-2-receptor levels (131), ANA patterns (132), antiendothelial-cell antibody levels (133), plasma thrombomodulin (134), antiheparin sulphate reactivity (135), decreased interleukin-1 receptor antagonist (136), antiribosomal P (137), and measurement of the activation and degradation components of complement (see Chapter 13 , Complement and Systemic Lupus Erythematosus). No one test (except for a dramatic change in serum creatinine or perhaps C3 complement) prompts the practitioner to action unless it is consistently abnormal, supported by other confirmatory laboratory tests and the clinical picture.

Measurements of Renal Function

The principal tests to evaluate renal function are blood urea nitrogen (BUN), serum creatinine, and creatinine clearance. The utility of the BUN is limited by its alteration with hydration status, bleeding, hepatic and dietary conditions. In clinical practice, the most convenient serial measurement of renal function is the serum creatinine. Serum creatinine level can vary with body weight, muscle mass, and state of hydration, and it tends to overestimate renal function by as much as 20% as it does not account for proximal tubular creatinine secretion (138 ,139). Creatinine is hypersecreted by injured tubules in patients with glomerulopathy. One study suggests that giving cimetidine (400 mg) tablets four times a day for 2 days blocks tubular secretion of creatinine and provides a more reliable measure of GFR (140). Because creatinine is calculated on a logarithmic scale, a rise from 1 to 2 mg/dL represents a 50% change, whereas a rise from 6 to 7 mg/dL reflects only a 3% change. Because determining a true, reliable renal function is vital in SLE clinical research, GFR measurements have

become the gold standard. Calculated by the standard formula (U cr V)/P, GFRs that are derived by inulin clearance, iothalamate clearance, and Tc99-DTPA clearance have proven to be reliable, but expensive and inconvenient (140 ,141). Hughes' group recently suggested that chromium-51 labeled EDTA-GFR may be a better predictor of nephritis than GFR indices alone (142). Reductions of GFR out of proportion to renal plasma flow indicate a low filtration fraction and greater disease severity (143).

Twenty-Four-Hour Urine Proteins

Twenty-four-hour urine proteins are valuable to follow only if they are elevated (100 ,137 ,143). Levels below 200 mg per 24 hours are normal; values of up to 1,000 mg can be seen in healthy subjects after vigorous exercise. When more than 3,500 mg per 24 hours are recorded, the patient usually has nephrotic syndrome, and ankle edema is present. Anasarca can be observed in patients who have more than 7,000 mg per 24 hours. In general, 24-hour urine proteins do not correlate well with disease activity, although this is not always the case (100). Decreases in 24-hour urine protein values usually reflect clinical improvement unless because of declining glomerular filtration. In this circumstance, dropping levels are a sign of renal failure.

Urinary Proteins and Sediment

Ropes (144) was the first rheumatologist to attach importance to following urinary sediment and protein level. She noted that 15 of 68 patients (22%) who had proteinuria and were not given corticosteroids had spontaneous disappearance of the proteinuria up to 14 years later. Table 56-2 summarizes Dubois' findings in 520 patients who were seen between 1950 and 1963 and who had multiple urine evaluations (145). Hematuria probably results from the escape of red cells through a gap in the glomerular basement membrane (146). Other reports in patients with nephritis have found microscopic hematuria in 33% to 78% (12 ,30 ,99 ,100 ,147 ,148), fat bodies in 33% to 48% (30 ,37), cellular casts in 34% to 40% (100 ,149), and greater than 1 g of urinary protein per 24 hours in 26% to 87%. Given the difficulty for most patients and settings in acquiring a valid 24 hour urine collection, reports have suggested that a random spot urine collection protein-to-creatinine ratio (corrected for a BSA of 1.75 m^2 BSA) has an excellent correlation with and may be more reliable than a 24-hour urine collection (150). Although the urinalysis may be normal despite abnormal findings on a renal biopsy, nearly all patients with clinically important renal disease have microscopic urine findings. The appearance of five or more leukocytes or red cells in a clean midstream urine specimen without infection, renal stones or other causes, especially with at least a trace of albumin, suggests active nephritis (151 ,152 ,153). Many patients may erroneously be considered to have urinary tract infections, and frequently have been given multiple courses of antibiotics. Confusing the picture, lupus patients may commonly experience increased frequency of urinary symptoms, or hematuria with menses and these circumstances must be excluded before assuming nephritis. As lupus damage advances, increased proteinuria and hyaline and fine granular casts, followed by coarse granular casts, red-cell casts, and white-cell casts are found. If nephrotic syndrome is present, urinary protein may be as high as 30 g per 24 hours, with good renal function. With further progression of renal disease, the numbers of all types of casts increase, waxy casts, broad renal failure casts appear, and a telescoped sediment becomes evident. Herbert et al. (154) found that a relapse of lupus nephritis can be predicted best by cellular casts followed by hematuria and white cells in the urine; these were more reliable than a drop in C3 complement.

Table 56-2: Abnormal Urinary Findings in 520 Cases of SLE

	Cases (n)	%
Albuminuria	240	46.1
WBCs in urine (more than 6/HPF in clean specimen)	185	35.5
Hematuria	170	32.6
Granular casts	164	31.5
Hyaline casts	148	28.4
RBC casts	39	7.5
Fatty casts	32	6.1
Oval fat bodies	23	4.4
Double refractile bodies	10	1.9
Waxy casts	9	1.7
Mixed fatty casts	6	1.2

HPF, High-powered field; RBC, red blood cell; WBC, white blood cell.

Analysis of Urine Protein Components

Albumin

Urine protein can be separated into albumin and gamma globulin fractions. Measurements of urinary albumin excretion by radioimmunoassay can pick up larger amounts than normally would be detected. Though not generally clinically useful, diminution in albumin excretion correlates with clinical response to treatment (155 ,156 ,157). Microalbuminuria is associated with mesangial disease (157), does not predict the development of nephritis (158), whereas polymeric albumin is associated with more serious disease (159).

γ Globulins

Urinary protein electrophoresis demonstrates increased γ-globulin levels during active disease; levels decrease with therapeutic response (160 ,161). No specific patterns are observed in SLE. Quantitative urine-protein analysis with can detect glomerular versus nonglomerular proteinuria (162).

Several groups have shown associations between levels of free immunoglobulin light chains and lupus nephritis activity (163 ,164 ,165 ,166 ,167 ,168 ,169).

Other Urinary Findings

Dubois observed bacterial cystitis or pyelonephritis in 22.5% of his 520 patients (145). Fries and Holman (30) reported dysuria in 14 of their 193 patients. Ropes reported urinary tract infections in 47% of her 150 patients (144). Meryhew et al. (170) studied urinary ANAs. Positive tests were found using both mouse kidney cells and Hep-2 cell substrates. IgG ANA was most frequently seen; one half had more than one immunoglobulin class detected. Anti-Sm, antiribonucleoprotein (anti-RNP), anti-Ro/SSA, and anti-dsDNA also were detected. The presence of anti-dsDNA and ANA correlated with increased clinical severity. ANA might appear in the urine as a result of decreased tubular reabsorption, antigen deposition, or genitourinary tract inflammation, but it probably is representative of glomerular leakage.

Nephrotic syndrome is associated with false-positive urine pregnancy tests (171). Numerous reports note urinary substances are increased with active lupus nephritis and are good markers of clinical activity including ferritin (172), anti-RNA polymerase I antibodies (173), neopterin (174 ,175), acid mucopolysaccharides (176), histuria (177), fibrin degradation products (178 ,179), several gastrointestinal enzymes (180), IL-6 (181 ,182), anti-DNA (183), soluble interleukin-2 receptors (184 ,185), urinary C4 (186), monocyte chemotactic and activating factor (187), retinal-binding protein (188), tumor necrosis factor-α and adhesion molecules (189), and low molecular weight C3 fragments (190), MCP-1, and CR1 (191 ,192).

Chapter 55 , Lupus Nephritis: Pathology, Pathogenesis, Clinical Correlations, and Prognosis discuss urinary prostaglandins, renal-tubular acidosis, aldosterone, the syndrome of inappropriate antidiuretic hormone, and renin activity measurements.

Clinicopathologic Laboratory Correlates

The six major parameters that are used to follow lupus nephritis disease activity are: (1) serum creatinine, (2) assessment of 24-hour protein excretion, (3) creatinine clearance, (4) C3 complement, (5) urine sediment, and (6) anti-dsDNA. Each of these tests tells the clinician different things; therapeutic decisions are based on considering the results for all of these values. Clinical trials also use other outcome criteria that are less important in a community practice. These include rigorous definitions for remissions, flares, relapses, exacerbations, and lupus activity scores (reviewed in reference (193)). Serum creatinine is an insensitive measure of the level of renal function; as the proximal tubule actively reabsorbs creatinine until the mechanism is saturated, a patient may have lost 50% of creatinine clearance with an increase in serum creatinine that remains within the normal range. Normalization of the creatinine level is associated with a favorable prognosis (98 ,141). A creatinine clearance of less than 10 mL/hr, or a serum creatinine of over 7 mg/dL, with uremic symptoms usually is an indication for dialysis. As mentioned earlier, hydration status, obstruction, severe infection, acute tubular necrosis, contrast-induced nephropathy and certain medications (especially nonsteroidal anti-inflammatory agents) can temporarily raise serum creatinine levels. Petri's group associated elevated serum creatinine with ages younger than 20 or older than 40, disease duration, and proteinuria, but not socioeconomic status, race, autoantibodies, or complement levels (56).

Renal Vein Thrombosis

Thrombosis of the renal veins complicating lupus nephritis was first reported in 1968 (194) and has been described in numerous cases since. It should be strongly considered in patients with nephrotic syndrome and/or antiphospholipid antibodies who present with flank pain and fever, thrombophlebitis, or pulmonary emboli (195 ,196 ,197 ,198). Bradley et al. (199) found renal-vein thrombosis in 11 of 280 patients with membranous glomerulonephritis or lupus glomerulonephritis. All 11 (three of whom had SLE) also had nephrotic syndrome, and 10 had pulmonary emboli. Six of 625 Taiwanese SLE patients developed renal-vein thrombosis; all were nephrotic (200). Renal-vein thrombosis needs to be differentiated from renal arteriolar thrombi seen on biopsy not correlated with antiphospholipid antibodies, but with a thrombotic microangiopathic picture (200 ,201). The clinical scenarios of accelerated or malignant hypertension or superimposed thrombotic thrombocytopenic purpura may also produce these findings. Renal-vein thrombosis was found in 27% of 11 patients with nephrotic syndrome, 62% of 13 with a history of thrombophlebitis, and none of 20 controls with SLE. Mintz's group concluded that a hypercoagulable state is a greater risk factor than the presence of nephrotic syndrome for renal vein thrombosis (197). Although the antiphospholipid antibodies predispose one to renal-vein thrombosis, their presence is not mandatory (202 ,203). Renal-vein thrombosis also has been reported in patients with SLE who have received renal allografts (204). If reasonable clinical suspicions exist (e.g., flank pain, hematuria, oliguria, peripheral edema), a magnetic resonance angiogram or renal ultrasound with Doppler blood flow assessment should be performed. Renal-vein thrombosis must be treated promptly with anticoagulants. Renal failure and pulmonary emboli are its most serious complications. Purified Malayan pit-viper venom (ancrod) may be useful as a defibrinator (205), but it generally is not available. Thrombolytic therapies have been successfully used for renal vein thrombosis in patients without SLE (206 ,207 ,208 ,209 ,210).

Antiphospholipid Antibodies, Renal Thrombotic Microangiopathy, and Lupus Nephritis

See Chapter 55 , Lupus Nephritis: Pathology, Pathogenesis, Clinical Correlations, and Prognosis, and Chapter 65 ,

Clinical and Management Aspects of the Antiphospholipid Antibody Syndrome.

Management of Lupus Nephritis

Therapeutic decisions for individual patients with lupus nephritis should be based on consideration of their clinical presentation, laboratory features, and histologic findings on biopsy. The principal goals of therapy are, first, to improve or prevent progressive loss of renal function. Prevention of ESRD is important because of the morbidity and mortality associated with its treatment. Mortality rates among lupus patients on dialysis do not differ from the overall dialysis population (10% per year), but the lupus patients are significantly younger, more frequently female and have fewer comorbidities such as diabetes. Second, because ESRD may be managed by dialysis or transplantation, treatment must do the overall patients as little harm as possible. Table 56-3 lists the toxicities of various therapies.

The general concepts and specific therapies outlined here have evolved over a nearly 40-year period with increasing rapidity recently as new data become available. The outcome of lupus nephritis has improved in some populations to a 10-year survival rate of over 80% with the guidance of the NIH recommendations for intravenous cyclophosphamide. The guidelines are by no means absolute, however, and many highly qualified rheumatologists and nephrologists treat lupus nephritis differently.

Table 56-3: Toxicities of Aggressive Regimens Used to Treat Proliferative Nephritis that May Occur >5% of the Time

I.	Prolonged high-dose oral prednisone therapy (1 mg/kg/day equivalent >6 weeks)
	Accelerated development of cataracts, glaucoma, hypertension, osteoporosis
	Diabetes mellitus
	Avascular necrosis of bone
	Diffuse ecchymoses
	Weight gain and marked cushingoid appearance
	Diplopia
	Emotional lability, mood changes
	Dyspepsia, ulcer risk
	Increased infection risk
	Menstrual irregularities
II.	Cyclophosphamide (more common in oral doses)
	Alopecia
	Amenorrhea, infertility
	Hemorrhagic cystitis
	Risk of malignancy
	Severe nausea and vomiting
	Increased risk of infection
	Teratogenicity
	Anemia, leukopenia, thrombocytopenia
III.	Azathioprine
	Nausea and vomiting
	Abnormal liver function tests
	Increased risk of infection

Intravenous Cyclophosphamide

Intravenous cyclophosphamide is given once a month for 6 consecutive months, starting at a dose of 0.5 to 0.75 g/m^2 body surface area (BSA) and increasing by 0.25 g/m^2 BSA on successive treatments (not to exceed 1g/m^2 BSA), provided that the 2-week leukocyte count remains above 3,000 cell/mm^3. As per the traditional NIH protocol, after the first 6 months, pulse cyclophosphamide is given every 3 months for a total of 24 months. Patients with significant renal impairment need a reduction in the dose of parenteral cyclophosphamide. The role for intermittent intravenous cyclophosphamide therapy was established by prospective, controlled clinical trials performed at the NIH (211 ,212 ,213 ,214). These trials demonstrated greater long-term renal survival with the use of cyclophosphamide as compared to corticosteroids alone. Academic community reports have also noted efficacy (215). Continuing quarterly pulse cyclophosphamide for at least 1 year after renal remission decreases the frequency of nephritic relapses and the risk of renal function deterioration (216 ,217 ,218).

Pulse Methylprednisolone

Pulse methylprednisolone as a single agent is not superior to cyclophosphamide-containing regimens (214). Typically at our institution, treatment for class IV or severe class III lupus nephritis is initiated with pulse methylprednisolone (7 mg/kg/day for 3 days) followed by intermittent intravenous cyclophosphamide and oral prednisone. Prednisone is started at a dose of 1 mg/kg/day (not exceeding 60 mg daily for adults) for the first month, followed by a gradual taper over the following 3 to 4 months. In two controlled trials for the treatment of proliferative lupus nephritis the combination of cyclophosphamide and pulse methylprednisolone monthly afforded a more rapid response and greater probability of renal remission, though at the cost of greater toxicity (212 ,214).

Azathioprine

The role of azathioprine in the treatment of proliferative lupus nephritis is less well established than that of cyclophosphamide. Early studies suggested improved outcomes with the use of azathioprine in combination with corticosteroids over corticosteroids alone, whereas in the NIH randomized, controlled trial azathioprine was no more effective than prednisone alone in reducing renal failure (211). However, azathioprine has fewer side effects than cyclophosphamide and may be considered for patients with focal proliferative (WHO class III) nephritis without markers associated with greater risk of ESRD (such as histologic findings

of necrosis, cellular crescents, or significant chronicity). In recent years, azathioprine has also been proposed as an effective maintenance regimen for patients with lupus nephritis following 6 months therapy with intravenous cyclophosphamide (219 ,220 ,221 ,222). Recently, measurement of azathioprine metabolites has become clinically available, allowing titration of the dose for individual patients to maximize therapeutic response and minimize the risk of toxicity (223). An ongoing study is evaluating the efficacy of this approach (J. Buyon, personal communication). In addition, patients lacking 6MMPT can be spared exposure to the drug (224).

Mycophenolate Mofetil

More recently, clinicians are choosing to shorten intravenous cyclophosphamide exposure to 6 months followed by a maintenance regimen of a purine synthesis inhibitor such as azathioprine or mycophenolate mofetil (MMF). Recently, Contreras et al. have shown improved overall and renal 5 year survival in patients receiving MMF or azathioprine following induction with 6 to 7 doses of IVC (219). In Europe, physicians have often employed shorter exposure to cyclophosphamide and use of maintenance with alternative immunosuppressants, or avoidance of IVC entirely. Euro-Lupus Nephritis Trial (ELNT) compared a modified NIH induction regimen (six monthly pulses followed by two quarterly pulses) to low dose (six biweekly pulses of 500 mg) IVC, followed in both treatment arms by maintenance with oral AZA at 1 mg/kg/day (225). Remission occurred in 71% of the low dose and 54% of the high dose group; the rate of long-term flares did not differ. Over a median follow up of 73 months, the probability of end-stage renal disease, doubling of serum creatinine, or death were not significantly different between patients in the low- versus high-dose IVC groups. Repeat renal biopsy in 20 of these patients after a mean of 27 months did not differ between treatment groups (226). On the whole, ELNT patients had less renal impairment at study entry compared with the NIH studies. Several reports and case series have noted efficacy of MMF in patients with resistant or relapsing lupus nephritis (227 ,228 ,229 ,230 ,231 ,232 ,233). Chan et al. noted comparable efficacy of MMF to oral Cytoxan therapy for severe nephritis (234). More recently, Ginzler et al. noted promising results in the use of MMF compared to IVC as induction therapy in a high risk population of U.S. patients with lupus nephritis (235). Ong et al. also noted the superiority of MMF to IVC as induction for severe lupus nephritis (236). Despite these encouraging results, it is currently premature to advocate the use of MMF as a first-line drug in the treatment of class IV lupus nephritis as data on duration of therapy required and long-term outcomes are still lacking.

Alternative Therapies

Cyclosporin has been shown to be effective in reducing clinical and histologic activity in proliferative lupus nephritis. Autoantibody formation and hypocomplementemia do not uniformly improve and the frequent occurrence of hypertension and nephrotoxicity limit the utility of this therapy (237 ,238). Case reports and series of efficacy of intravenous γ globulin in refractory severe lupus including nephritis have been noted (239 ,240). However, reports have also noted severe exacerbation of lupus with development of vasculitis have been described as toxicities following intravenous immunoglobulin (IVIG). Nephrotoxicity can be a serious rare complication of IVIG therapy because of osmotic nephrosis when sucrose is used in the preparation. Pre-existing renal disease, volume depletion, and older age are risk factors for such toxicity. Additionally, hyperviscosity complicated by neurologic events may occur. Previous variable results of IVIG treatment in SLE could be related to variable enrichment of different lots of IVIG in suppressive antipathogenic Id antibodies.

A controlled clinical trial showed no additional benefit of plasmapheresis for lupus nephritis compared with corticosteroids and short-course oral cyclophosphamide therapy alone (241). Plasmapheresis may however have a role in the treatment of patients with overwhelming disease in whom standard therapy is failing. The incidence of thrombotic thrombocytopenic purpura is increased in SLE. In the GDCN nephropathology database TTP occurs in 10% of patients with severe class IV lupus nephritis. In this disorder, plasmapheresis is lifesaving.

Renal Transplantation in Patients with SLE

Studies addressing the outcome of renal transplantation in patients with SLE compared to that of non-SLE patients have led to conflicting results (242 ,243 ,244 ,245 ,246). A case control study of 97 renal transplant recipients with SLE, matched for age, gender, race, type of allograft, number of previous transplants, and year of transplantation, revealed relative risk of graft loss of 2 when SLE was the cause of ESRD (242). Conversely, recent data from the United States Renal Data System compared the outcomes of 772 adults with ESRD from lupus nephritis and 32,644 adults with ESRD because of other causes who received a transplant between 1987 and 1994. After adjusting for potential confounding factors, the risk of graft failure or patient mortality was not increased in patients with SLE after first cadaveric and first living-related renal transplant (243). The reported rates of recurrent SLE disease post transplantation are also widely variable; however recurrent lupus nephritis appears to account for graft loss in <4% of grafts. The presence of antiphospholipid antibodies may be associated with an elevated risk of thrombotic complications and graft loss raising the question as to whether patients with a history of antiphospholipid syndrome should receive anticoagulant therapy in the immediate posttransplant period.

Clinical Guidelines for Evaluation and Treatment of Lupus Nephritis

- All patients who present with lupus nephritis should have a renal biopsy providing no contraindications exist (severe thrombocytopenia, refusal of blood

products, uncorrectable coagulopathy) and a physician who is expert in biopsy is available. Because therapy often differs greatly for different histopathologic classes, tissue evaluation is essential. In addition to classifying the lesion, activity and chronicity indices should be described with attention to high risk features such as crescent formation, karyorrhexis, or necrosis. A repeat renal biopsy may be required in patients with a changing clinical course in whom additional, more aggressive therapy is being considered.

- Evaluating renal activity should include: urine sediment appearance, serum creatinine, blood pressure, serum albumin, C3 complement determination, anti-DNA, proteinuria (often estimated by protein to creatinine ratio), and creatinine clearance. These may be monitored as the clinical situation dictates. Daily measurement of serum creatinine may be useful in rapidly progressive disease; other parameters require 1 to 2 weeks to change.
- Patients with antiphospholipid antibodies and nephritis have a poorer renal outcome, more histologic thrombotic microangiopathy, and increased complications with dialysis and transplantation. At a minimum, low-dose aspirin should be given; individuals with a history of a thrombotic event should be on lifelong warfarin or an equivalent thromboprophylactic regimen. This may complicate renal biopsy as anticoagulation is typically suspended for a time following biopsy to decrease the risk of bleeding at the biopsy site.
- Hypertension must be aggressively treated. With lupus nephritis the goal should be age-appropriate blood pressure (especially important in young patients). The target blood pressure for patients with a history of glomerulonephritis should be less than or equal to 120/80 mm Hg as for patients with other glomerulonephritides. The entire spectrum of antihypertensive agents have been used in patients with lupus, but there is ongoing interest in the benefit of angiotensin-converting enzyme (ACE) inhibitors, especially in patients with persistent proteinuria. ACE inhibitors may have renal protective properties, over and above their antihypertensive effects (247). The use of ACE blockers alone or in combination with ACE inhibitors is also commonly employed. Dietary restriction of salt is recommended in all patients with hypertension accompanying active lupus nephritis. Loop diuretics are used to diminish edema and control hypertension as needed, with appropriate electrolyte monitoring. Diuretics are often essential to control blood pressure while on high dose steroids with associated sodium retention. With nephrosis and hypoalbuminemia, torsemide may be more effective than furosemide. However, whenever possible, thiazide diuretics should be employed as they avoid the increased calciuria produced by loop diuretics, decreasing the risk of osteoporosis.
- Hypercholesterolemia must be controlled both to reduce the risk of premature atherosclerosis and to prevent decreased renal function. Fat intake should be restricted if hyperlipidemia is present or the patient is nephrotic. The new American Heart Association (AHA) guidelines of serum cholesterol below 180, rather than 200 should be the target for therapy, given the increase in cardiovascular disease with SLE. Although there are yet no prospective controlled studies published demonstrating improved outcome in lupus patients, such studies are underway in an inception cohort followed in the Systemic Lupus International Cooperating Clinics (248). Clinically, it is recommended that patients follow a low cholesterol, low-fat diet and when hyperlipidemia is persistent receive lipid lowering agents such as the HMG Co-A reductase inhibitors. Many clinicians, recognizing the increased risk of atherosclerosis in lupus patients, will advise patients to take an aspirin daily and folic supplementation of 1 to 5 mg daily. Plaquenil has also been associated with fewer cardiovascular events, particularly in patients who have APL.

  Additionally, patients with renal insufficiency may be placed on a low-protein diet to reduce the adverse effects of protein on renal hemodynamics and hyperfiltration. Supplementation with vitamin D 1,25 or erythropoietin may be required as patients develop chronic renal insufficiency as despite the best of efforts some patients will develop ESRD.

- Compulsive attention must be paid to the early detection and aggressive treatment of infections as they account for about 20% of deaths among patients with SLE.
- Whenever corticosteroids are used, measures must be taken to minimize the development of osteoporosis (following ACR guidelines to prevent steroid induced OP (249)). These include calcium and vitamin D supplementation, weightbearing exercise as tolerated and potential therapy with pharmacologic agents including calcitonin if renally impaired, bisphosphonates (unless contraindicated by azotemia or gastrointestinal toxicity) or recombinant PTH. (See Chapter 60 , Adjunctive Measures and Issues: Allergies, Antibiotics, Vaccines, Osteoporosis, and Disability).
- The following parameters are essential to monitor toxicity associated with corticosteroids, diuretics, and cytotoxic agents: blood pressure, complete blood count, platelet count, potassium, glucose, cholesterol, liver function tests, weight, muscle strength, gonadal function, and bone density. These are closely monitored as the clinical situation requires.
- Patients are instructed to avoid therapeutic doses of salicylates and nonsteroidal antiinflammatory agents, because they may impair renal function, exacerbate edema and hypertension, and increase risk of gastrointestinal toxicity (particularly in combination with corticosteroids and immunosuppressive agents). If absolutely necessary during the course of treatment for nephritis, they should be given for short periods at low doses with careful supervision. The cardiovascular risks with NSAIDs is presently unknown.
- Pregnancy should be discouraged in patients with active nephritis as the risks for maternal and fetal

morbidity and mortality, including renal failure, are increased. Pregnancy while requiring dialysis is high risk to both mother and fetus, has a low success rate and requires near daily treatments. Contraception, fertility, and pregnancy are important issues in this predominately female patient population. Advice on the choice of contraceptive method should be given, keeping in mind additive thrombotic risk factors, including the presence of antiphospholipid antibodies (aPL), hypertension, and nephrotic syndrome. Clinical trials of estrogen containing oral contraceptives in APL negative premenopausal women with SLE have recently been published, showing no increase in lupus flares (249 ,250). In postmenopausal women, an increase of 10% in risk of mild to moderate but not severe flares was noted with hormone replacement therapy (251). In small pilot studies of women with SLE, the use of the GnRH agonist leuprolide acetate appeared to prevent cyclophosphamide-induced ovarian failure (252 ,253 ,254). However, as this agent is an agonist, in the first few days levels of estrogen may be increased, raising the risks of ovarian hyperstimulation syndrome, multiple birth if pregnancy occurs and possible blood clots. These risks must be reviewed carefully with the patient and referring physicians: In young women with multiple risks or with a history of clotting, consideration of subcutaneous heparin until estrogen production is suppressed is wise. Of course, bone density must be assessed and maintained (254).

- Antimalarials may be given or continued for active skin disease or to reduce risks of antiphospholipid antibody syndrome, but will not impact nephritis.

Treatment of lupus nephritis requires an understanding of the immunopathogenesis, risk stratification by renal biopsy classification, appropriateness of renal biopsy, and finally, familiarity with the specific therapeutic modalities. Seasoned clinicians, familiar with the experience with hemo and peritoneal dialysis as well as renal transplantation in lupus patients, recognize the need to abandon immunosuppressive therapy once advanced glomerulosclerosis has developed. It is useful to discuss the various therapeutic agents for lupus nephritis in the context of the specific class of lupus renal disease. The following therapies are advised for specific biopsy patterns (Fig. 56-1):

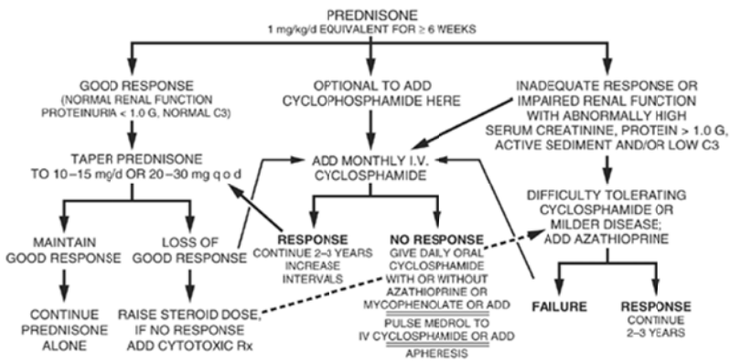

Figure 56-1. Algorithm for the treatment of proliferative (class III or IV) nephritis.

- ISN Class I (WHO class II): Many mesangial lesions do not need specific therapy. In patients with WHO class II patterns and over 1 g of proteinuria, high anti-dsDNA, and low C3 complement, we usually administer prednisone and hydroxychloroquine in accordance with the degree of extrarenal clinical activity.
- ISN III or IV (WHO class III and IV): These are treated similarly and have similar prognoses, especially when high risk features such as crescents or necrosis are present. (Fig. 56-1). Because the risk of end-stage renal disease in 10 years may exceed 50% especially in a majority African-American population, aggressive management is advised. We recommend the following:
  - Administration of 1 mg/kg/day of prednisone equivalent for at least 4 weeks, depending on clinical response. The race and age of the patient will impact steroid therapy. Children and young adults into the early 20s may be more steroid sensitive and require higher doses of prednisone than older patients. For issues in the treatment of lupus nephritis in children, see Chapter 41 , Systemic Lupus Erythematosus in Childhood and Adolescence. Pediatric rheumatologists not uncommonly employ high dose prednisone as 2 mg/kg/day. Cytotoxic drugs often take months to become effective, and glucocorticoids stabilize the patient in the interim. Prednisone is tapered over 3 to 4 months. Doses are decreased to a

maintenance of less than 15 mg of prednisone equivalent daily as needed for extrarenal activity.

- Prednisone treatment by itself can suppress evidence of proliferative disease, especially renal sediment abnormalities, but this course of action is associated with more renal scarring, and steroid related complications (211 ,212). Depending on the individual clinical situation, cytotoxic drugs should be added at the onset of therapy. Published studies suggest that the timely addition of cytotoxic drugs is associated with an increased probability of avoiding end-stage renal disease, as well as being steroid-sparing (255). Such benefit may require administration of cytotoxics for 2 years or longer in many patients.
- Intravenous cyclophosphamide administration is given monthly for 6 months as per the NIH regimen. We currently do not administer this therapy consecutively for more than 6 months. 2-Mercaptoethane sulfonate sodium (Mesna, Bristol-Myers Squibb Oncology/Immunology) can be given with infusions to minimize bladder toxicity, and ondansetron (Zofran, Glaxo Welcome Oncology/HIV) or granisetron (Kytril, SmithKline Beecham) minimize nausea. Based on the Contreras study (219), we follow induction with cyclophosphamide with up to 5 years of MMF or azathioprine. A current clinical trial is evaluating MMF versus cyclophosphamide therapy for 6 months' induction followed by a comparison of MMF versus azathioprine for maintenance therapy.
- Patients refractory to cyclophosphamide therapy. After 6 months of cyclophosphamide therapy, somewhere 10% and 40% of patients, especially minority patients (African American and Hispanic) and those with continued nephritic urinary sediment, will be refractory to prednisone plus intravenous cyclophosphamide (49). The following strategies may be considered:
  - Monthly pulse doses of methylprednisolone may be added to the intravenous cyclophosphamide for patients with severe renal damage, but should not be substituted for it (215).
  - Change to oral immunosuppressive with azathioprine, MMF, cyclosporin, cyclophosphamide, or combinations of these drugs.
  - Raising daily corticosteroid doses after induction is discouraged for renal indications as the higher dose may improve sediment abnormalities but does not improve long-term renal outcome.
  - Consideration of experimental therapies including B cell depletion with rituximab (anti-CD20), intravenous gamma globulin, addition of CTLA4-Ig to cyclophosphamide therapy or bone marrow transplantation.
- Acute flares with renal deterioration can be managed with pulse methylprednisolone and consideration of a new immunosuppressive regimen. Apheresis may be useful if the patient has cryoglobulinemia, hyperviscosity, catastrophic antiphospholipid antibody syndrome or thrombotic thrombocytopenic purpura.
- Special circumstances may warrant adjustments or changes in the above regimen. These include:
  - Corticosteroids: uncontrollable diabetes or hypertension, multiple sites of painful avascular necrosis, severe osteoporosis, steroid psychosis, life-threatening infection, severe myopathy.
  - Cyclophosphamide: refractory hemorrhagic cystitis despite Mesna therapy, severe nausea and/or vomiting, refusal to accept the possibility of infertility, prior radiation therapy, history of malignancy, cytopenia as a result of marrow suppression (cytopenias as a result of peripheral destruction are not contraindications).

  Azathioprine or mycophenolate mofetil usually is the second-line agent of choice. Infrequently, cyclosporine, chlorambucil, 2-CDA or nitrogen mustard (256) may be advised.

- Class V: Patients may be treated with 1 mg/kg/day of prednisone equivalent for 6 to 12 weeks, followed by its discontinuation if there is no response or tapering to a maintenance of 10 mg prednisone equivalent a day for 1 to 2 years if there is a response. Others may employ the Ponticuli protocol of alternate day prednisone. Cytotoxic drugs generally are not used unless patients have severe nephrosis (>10 g proteinuria daily) or developing renal insufficiency is present. Pure membranous lesions are uncommon, composing 15% to 30% of all biopsies. Recently, evidence has been presented suggesting that cyclosporine is effective in managing membranous nephritis, though this is controversial.
- Patients with a long-standing creatinine level over 3 mg/dL and/or a high chronicity index.
  - Aggressive management usually is not advised unless a high activity index also is present or extrarenal disease warranting cytotoxic therapy is evident. It is better to plan for dialysis and/or transplantation. Chronic renal insufficiency evaluation should include measurements of erythropoietin, vitamin D 1,25, calcium, PTH, and phosphorus levels.
  - Patients may be maintained on 5 to 10 mg of prednisone equivalent daily if needed to control extrarenal lupus, bearing in mind the increased risk of infection if the patient requires a peritoneal or hemodialysis catheter rather than a shunt or graft for dialysis access.
- Class VI. Patients who should not be treated include those with significant renal scarring or other evidence of irreversible disease. There is little benefit in aggressively managing patients with a stable creatinine level above 5 mg/dL; it frequently produces more harm than good. The reader is referred to Chapter 62 , Nonpharmacologic Therapeutic Methods, for a discussion of the management of patients with lupus and end-stage renal disease (specifically, dialysis and transplantation).

When Should a Renal Biopsy Be Performed?

An important issue in the evaluation and treatment of lupus renal disease is the necessity and timing of a renal biopsy. The strongest argument for a renal biopsy is the likelihood that the histopathologic findings will influence initiation, selection or discontinuation of therapeutic agents. Dubois noted two primary reasons to obtain a renal biopsy: confirmation of diagnosis in equivocal cases and determination, in advanced cases, whether further treatment was indicated. Diffuse scarring with little or no inflammation would prompt conservative management alone (256). Fries et al. (257) reviewed 177 renal biopsies, concluding that the information provided prognostic but added little clinical information. It was not cost-effective and had some inherent risks. Other reports appearing between 1978 and 1985 questioned the interobserver reliability between pathologic scoring, failed to correlate a clinical nephritis index with histologic patterns, and showed that the World Health Organization (WHO) histologic classification did not predict results of therapy at rebiopsy 12 months later (258 ,259 ,260 ,261 ,262 ,263 ,264).

Several advances have increased the frequency and safety of renal biopsies. The development of activity and chronicity indices by the NIH allowed comparison of lesions for outcome and for changes in response to treatment (265). High chronicity scores clearly are associated with a poor outcome and lack of response to immunosuppression. High activity indices, especially with more than 30% crescents, also are associated with poor outcomes but often are reversible with aggressive treatment (Table 56-4). However, some have questioned their value or reproducibility in a community setting (266 ,267). Availability of an improved renal biopsy needle, and real-time ultrasound guidance decreases the risk of significant bleeding. Evidence that tubulointerstitial disease, diagnosed only at biopsy, is of prognostic importance (268). Clinically, mesangial disease can present identically to proliferative disease. Because the therapies are different and only biopsy can distinguish the two, biopsy is desirable. Mesangial disease does not require cytotoxic therapy (146 ,153). Pure membranous disease has a different treatment and outcome than proliferative disease and can only be diagnosed by biopsy (269 ,270). Mixed membranous and proliferative nephritis may have a worse outcome than proliferative disease alone (271).Three well-designed studies document that biopsy patterns have prognostic importance and help to predict outcomes (264 ,272 ,273).

Table 56-4: Renal Pathology Scoring System[a]

Activity Index	Chronicity Index
Glomerular abnormalities	
1. Cellular proliferation	1. Glomerular sclerosis
2. Fibrinoid necrosis, karyorrhexis	2. Fibrous crescents
3. Cellular crescents	
4. Hyaline thrombi, wire loops	
5. Leukocyte infiltration	
Tubulointerstitial abnormalities	
1. Mononuclear cell infiltration	1. Interstitial fibrosis
	2. Tubular atrophy

[a]Fibrinoid necrosis and cellular crescents are weighted by a factor of 2. The maximum score of the activity index is 24; that of the chronicity index is 12. *Source:* From Austin et al. (69); with permission.

Despite these general correlations, there is substantial overlap in the clinical presentation of patients with the various histopathologic findings and it is very difficult to ascertain the type or severity of renal disease based on clinical grounds alone. For this reason, a renal biopsy is very useful—if not essential—in the management of patients with suspected lupus nephritis. It provides an invaluable guide to therapy by clarifying the clinicopathologic syndrome, and assessing the relative degrees of active inflammation and chronic scarring. It may also identify unsuspected causes for an acute worsening in renal function such as the development of a thrombotic microangiopathy, or a drug-induced tubulointerstitial nephritis.

Thrombotic microangiopathy is increased in frequency in SLE sometimes associated with antiphospholipid antibodies or with an overlap syndrome such as systemic sclerosis. Another difficulty in managing patients with lupus nephritis lies in the fact that the pathologic lesion may change from one form of glomerular injury to another. As reviewed in Chapter 55 , up to 30% of patients undergoing second biopsies transform to different patterns; Chapter 55 reviews the various patterns and their clinicopathologic significance.

Course of Lupus Nephritis

Over the last five decades, a tremendous change has evolved in the approach to lupus nephritis, and this has greatly altered its outcome (2).

Studies From 1950 to 1989

In the early 1950s, low-dose corticosteroids were used, with 5-year survival rates of close to zero (274). By the late 1950s and early 1960s, prolonged high-dose corticosteroids were employed (with a few centers using nitrogen mustard), and the overall 5-year survival rose to 25% (275). The mid to late 1960s were characterized by the availability of hemodialysis, moderate-dose steroid usage, and widespread use of high doses of azathioprine and oral cyclophosphamide. The overall 5-year survival then was 40% to 70%. By the early 1970s, physicians temporized their use of cytotoxic drugs and took advantage of newer antibiotics and antihypertensive agents, resulting in 60 to 80% 5-year survivals. (In the Dubois/Wallace series (20), 10-year survivals for middle-class patients diagnosed in the decades beginning in 1950 were 65% and then 60% [1960], 76% [1970], and 92% [1980]). The WHO classification system helped to stratify renal disease into pathologic subsets that

helped tailor therapy. The 1980s saw the introduction of intermittent, parenteral cyclophosphamide combined with corticosteroids. Activity and chronicity indices were described, and interventions with pulse-dose corticosteroids and apheresis became more common. In 1989, Esdaile et al. (276) were able to document 85% and 73% 5- and 10-year survivals, respectively, among 87 patients followed for a mean of 8.4 years. Unfortunately, in the United States, this improvement in mortality has not been experienced uniformly. Although the survival rates in Caucasians have improved, mortality rates continue to rise in African-American patients (1).

Recent and Cumulative Insights

Along the way, new insights were derived that allowed investigators to determine prognostic subsets and assess the impact of various therapies. Associated with a poorer outcome were nephrotic syndrome, nephritis with class IV lesions, high chronicity, hypertension, interstitial disease, smoking, infections, thrombocytopenia, and childhood onset of nephritis (20 ,27 ,62 ,70 ,207 ,208 ,209 ,277). The NIH group correlated corticosteroid therapy with progressive renal scarring and a worse prognosis than in those given corticosteroids plus cytotoxic treatments (211). Blacks, race, younger age at onset, low complements, anemia, and crescents on biopsy were similarly associated with a poor prognosis (21 ,49). Efforts also were made to correlate prognosis with biopsy pattern. Mcclusky (211) in 1975 and Pollak and Kant (212) in 1981 summarized several studies and found the 5-year survival rate of patients with minimal lesions to be 80% to 90%, with mesangial lesions to be 68%, mild proliferative lesions to be 40% to 80%, severe proliferative lesions to be 25% to 40%, and membranous lesions with proliferative changes to be 60% to 80%. The worst prognostic subset of lupus nephritis is nephrotic syndrome, in which one half were dead within 10 years (20 ,213). The most common causes of death were and continue to be complications of renal disease and sepsis (22). Papers in the last few years for the first time have mentioned discontinuing therapy after successful treatment (214 ,215), and more emphasis is being placed on decreasing the risk of evolving end-stage renal disease. In clinical research, emphasis is focusing on genetic susceptibility to severe nephritis and potential biomarkers of disease activity and response (278 ,279 ,280). Despite all these advances, however, certain subsets of patients with focal or diffuse proliferative lesions and scarring glomerular and tubulointerstitial regions still have a 50% chance of evolving into end-stage renal disease within 5 years, and aggressive management appears to be warranted (49 ,216 ,217 ,218 ,219). Of 150 patients who were seen by one of us, 10%, 19%, and 30% developed end-stage disease at 5, 10, and 15 years, respectively (27). Piette's French group followed 180 lupus nephritis patients at a single center. In 1999, their 5-, 10-, and 15-year renal survivals were 95%, 89% and 76%, respectively (2). Mok et al. followed 183 nephritis patients in Hong Kong. In 1999, their 5, 10, and 15 year renal survivals were 94%, 92%, and 75% (64). Remissions after initial therapy for nephritis correlate with an improved renal and patient survival (224).

Summary and Future Directions

In summary, lupus nephritis has evolved from a frequently terminal process to one in which a fairly normal quality of life and good outcome are possible. First, the treating physician must accurately stage the disease with laboratory and tissue evaluations. Next, therapy is fashioned for the specific disease subsets that are involved. Third, both side effects and the complications of treatment must be managed, along with frequent assessments and modifications of therapy depending on the patient's response. At present, many centers are reassessing the traditional 2-year NIH regimen of cyclophosphamide for severe lupus nephritis, reducing exposure to this toxic therapy and employing long-term maintenance with azathioprine or MMF. There are currently more than eight clinical trials of agents for treatment of lupus, including the introduction of biologic therapies (Discussed in Chapter 66 , Experimental Therapies in Systemic Lupus Erythematosus). These include monoclonal antibodies directed against anti-CD40 ligand, BLYS, BAFF, complement component C5, B cell depletion with anti-CD20 chimeric antibody, and T cell tolerogen CTLA4-Ig and anti-DNAase (reviewed in Table 56-1). None have so far been sufficiently evaluated to warrant their use outside of controlled clinical trials.

It is critical for expert clinicians to interpret this rapidly changing field to best advise individual physicians and patients on the optimal individual therapeutic regimen.

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Section VI
The Management of Lupus

Chapter 57
Principles of Therapy and Local Measures

Daniel J Wallace

Formulation Overview

One of the most difficult and misunderstood aspects of lupus is its management. Before therapy is initiated, the practitioner must determine the type of lupus and, on this basis, formulate a treatment program. Because the prognosis of each clinical subset differs widely, it is essential that the patient database be completed before an educational session is initiated. Have all blood tests, radiographic measures, biopsies, and scans providing information that can affect treatment been performed? Once these prerequisites have been met, the physician should be able to answer the following questions:

- Does the patient meet the American College of Rheumatology (ACR) criteria for systemic lupus erythematosus (SLE)?
  - If not, does the patient meet biopsy criteria for discoid lupus or subacute cutaneous lupus? If not, is the physician satisfied that the patient has SLE, in spite of lacking four criteria? (This distinction is a matter of clinical judgment.)
  - If not, does the patient have an undifferentiated connective-tissue disease (UCTD)? Some patients with clear-cut inflammatory arthritis, a positive antinuclear antibody (ANA), and constitutional symptoms are treated similarly to lupus patients (see Chapter 49). About 14% with UCTD evolve to classical SLE.
  - If so, are related disorders such as mixed connective-tissue disease (MCTD), scleroderma, and dermatopolymyositis excluded?
- If the patient has SLE, is life-threatening organ involvement present? If not, does the patient have mild SLE?
- Which subset best describes the disease? Does a particular aspect of the patient's disease require specific considerations, intervention, or counseling (e.g., antiphospholipid syndrome, Ro (SSA) positivity, seizures, inappropriate behavior, concurrent fibromyalgia)?

Some patients have been labeled as having SLE, when in fact they do not. The implications of telling a patient that he or she has lupus are tremendous. The emotional and psychologic effects of receiving this diagnosis open up new worlds of powerful and expensive medications, influence career planning and family life, alter one's productivity and lifestyle, and in the United States, makes it difficult to obtain health, life, or disability insurance. If a patient is not certain of the diagnosis, they should not lock themselves in (1).

The Educational Session

All newly diagnosed patients, as well as those who are new to the treating physician, deserve an educational session that includes concerned family members and loved ones. The session is supervised by the physician and may involve other health professionals or use audiovisual aids. Several studies have demonstrated that socioeconomic differences account for the widely divergent outcomes in those with SLE (2 ,3) (see Chapter 69). It is critical that the patient establish a relationship and a rapport with the physician, speak a common language, keep appointments, take medication as prescribed, have transportation to the medical office, and have access to medical assistance or advice 24 hours a day. Educational and informational literature relating to various aspects of the disease, including therapy, are available from organizations such as the Arthritis Foundation and the Lupus Foundation of America (see Appendix 2 for additional resource information).

The treatment of lupus erythematosus is divided into three categories: (1) physical and psychologic measures, (2) drug therapy, and (3) surgery. The latter is employed occasionally, and the first is infrequently discussed. Table 57-1 summarizes the issues that should be discussed with the patient and family during the educational session.

General Therapeutic Considerations

Rest, Sleep, and the Treatment of Fatigue

Fatigue is present in at least one half and up to 87% of all patients with SLE, and it can be their most disabling symptom (4 ,5). Potentially reversible causes of fatigue should be ruled out first. These include anemia, fever, infection, hypothyroidism, hormonal deficiencies, hyperglycemia, and complications from medication. In SLE, fatigue may be related to cytokine dysfunction as well as to inflammation.

The administration of certain cytokines is known to induce fatigue (6), and hypoimmune fatigue syndromes could reflect decreases of the stress response (7) (see Chapters 17 and 38).

Table 57-1: Issues to Be Covered in the Educational Session

1. What is lupus, and what are its causes?
2. Many types of lupus exist. You have (cutaneous, mild, organ-threatening) lupus erythematosus, which has a (fair, good, excellent) prognosis with treatment.
3. Physical measures include the use of heat, exercise, and diet. Physical, occupational, or vocational therapy may be helpful. Discuss general preventive strategies that relate to osteoporosis, avoiding infections, and immunizations.
4. Psychologic measures include dealing with fatigue, emotional stress (in appropriate cases), physical trauma, family and job problems, and pain. Genetic counseling and a discussion of lupus and pregnancy may be needed.
5. Medication includes salicylates, nonsteroidal antiinflammatory drugs, antimalarials, corticosteroids, cytotoxic drugs, and innovative therapies. Adjunctive measures such as vitamins, birth control, and the prevention of antiphospholipid antibody complications may be helpful.
6. Resource information includes patient education materials, counseling availability, self-help, websites, exercise groups, and useful telephone numbers.

Reduced muscle aerobic capacity may play a role (8). The concept of pacing is paramount in managing fatigue. Total bed rest can worsen fatigue as well as promote osteoporosis, muscle disuse, atrophy, and contractures. Overexertion and fatigue denial also are counterproductive. Patients should pace themselves. An hour or two of morning activity should be followed by a midmorning break. A couple of hours of late-morning activity could be followed by a restful lunch break. Periods of activity followed by periods of rest usually permit most patients with lupus to attain an improved level of functioning and productivity.

The quality of sleep in SLE is impaired compared with healthy controls. This is most likely because of depressed mood, fibromyalgia, steroid therapy, and lack of exercise (9 ,10).

Treatment of fatigue requires consideration of the source and contributory factors. Iron deficiency anemia is common because of dietary deficiency, heavy menstrual periods, and/or blood loss resulting from the use of salicylates and nonsteroidal anti-inflammatory drugs (NSAIDs). If the fatigue is caused by parenchymal pulmonary disease, oxygen may be helpful; if it is secondary to inflammation, anti-inflammatory drugs are used. In addition to corticosteroids, quinacrine and hydroxychloroquine (Plaquenil, Sanofi) are cortical stimulants and may decrease fatigue in patients without organ-threatening involvement (11 ,12). Dehydroepiandrosterone (DHEA), modafinil (Provigil, Cephalon) and selective serotonin reuptake inhibitors (e.g., fluoxetine [Prozac, Dista)] can be useful. Many patients with SLE who have minimal disease activity and normal blood work (other than a positive ANA) complain of profound fatigue. Depression, fibromyalgia, and emotional stress must be excluded as causes. Three surveys have suggested that fibromyalgia or depression are the most common causes of fatigue in SLE (13 ,14 ,15). Secondary fibromyalgia with a concomitant sleep disorder is not uncommon (16). Some physicians empirically prescribe low doses of thyroid, vitamin B12 injections, or amphetamines for the nonspecific fatigue of SLE; however, the routine use of these agents should be discouraged. Occasionally, I have found methylphenidate hydrochloride (Ritalin, Novartis) to be useful in severe cases. In contrast, the use of agents that promote restorative sleep should be considered.

Exercise, Physical Therapy, and Rehabilitation

A major cause of fatigue in SLE is from deconditioning. Aerobic capacity is 62% of the average expected in healthy, age-matched females (10). Inflamed peripheral muscles in SLE may result in decreased aerobic exercise capacity (8 ,17). Exercise regimens improved physical functioning and fatigue (18 ,19 ,20 ,21). The patient with SLE should remain physically active and avoid excessive bed rest. Exercises that strengthen muscles and improve endurance while avoiding undue stress to inflamed joints are desirable. Activities such as swimming, walking, low-impact aerobics, and bicycling should be encouraged. Recreational activities involving fine-motor movements and placing stress on certain ligamentous and other supporting structures (e.g., bowling, rowing, weight lifting, golf, tennis, jogging) should be considered on an individual basis. Exercises involving sustained isometric contractions increase muscle strength more than isotonic exercises do (e.g., stretching, Pilates). Physical measures, such as the use of local moist heat or cold, decrease joint pain and inflammation. Many patients benefit from a whirlpool bath (Jacuzzi), hot tub, or therapy pool, or from merely soaking in a tub of hot water.

Physical therapists instruct patients in strengthening and toning exercises, improved body mechanics, and gait training. No specific measures or treatment approaches are unique for patients with lupus. Joint deformities develop in approximately 10% of patients; physical and occupational therapies to minimize deformities are desirable in this group. Splints are useful for most patients with carpal-tunnel syndrome related to SLE. Corrective-tendon surgery and joint replacement are helpful in advanced cases.

Occupational therapists instruct patients in the principles of energy conservation and joint protection. They evaluate activities of daily living and advise on the use of devices or aids, such as wrist splints, comb handles, and raised toilet seats, when needed.

Vocational rehabilitation may be important in retraining a patient with SLE who can no longer work in the sun

(e.g., farmer, construction worker, fishermen) or perform tasks requiring fine hand-motor function (e.g., computer ergonomics).

Tobacco Smoke and Alcohol

Smoking impairs oxygenation, raises blood pressure, and worsens Raynaud among other adverse actions. Two studies have associated smoking with the formation of autoantibodies, including ANA (22 ,23). A study of 56 patients with SLE failed to show an immunosuppressive effect of tobacco, which has been held to improve ulcerative colitis (24). Three reports (25 ,26 ,27) correlated tobacco use with worse cutaneous-lupus or disease activity than among nonsmokers. Additional comparisons have confirmed that chronic, cutaneous lupus is more common in lupus than in nonsmokers (28), as is SLE (29 ,30). Two epidemiologic surveys (31 ,32) associated smoking with a 2.3 and 6.69 odds ratio for developing SLE. Because tobacco smoke contains potentially lipogenic hydrazines (see Chapter 3 , Environmental Aspects of Lupus), abstinence and avoiding second-hand smoke are important. The efficacy of antimalarials may be decreased in smokers, perhaps as a result of the effect of tobacco upon the cytochrome P450 enzyme system that metabolizes chloroquines (33 ,34).

Although alcohol can worsen reflux esophagitis common in SLE and is not advised in patients taking methotrexate, two studies have concluded that moderate alcohol use was inversely correlated with the presence of SLE (29 ,30 ,31), and two showed no influence on its development (27 ,32). A meta-analysis of 7 case-controlled and 2 cohort studies found an overall 1.5 odds ratio for developing SLE (35).

Weather and Seasons

Changes in barometric pressure can aggravate stiffness and aching in patients with inflammatory arthritis (36). In other words, whether a climate is hot or cold or wet or dry per se does not influence joint symptoms; barometric alterations do (e.g., hot to cold or wet to dry). Patients with lupus should expect some increased stiffness and aching in these circumstances and not to feel that they have done anything wrong.

Does increased summer sunlight or seasonal changes affect SLE? In Norway and Finland, there are fewer flares in January and more sun sensitive rashes during the summer (37 ,38). Sixty-six French lupus patients had significantly more organ-threatening flares during the summer (39). Israeli patients had more phototoxicity in summer months (40), but more joint pains, weakness, fatigue, and Raynaud during the winter (41).

Pain Management

Patients with lupus have an increased prevalence of pain management problems (42 ,43). Patients with inflammatory arthritis respond poorly to analgesics with no anti-inflammatory effects. The use of propoxyphene, codeine, or pentazocine in SLE should be limited to postoperative management and other situations. These drugs can induce dependence, have short-lived effects, and do not address the underlying problem. Anti-inflammatory drugs (e.g., salicylates, NSAIDs, corticosteroids), therefore, are more effective in treating pain symptoms in SLE. Some patients with chronic pain problems who are unresponsive to simple measures should be referred to pain-management centers, which use measures such as acupuncture, transcutaneous electrical nerve stimulation units, biofeedback, psychologic counseling, and physical therapy to alleviate pain and eliminate narcotic dependence. Other causes of pain in patients with SLE include avascular necrosis, headache, steroid-induced hyperesthesia, and fibromyalgia.

Role of Stress and Trauma

Many studies have shown that certain forms of emotional stress, including depression and bereavement, as well as physical trauma can affect the immune system for example, causing decreased lymphocyte mitogenic responsiveness, lymphocyte cytotoxicity, increased natural killer-cell activity, skin homograft rejection, graft-versus-host response, and delayed hypersensitivity (44 ,45 ,46). Stress, unfortunately, is difficult to quantitate for evaluation of its clinical effects. Could the impairment in T-cell immune functions be responsible for a clinical flare of lupus that is mediated by B-cell hyperreactivity? Acute stress in SLE may correlate with increased urine neopterin levels, IL-4 levels, decreased natural killer-cell response, and increased numbers of β_2 adrenoreceptors on mononuclear cells with or without a clinical disease flare (47 ,48 ,49).

Have these immune abnormalities been reproduced in patients with lupus? Can the neuroendocrine axis influence immune responses? Chapters 17 and 18 review the results of basic science, animal studies, and psycho-neuro-hormonal-immune links in this area.

Can Stress Induce Lupus?

In 1955, McLeary et al. (50) related the onset of disease to significant crises in interpersonal relationships in 13 of 14 patients with SLE. In 1967, Otto and Mackay (51) compared 20 patients with SLE to 20 controls. The SLE-hospitalized group experienced significantly more stress than other hospitalized, seriously ill controls before the onset of disease. All patients thought that stress provoked their illness. In another study, 18 of 36 patients with SLE (50%) who were interviewed believed that psychologic factors triggered disease onset, and an additional 25 thought that it was possible (52).

Can Stress Exacerbate Pre-Existing SLE?

Ropes observed 45 serious disease flares in her 160-patient cohort over a 40-year period. Of the 45 patients, 41 believed that emotional stress precipitated their flare (53).

Hinrichsen et al. (54) exposed 14 women with SLE, 14 healthy controls, and 12 sarcoidosis patients to acoustic stress. Significant increases in polymorphonuclear leukocyte and lymphocyte counts as well as significant elevations of circulating B and $CD8^+$ T lymphocytes, with a relative reduction in $CD4^+$ T lymphocytes, were noted in the healthy controls but not in the patients with SLE or sarcoidosis. The difference in effects was not steroid related. A follow-up study by this group (55) suggested that patients with SLE have significantly reduced cell mobilization to psychologic stress compared with that of controls.

Recently, Dobkin et al. reviewed various ascertainment methods for evaluating psychosocial distress and were able to correlate it with increased disease activity among 44 lupus patients (56). Recent studies have correlated clinical flares with increased psychological stress and improvements with a stress reduction program (57 ,58). One study suggested that sexual abuse (a form of chronic stress) raises ANA titers (59), and another correlated daily stress rather than stressful life events with lupus activity (60). Patients with lupus vary widely in their responses to stress (61). Nevertheless, stress reduction is a helpful measure in the overall management of SLE.

Can Physical Trauma Cause or Exacerbate Lupus?

No evidence has shown that physical trauma is related to the causation or exacerbation of SLE. Many patients' conditions, however, appeared to worsen after major vehicular accidents. Discoid lupus erythematosus (DLE) can develop as a result of physical trauma; King-Smith (62) first observed this in 1926. In 1956, Kern and Schiff (63) reported on five well-documented cases and sent a questionnaire to 400 dermatologists. Of these, 54 reported having treated 78 patients. The most common causes of DLE were blows from various objects, lacerations, and scars. In 1963, Lodin (64) confirmed these findings and noted that 10 of 458 Swedish patients (2.2%) with DLE had a documented preceding trauma. These observations were reinforced by Eskreis et al. (65) in 1988 and de Boer et al. in 1997 (66). On the other hand, profound stress or trauma may improve lupus. Ten patients of mine with diffuse, proliferative nephritis living near the epicenter of the 1994 Los Angeles earthquake showed improvements in their sedimentation rates, anti-DNA levels, and 24-hour urine proteins 30 to 45 days after the quake occurred (67), but this was not confirmed in a study from Taiwan after a 1999 earthquake (68). One report of catastrophic antiphospholipid syndrome provoked by trauma has appeared (69).

In summary, several authors have implicated stress as a factor that can induce or exacerbate SLE. However, a definitive study using large numbers of patients and controls with a similar chronic illness is needed before the association can be considered established. Until then, stress reduction is both prudent and important.

Is There a Lupus Diet?

Patients with SLE should eat three well-balanced, nutritious meals daily. Animal studies suggesting that fish-oil intake might be beneficial in the treatment of autoimmune diseases have led to several human clinical trials based on the findings that eicosapentaenoic acid and docosahexaenoic acid inhibit platelet aggregation, leukotriene B4 production by polymorphs, and 5-lipoxygenase products in polymorphs and monocytes (70 ,71 ,72). Although a small-scale, open-label study suggested immunologic benefits (73) and another an improved sense of well-being (74), in contrast to rheumatoid arthritis, three of four double-blind, placebo-controlled studies of SLE failed to show any anti-inflammatory actions or other clinical benefits, except for a slight lowering in plasma triglyceride and an elevation of high-density lipoprotein levels (75 ,76 ,77 ,78 ,79). Fish-oil derivatives may prevent miscarriages associated with antiphospholipid antibodies (80). Flaxseed does not benefit lupus nephritis (81). Substituting dietary polyunsaturated fatty acids with saturated fats (which may not be such a good idea according to this chapter's author) was suggested in one uncontrolled study (82). A diet high in fatty meats may be associated with more severe disease (83). The reader is referred to an excellent critical review of the subject (84).

The ingestion of alfalfa sprouts can induce lupus in primates and might exacerbate human SLE; thus, alfalfa sprouts should be avoided (85 ,86 ,87 ,88 ,89 ,90). The offending agent is probably an amino acid, L-canavanine, which has been shown to alter both T- and B-cell responses (85).

Milk may decrease the risk of developing SLE (31). Two controlled studies have found patients with SLE to have increased food allergies (91 ,92), but the clinical significance of this observation is uncertain (see Chapter 67 , Adjunctive Measures and Issues: Allergies, Antibiotics, Vaccines, Osteoporosis, and Disability).

Patients taking large doses of corticosteroids and those who are hypertensive should restrict their salt intake. Some patients with nephritis need to be salt, potassium, and protein restricted. Potassium supplementation may be needed for some patients on diuretics, and patients with anemia often benefit from foods with a high iron content (e.g., red meat). Steroids can increase lipid levels and induce a chemical diabetes, and a low-fat or diabetic diet should be implemented if this occurs (93).

In summary, patients with lupus should have a healthy, well-balanced diet. Avoidance of alfalfa sprouts and eating two fish meals a week may be prudent. More restricted diets are mandated among those taking corticosteroids and patients with renal impairment.

Do Vitamins Play a Role in Managing Lupus?

Almost all of my patients have asked about the efficacy of vitamins in SLE. No controlled studies have been published demonstrating any clear-cut benefits from their use. Low homocysteine levels are more common in

SLE, are associated with increased atheroembolic complications, and are treated with the administration of folic acid (see Chapter 44). Vitamin B12 and folic acid can be used to treat specific types of anemias, and vitamin E may improve wound healing. Vitamin B6 (pyridoxine) is a diuretic and has been used in carpal-tunnel syndrome as an adjunctive agent. In a controlled study, the administration of Vitamins C and E to lupus patients was associated with decreased lipid peroxidation after 3 months, but did not affect endothelial cell function (94). Vitamin C may decrease lupus activity (95).

In 4 studies, lupus patients had low serum 25-hydroxyvitamin D3 levels (96 ,97 ,98 ,99). This is associated with renal impairment and hydroxychloroquine use (which inhibits the conversion of 25 (OH)- to 1,25 (OH) vitamin D. Vitamin D, with calcium supplementation, may retard the osteoporosis that is induced by corticosteroids. Other than these nonspecific measures, the judicious use of vitamins by patients with lupus is probably harmless and often has a placebo effect as long as intake is not excessive.

The older literature is replete with references to the successful use of many systemic drugs. Vitamin B12 and pantothenic acid have been reported in controlled studies to be of benefit (100 ,101 ,102). Authorities such as Sulzberger (103) have recommended the use of vitamin B12, liver extract, and bismuth (104 ,105 ,106) for the treatment of discoid LE. Ayres and Mihan (107 ,108) reviewed ten papers that evaluated the use of the antioxidant vitamin E for discoid lesions. Nine were published prior to 1955, and a recent report suggested no benefits (109).

How Important Is Patient Compliance and Treatment Adherence?

Part of the patient educational session with a new lupus patient must be a discussion relating to compliance. In the Lupus in Minority populations: Nature Vs Nurture (LUMINA) Texas- and Alabama-based cohort, nearly half of the patients were noncompliant. They tended to be young, unmarried, African American, and ill (110 ,111). Adherence to treatment regimens tends to be problematic. One third of 195 Canadian lupus patients did not participate in mandated annual eye examinations to monitor antimalarial therapies (112). Compliance problems among 112 lupus patients in Detroit were related to depression, medication concerns, physical symptoms, short-term memory problems, and the need to child or elder care (113). Failure to comply with physician recommendations was shown to be the cause of renal failure in 5 of the 17 patients at the University of Toronto between 1995 and 1998 (114). In Great Britain, the rate of compliance was treatment specific among 50 females with SLE, ranging from 41% for using sun protection, 83% for hydroxychloroquine, to 94% for steroids and 100% for azathioprine therapy (115). Ninety-five patients at the University of Cincinnati had a similar outcome when one examined pharmacy refill records (116). Adherence to treatment plans in a critical component in managing SLE.

Sun Avoidance and Phototoxicity

UV light consists of three bands, two of which are important in SLE. UVA light (i.e., 320 to 400 nm) is responsible for drug-induced photosensitivity (i.e., photoallergic reactions) and delayed tanning, and it is constant during the day. It takes approximately 1 hour of UVA exposure to induce sunburn. UVB light (i.e., 290 to 320 nm) is more significant in SLE. It is more pronounced during midday (10 am to 3 pm) and causes sunburn readily (i.e., phototoxic reactions).

Hundreds of prescription drugs can cause photoallergic and/or phototoxic reactions. The most common are phenothiazines, tetracyclines, nalidixic acid, sulfa-containing agents, piroxicam, methotrexate, amiodarone, psoralens, phenytoin, and carprofen (117). Photosensitizing chemicals are found in certain perfumes, mercury-vapor lamps, xenon-arc lamps, tungsten iodide light sources, discotheques, color television sets, halogen lamps (118), and photocopier machines (119).

Although 93% of lupus patients have abnormal phototest results (120), clinical and self-reported sun sensitivity is reported less often. One study of 125 patients with SLE noted that 73 are sun-sensitive (121). In 42 of patients, sun exposure exacerbated systemic symptoms, and in 35, it had a significant effect on lifestyle. The mechanism by which this occurs is probably related to the action of ultraviolet (UV) light on epidermal DNA, which enhances its antigenicity, allowing anti-Ro to be exposed to the cell surface, which promotes an inflammatory response, and skin production of cytokines, prostaglandins, and oxygen free radicals (see Chapters 29 ,30 ,31).

The presence of anti-Ro/SSA antibody is associated with photosensitivity in more than 90% of white patients with SLE (122). Fluorescent lights are a source of UVA and UVB, but only rarely might their avoidance be beneficial (123). Clear jacket and bulk covers that control UV emanation without reducing visibility are available and, for all practical purposes, eliminate any risks (124 ,125).

Which Sunscreen Should Be Used in Lupus?

Although the UV end of the spectrum is the most damaging to lupus skin lesions, heat and infrared exposure also can cause exacerbations. The flares produced by infrared exposure are characterized by a marked increase in erythema of short duration. These are frequently experienced by patients with SLE who work near a hot stove, oven, or furnace for any length of time. One characteristic of DLE and SLE is that skin burns and scalds can produce localized lesions of DLE at the site of trauma (i.e., the Koebner phenomenon) even in apparently normal skin (62 ,63 ,64). Sunscreens are UV light-absorbing chemical agents in a cream, oil, lotion, alcohol, or gel vehicle. These chemicals can block UVA, UVB, or both. They include aminobenzoic-acid esters (UVB), cinnamates (UVB), salicylates (UVB), benzophenones (UVA, UVB), avobenzone (UVA), anthralites (UVA, UVB), and butylmethoxydibenzomethanes (UVA, UVB).

Physical sun blocks containing titanium dioxide and zinc oxide scatter light. A sun protection factor (SPF) value is the ratio of the time that is required to produce erythema through a UVB sunscreen product to the time that is required to produce the same degree of erythema without it. The SPF ranges from 2 (i.e., minimal protection) to 50 (i.e., highest protection). We advise outpatients to use agents with a high SPF (i.e., at least 15). A sunscreen with SPF of 15 will block 93% of UVB, whereas one with SPF 50 will block only 5% more. The Food and Drug Administration (FDA) permits sunscreen manufacturers to claim broad-spectrum protection if their products block at least part of UVA-2 light in addition to UVB. Table 57-2 lists some of the best agents. Although few of these have been tested in lupus patients, the reader is referred to the only critical, brand-name comparison study published (126).

Unfortunately, because of irritation, contact dermatitis, and occasional photosensitivity, patient compliance is poor, and it may be necessary to try several compounds before an acceptable block is found. Particularly, the alcohol base in p-aminobenzoic acid (PABA) and PABA esters may sting and dry the skin. Wind, heat, humidity, and altitude can decrease an agent's protective effect.

Table 57-2: Useful Sunscreens Currently Available for Lupus Patients

Broad-spectrum UVA/UVB sunscreens containing parsol 1789
Cetaphil Daily Moisturizer SPF 15
Coppertone Shade Spray Mist SPF 30
Coppertone Shade Sunblock Lotion SPF 30/45
La Roche-Posay Anthelios ‘L’ Cream SPF 60
Ombrelle Sunscreen Lotion/Spray SPF 15/30
Presun Ultra Lotion/Gel SPF 15/30
Solbar AVO SPF 32
Sunscreens for very sensitive skin (generally contain titanium dioxide or zinc oxide)
Clinique City Block SPF 15/25
DuraScreen SPF 30
Elta Block SPF 30/32
Estee Lauder Sunblock SPF 15/30
MD Forte Total Daily Protector SPF 15
Neutrogena Sensitive Skin Sunblocker SPF 17
Presun Sensitive Block SPF 28
Vanicream SPF 15/35
Sunscreen for lips or eyelids
Chapstick Ultra SPF 15/30
Coppertone Lipkote SPF 15
Coppertone Shade Sunblock Stick SPF 30
La Roche-Posay Antherpos Ceralip SPF 50
Neutrogena Lip Moisturizer SPF 15
Neutrogena Sunblock Stick SPF 25
Presun Lip Protector SPF 15
Moisturizer/sunscreen combinations
Cetaphil Daily Facial Moisturizer SPF 15
Elta Block SPF 30/32
Eucerin Daily Lotion SPF 15/25
Keri Skin Renewal SPF 15
Lubriderm Daily UV Lotion with Sunscreen SPF 15
Neutrogena Healthy Skin SPF 15
Neutrogena Moisture SPF 15
Oil of Olay Daily UV Protectant SPF 15
Purpose Dual Moisturizer Lotion/Cream SPF 15
Waterproof/sweat-resistant sunscreens
Coppertone Shade Spray Mist SPF 30
Coppertone Sport Spray/Stick SPF 15/30
Elta Block Super Waterproof SPF 30
La Roche-Posay Anthelios ‘S’ Cream SPF 30
Neutrogena Sunblock Spray/Stick SPF 20/25
Presun Ultra Spray SPF 27
Solbar Cream SPF 50
Oil-free sunscreens
Clinique Oil Free Sunblock SPF 15
Coppertone Shade Oil-Free Gel SPF 30
Neutrogena Oil Free Sunblock Lotion SPF 30
Neutrogena Sunblock Spray SPF 20
Ombrelle Sunscreen Spray SPF 15

Recommendation of sunscreens currently available commercially in the United States. (This list was adapted from a list published on the RxDerm-L Internet chat group in 1997. It has been updated by the authors for this review). It should be noted that this list was generated as a result of the “expert opinion” of a number of practicing dermatologists across the United States. Unfortunately, there are virtually no current published data resulting from systematic comparisons of the efficacy of various commercial products in the categories indicated in this table. Please note that LE patients are recommended to use a product containing an SPF of 30 or greater whenever possible.

Sunscreens should be applied over active and healed lesions and to areas that may burn, including the cheeks, nose, lips, and arms approximately 30 minutes before sun exposure. They can be applied over the scalp hair before going outdoors, and cosmetics may be applied over sunscreens. Two forms of UV light exposure often are overlooked. Skin lesions frequently are more intense on the left cheek and the lateral aspect of the left arm because of UVA exposure while driving a car. If the lesions are primarily distributed in these areas, the physician should inquire whether such exposure might be responsible and advise the patient on avoiding it (127). Merely keeping the window closed or tinting the window may filter the sunlight sufficiently. Automotive glass blocks UVB effectively but not UVA. Another unnoticed source of exposure is UV light that is reflected off the surface of sand, water, cement, or snow, and UV radiation is greater at higher altitudes. For example, the intensity at 5,000 feet is 20% higher than that at sea level. Patients should be cautioned about these sources of danger. A cloudy day only decreases UV exposure by 20% to 40%.

Sunscreens block vitamin D activation in the skin, and oral supplementation may be required. An occasional patient develops eye sensitivity to UV light that is not responsive to wearing ordinary sunglasses. Special coated lenses to protect

the eyes are available (128). In patients with lupus erythematosus and a definite UV sensitivity, walking a few blocks without any protection usually is permitted. If further exposure is necessary, general measures such as wearing a broad-brimmed hat (4-inch or greater is advised) and long sleeves, as well as using an umbrella can be used; these decrease UV exposure by 30% to 50%. When a remission occurs, either spontaneously or induced, greater freedom of sun exposure is permitted. Frequently, otherwise asymptomatic patients have a persistent butterfly erythema that is aggravated by sun exposure, and use of antimalarials and local sunscreens usually controls this if it is severe enough to warrant therapy. Numerous web sites offer information related to specialized sun protective clothing, local daily UV indices and guides for sun protection. They can be referenced via Dr. Richard Sontheimer or the Lupus Foundation of America.

Avoidance of UV exposure has been so overemphasized that many patients are irrational about going out during the day. Unless definite evidence of exacerbations that are provoked by such exposure is noted, normal activities need not be restricted or curtailed. Although it is advisable to caution the patient that sun exposure may cause increased local erythema or development of new skin lesions, the physician should avoid causing a sunlight phobia. The average patient, even one who is photosensitive, usually can walk a few blocks at midday without protection and experience no ill effects. The question of how limited light exposure should be must be determined on an individual basis. The physician must use judgment so that the patient's way of life is interrupted as little as possible. Because sun exposure is greatest midday, outdoor activities should be undertaken in the morning or later in the afternoon.

Antimalarial therapy increases the patient's tolerance to sun exposure even in those who were extremely sensitive to UV light before taking them (129 ,130 ,131 ,132). The degree of limitation must be reevaluated frequently, because the tendency to sunlight-induced exacerbation of skin lesions can subside, particularly with disease remission (either spontaneous or drug-induced). Even NSAIDs can be photoprotective (133).

Local Therapy for Cutaneous Lupus Erythematosus

Local treatment is used for isolated lesions of DLE or for refractory skin lesions in patients with DLE or SLE. The most effective, safe, and least scarring type of local therapy is the use of various steroid preparations. These can be fluorinated or nonfluorinated, and they may be of low, intermediate, or high potency (Table 57-3). Most nonfluorinated steroids include hydrocortisone cream or ointment and now are available as over-the-counter (OTC) preparations in strengths of less than 1%. These agents are less expensive but less potent than the fluorinated preparations, which produce more stinging, dermal atrophy, depigmentation, striae, telangiectasia, acne, folliculitis, and Candida superinfection (134). Fluorinated steroids cannot be applied to the face for more than 2 weeks at a time without expecting cutaneous side effects. Studies show that betamethasone dipropionate 0.05% (Diprolene, Schering) and clobetasol propionate 0.05% (Temovate, Glaxo Wellcome) creams or ointments are the most effective dermatologic agents for short-term treatment of discoid lesions, especially in conjunction with antimalarials (135).

These preparations should be used three or four times daily for optimal effectiveness and only applied directly over the lesions. Patients should be warned not to use them on normal skin, because they induce atrophy. Improvement usually is noted within a few days. Unfortunately, recurrences frequently appear within a few days to weeks after the cessation of treatment, but small lesions can be controlled adequately and indefinitely by the intermittent use of this method. Old, indurated, and chronically scaling lesions respond poorly to this form of treatment alone and require occlusive therapy (discussed later), intracutaneous injection, and/or antimalarials. Patients usually start on an intermediate-strength steroid cream or ointment and move up to high-potency agents for resistant lesions. Ointments generally are used for dry skin and creams for oily skin, but the ointment form is more effective than a cream, gel, or lotion. Fluorocarbon-propelled sprays are the least effective. Thin skin is more permeable to topical steroids as well.

The introduction of Actiderm (ConvaTec) patches allows for the improved absorption of high-potency steroids with less irritation. This should complement the use of translucent plastic, steroid-impregnated tape (Cordran Tape, Oclassen), and occlusive dressings such as plastic wrap, which increase percutaneous absorption by a factor of 100 and have been documented to be effective for those with severe DLE (136 ,137 ,138 ,139 ,140). Airtight occlusion of the skin causes obstruction of the sweat ducts, however, which may exacerbate pruritus and foster bacterial overgrowth on the skin surface. Jansen et al. (141) reported that topical fluocinolone acetonide cream 0.025% (Synalar, Medicis), when applied by massaging into the lesions four or five times daily or by using an occlusive dressing daily, was effective in 43 of 59 patients with DLE. It was necessary to supplement local therapy with antimalarials or corticosteroids in 11 patients. Of these 59 patients, five failed to respond, and 23 who had required antimalarials were able to discontinue them with the consistent use of local medication. All 24 patients who responded well to topical therapy and were followed through two summers did well, except for three who relapsed and required retreatment.

In another study (142), daily application of triamcinolone acetonide 0.5% in a flexible collodion base to recalcitrant DLE lesions was effective in six of seven patients compared with the use of nonmedicated collodion as a control. It may be especially helpful in the external ear, where occlusive dressings are impractical.

Intralesional therapy often is helpful when topical applications fail. Several studies have shown the value of intradermal injections of steroids. Callen, as well as other

investigators, have documented the superiority of this method to the use of topical or oral steroids in resistant lesions (140 ,143 ,144 ,145). Triamcinolone suspensions have been the most widely used; these include triamcinolone diacetate 1.25% or 2.5% suspension (Aristocort Diacetate Parenteral), triamcinolone acetonide 10 mg/mL (Kenalog Parenteral, Westwood-Squibb), and 1% or 2.5% aqueous suspension (Meticortelone Acetate Suspension).

Table 57-3: Topical Corticosteroids Ranked by Potency (65)*

Group	Generic Name	Brand Name
I. Superpotent	Clobetasol propionate cream, ointment, gel, or emollient, 0.05%	Temovate
	Betamethasone dipropionate cream or ointment, 0.05%	Diprolene
	Diflorasone diacetate ointment, 0.05%	Psorcon
	Halobetasol propionate cream of ointment, 0.05%	Ultravate
II. Potent		
	Amcinonide ointment, 0.1%	Cyclocort
	Betamethasone dipropionate cream, 0.05%	Diprolene
	Betamethasone dipropionate ointment, 0.05%	Diprosone
	Desoximetasone cream or ointment, 0.25% and gel, 0.05%	Topicort
	Diflorasone diacetate ointment, 0.05%	Maxiflor
	Fluocinonide cream, gel, or ointment, 0.05%	Lidex
	Halcinonide cream, 0.1%	Halog
	Mometasone furoate ointment, 0.1%	Elocon
III. Midpotent		
	Amcinonide cream or lotion, 0.1%	Cyclocort
	Betamethasone dipropionate cream, 0.05%	Diprosone
	Betamethasone valerate ointment, 0.1%	Valisone
	Diflorasone diacetate cream, 0.05%	Maxiflor
	Fluocinonide cream, 0.05%	Lidex-E
	Fluticasone propionate ointment, 0.005%	Cutivate
	Halcinonide ointment, 0.1%	Halog
	Triamcinolone acetonide ointment, 0.1%	Aristocort A
IV. Midpotent		
	Fluocinolone acetonide ointment, 0.025%	Synalar
	Flurandrenolide ointment, 0.05%	Cordran
	Hydrocortisone valerate ointment, 0.2%	Westcort
	Mometasone furoate cream, 0.1%	Elocon
	Triamcinolone acetonide cream, 0.1%	Kenalog
V. Midpotent		
	Betamethasone dipropionate lotion, 0.05%	Diprosone
	Betamethasone valerate cream, 0.1%	Valisone
	Fluocinolone acetonide cream, 0.025%	Synalar
	Flurandrenolide cream, 0.05%	Cordran
	Fluticasone propionate cream, 0.05%	Cutivate
	Hydrocortisone butyrate cream, 0.1%	Locoid
	Hydrocortisone valerate cream, 0.2%	Westcort
	Triamcinolone acetonide lotion, 0.1%	Kenalog
VI. Mild		
	Alclometasone dipropionate cream or ointment, 0.05%	Alcovate
	Betamethasone valerate lotion, 0.05%	Valisone
	Desonide cream, 0.05%	DesOwen
	Flumethasone pivalate cream, 0.03%	Locorten
	Fluocinolone acetonide cream or solution, 0.01%	Synalar
	Triamcinolone acetonide cream, 0.1%	Aristocort A
VII. Mild		
	Topicals with hydrocortisone, dexamethasone, flumethasone, prednisolone, and methylprednisolone	

*Group I compounds are arranged by potency.

Occasionally, acute swelling may occur at the site of injection, but this usually subsides within 24 hours. A local depression also may appear because of tissue reabsorption; this may be noted in five of patients (145) and usually disappears within several months. It is probably a pseudoatrophy resulting from true tissue destruction. James (145) reported nine patients with DLE who were treated in this manner: five had an excellent response, and the benefit was satisfactory in two. Rowell (146) treated 28 patients with multiple intralesional injections of triamcinolone at 3-week intervals. In 13 patients, the injected lesions cleared, and 13 other patients improved. Only two patients did not respond. Smith (147) also described favorable results in 13 patients with DLE. Biopsy studies before and after therapy showed a diminution of follicular plugging and hyperkeratosis in all patients, accompanied by some thinning of the epidermis. Vascular dilatation and cellular infiltration disappeared, and skin atrophy was not observed.

Topical Calcineurins for Cutaneous Lupus

The availability of 0.1% tacrolimus (Protopic) and pimecrolimus (Elidel) for eczema and allergic dermatitis has led to their anecdotal use for cutaneous lupus. In contrast to fluorinated steroids, these agents have the advantage of being applied facially without fear of inducing cutaneous atrophy. Six reports involving 20 patients report a collective 50% response rate among refractory patients (148 ,149 ,150 ,151 ,152 ,153) (see Chapter 66).

Skin Transplantation, Intralesional Antimalarials and Innovative Therapies

The transplantation of normal skin to sites of excised quiescent lesions has been successful in a small number of patients (154 ,155 ,156). Transplantation of 4-mm, hair-bearing punch grafts into active plaques of patients with DLE, however, showed recipient dominance, with a decrease in hair survival and the appearance of discoid lesions in the implanted skin (157).

Intralesional administration of quinacrine and chloroquine was used in the 1950s with good results (129 ,158 ,159), but it was abandoned because of the high incidence of hemorrhagic bullae, local discomfort, erythema, bleeding, and crusting of blood. A form of topical chloroquine clearly protects one from UVB (129). Caustic acids, intralesional gold, topical 5-fluorouracil, nitrogen mustard, BCNU, cryotherapy, imiquimod, apheresis, and topical retinoids and vitamin D analogs and solid carbon dioxide also have been used (160–167, see Chapter 66).

The reader is referred to an excellent review of the subject of topical therapies for cutaneous lupus (168).

Can Lupus Patients Undergo Topical Cosmetic Procedures?

There is no published, evidence-based material on the subject. Andrew Franks, a dermatolupologist at the New York University School of Medicine, published his anecdotal experience in the Lupus Foundation of America newsletter (Lupus News, Fall 2004, p 34–35). Lasers, collagen, hyaluronic acid gels, Botox, thermage, microdermabrasion, and sclerotherapy have been used for butterfly rashes, blemishes, telangiectasias, scars, skin tightening, blemishes and spider veins for both lupus and nonlupus-related purposes. If appropriate precautions are taken (e.g., waiting until steroids are stopped or are at their lowest possible dose, skin testing the patient with collagen first), these procedures can improve one's quality of life and appearance.

Topical Therapy for Systemic Lupus Erythematosus

Triamcinolone acetonide in an emollient dental paste (Kenalog in Orabase, Westwood-Squibb), used two or three times daily and at bedtime, is useful for sensitive lupus mucous membrane lesions. A buttermilk or hydrogen peroxide swish or gargle also is effective as an adjunctive agent. Over the long term, systemic antimalarials are more efficacious for lupus mucous membrane involvement.

Joint aches can be managed with topical nonsteroidal gels such as ketoprofen (20%) with or without muscle relaxants (e.g., 1% cyclobenzaprine) if fibromyalgia is also present. These preparations are available from compounding pharmacists.

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Chapter 58

Nonsteroidals and Salicylates for Systemic Lupus Erythematosus

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Nearly 80% of patients with systemic lupus erythematosus (SLE) are treated with nonsteroidal anti-inflammatory drugs (NSAIDs) for fever, arthritis, serositis, and headaches. This chapter reviews the literature on nonselective and selective inhibitors of cyclooxygenases, with an emphasis on the efficacy and safety profile reported in SLE patients. All NSAIDs, regardless of their cyclooxygenase selectivity, induce renal side effects including sodium retention and reduction in glomerular filtration rate. Additionally, lupus nephritis is a risk factor for NSAID-induced acute renal failure. Hepatic toxic effects to NSAIDs are increased in SLE patients as well as cutaneous and allergic reactions. Finally, aseptic meningitis has been reported more frequently in SLE patients with NSAID use. Nevertheless, and in spite of these caveats, NSAIDs can safely be prescribed to most lupus patients, provided they are evaluated and closely monitored on a regular basis.

Beginning with phenylbutazone in 1953, indomethacin in 1965, and ibuprofen in 1974, enormous quantities of NSAIDs have been tested, manufactured, and sold worldwide. They are among the most commonly prescribed drugs in the world, with estimates as high as $2 billion spent annually in the United States (1). Despite the fact that the Food and Drug Administration (FDA) has not approved a commercial preparation of NSAIDs in the management of SLE, these agents have been used widely for the treatment of SLE-associated fever, arthritis, pleuritis, and pericarditis. Although the number of well-controlled evidenced-based actual studies in SLE patients is low, this review will discuss the role of NSAIDs in the management of lupus patients, and evaluate some of the major side effects associated with their use.

Prostaglandin Synthesis and Metabolism

The cellular membrane bilayer provides the substrate for the synthesis of prostaglandins and thromboxanes. Arachidonic acid is initially produced in response to chemical or mechanical stimuli by the actions of the enzyme phospholipase A. Arachidonic acid is subsequently metabolized either by cyclooxygenase A to form an unstable endoperoxide called PGH2 or by 5-lipooxygenase to produce leukotrienes. PGH2, in turn, degrades to form prostaglandins PGI2, PGE2, PGD2, toxic oxygen radicals, and thromboxane A2. Prostaglandins induce a variety of inflammatory effects such as swelling, erythema, changes in vascular permeability, and neutrophil chemotaxis (2 ,3 ,4). Additionally, prostaglandins have a myriad of effects on multiple organ systems, including the renal, gastrointestinal (GI), and musculoskeletal systems.

NSAIDs inhibit the rate limiting step in the production of prostaglandins by binding to cyclooxygenase A. It is now recognized that there are three distinct proteins that process cyclooxygenase activity, known as COX-1, COX-2, and COX-3. COX-1 is ubiquitous throughout the body in renal collecting tubules, platelets, endothelial cells, smooth muscle, and gastric mucosa, whereas the COX-2 gene is expressed in a limited number of cells such as neurons, synoviocytes, and smooth muscle cells (5). COX-3, which is inhibited by high-dose acetaminophen, is constitutively expressed in brain and heart tissue and thought to be responsible for febrile reactions (6).

Both COX-1 and COX-2 enzymes use arachidonic acid to produce prostaglandins, which in turn, are modified to generate bioactive lipids. Since 1998, four COX-2 selective inhibitors have been introduced to the U.S. market. Rofecoxib, valdecoxib, and celecoxib have all been shown to be as effective as naproxen for pain control in rheumatoid arthritis and osteoarthritis patients. They are highly selective for COX-2 enzymes even at doses significantly higher than the manufacturers' recommended dosage. Initially designed to minimize GI toxicity, they were regarded as safer agents compared to many nonselective COX inhibitors. Nevertheless, they have been observed to cause serious gastroduodenal injury in large clinical trials at a rate greater than placebo but less than nonselective COX inhibitors (7 ,8 ,9). Most recently, there has been extensive debate regarding the safety profile of COX-2 inhibitors, including the increased cardiovascular risk associated with their use (10).

Clinical Efficacy in SLE

Nonsteroidals were first used in the treatment of SLE patients in 1953 by Langhof, who reported that phenylbutazone improved “subacute” lupus in four patients (11).

This was followed by observations from Harvey et al. in 1954; 7 of 19 patients experienced significant improvement with treatment doses of aspirin. Additionally, four patients experienced a lower febrile response without great clinical improvement (12). In 1956, Dubois noted a “remission” in 39% of his patients with the combined use of aspirin and bed rest (13). In 1966, he suggested treatment doses as high as 300 mg of phenylbutazone may improve the symptoms of SLE (14). Additionally in 1975, Dubois found that in seventeen patients given an average of 2,400 mg of ibuprofen, a 69% significant clinical improvement occurred (15).

Table 58-1: Summary Points Relating to the Use of NSAIDs in Systemic Lupus

1. No NSAIDs are currently approved by the FDA for use in SLE.
2. Over 70% of patients with SLE use an NSAID on a regular basis, mostly for fever, adenopathy, headache, myalgias, arthralgias/arthritis, and/or serositis.
3. Only one controlled trial has ever been conducted on the use of NSAIDs in SLE. In that study aspirin was superior to ibuprofen; a prospective celecoxib trial is in progress.
4. Lupus patients have more complications from NSAIDs than healthy persons without SLE; transaminitis, sun sensitized or sun-induced rashes, fluid retention, hypertension, gastrointestinal ulcerations and aseptic meningitis are probably more common in this population. It is not completely understood if disease activity, disease related risk factors (renal disease, for example) or concomitant medications play a supporting role in these events.
5. NSAIDs can safely be prescribed to most lupus patients provided they are closely monitored on a regular basis.
6. NSAIDs, especially COX-2 inhibitors, should be used with caution in women of childbearing age if pregnancy is a possibility.
7. The risks and benefits must be heavily weighed and discussed with the use of NSAIDs, especially of COX-2 inhibitors, in lupus patients. More long-term data are needed to fully appreciate the potential adverse cardiovascular and cerebrovascular events associated with their usage.

COX-2, cyclooxygenase-2; FDA, Food and Drug Administration; NSAIDs, nonsteroidal anti-inflammatory drugs; SLE, systemic lupus erythematosus.

The National Institutes of Health (NIH) conducted the first double-blinded, controlled study of NSAIDs for SLE. Nineteen patients with fever, arthritis, and serositis were randomized to receive 3,600 mg of aspirin or 2,400 mg of ibuprofen for 10 days. Seventeen patients completed the trial; seven of the nine on aspirin and two of eight on ibuprofen had dramatic improvement in swollen joints and joint pain. Two patients randomized to ibuprofen had decreases in creatinine clearance and an elevation in liver enzymes; these findings reversed upon drug cessation (16).

In 1989, the rheumatology division at Cedars-Sinai Medical Center found 77% of 925 lupus patients had been treated with NSAIDs, and 41% with salicylates. Though no direct evidence supported NSAIDs efficacy in SLE, its usage appeared to improve fever, headaches, arthralgias, and serositis associated with the disease (17). A similar finding was reported by Ramsay-Goldman et al. at the University of Pittsburgh, where 73% of the patients with SLE were taking NSAIDs (18). Moreover, in 1989, steroid sparing properties were reported in 30 Hungarian patients taking piroxicam for 3 months (19). In 1993, Espinoza et al. treated six patients who had refractory nephritic syndrome with indomethacin. A reduction in proteinuria and persistent modest improvements in serum albumin were noted in four of the six patients (20).

Several studies have led to aspirin use for the treatment of antiphospholipid syndrome. Patients with antiphospholipid syndrome are at increased risk for arterial and venous thrombosis, in addition to recurrent fetal loss. Gattenby et al. reported a decrease in fetal loss from 88% to 55% in SLE patients with positive antiphospholipid antibodies following treatment with low dose aspirin. Additionally, several other studies have shown similar findings in this population with low dose aspirin use. ASA is thought to stimulate interleukin-3 production, which in turn promotes placental and fetal development. Moreover, low-dose ASA blocks the production of thromboxane A2, thereby decreasing potential vasoconstriction and platelet aggregation. Most recently, Lander et al. showed in 50 SLE patients that the selective COX-2 inhibitor, celecoxib, was both safe and effective in a community-based SLE population with a predominance of musculoskeletal complaints and less severe organ involvement (21).

The number of randomized controlled clinical trials monitoring SLE patients with NSAIDs use, especially comparing those with renal involvement and those without, is extremely limited. Therefore, current clinical use of NSAIDs in SLE is based primarily on case reports and series as well as documented efficacy displayed in nonlupus patients with other rheumatic diseases, such as rheumatoid arthritis or osteoarthritis (Table 58-1).

Adverse Side Effects of NSAIDs in SLE

Although NSAIDs are often the first line of therapy in patients with SLE, there are risks associated with its use. Many variables, including comorbid conditions, multiple medication usage, baseline renal function, and age may all contribute to its potential toxicity.

Renal

The most common and worrisome side effect is acute renal insufficiency, typically occurring in patients with additional risk factors such as advanced age, intravascular volume contraction, or pre-existing renal insufficiency (22).

Currently it is felt that NSAIDs induce renal side effects by inhibiting prostaglandin synthesis which plays a key role in vasodilatory regulation (23). Prostaglandins affect vasodilatory effects on a cellular level with subsequent compensatory adjustments in renal perfusion. Documented in animal models with lupus nephritis, they are also thought to play a role in the inhibition of T lymphocyte induction and tissue damage (4). In 1977, Kimberly and Plotz noted a 58% reduction in creatinine clearance as well as a 163% increase in serum creatinine in 13 of 23 lupus patients after minimum of 7 days of aspirin therapy. These reversible changes were more prominent in patients with low complement levels and active nephritis (24). Additionally, there have been several studies detailing reductions in glomerular filtration rate (GFR) with NSAID use. Borg et al. noted a 15% reduction in GFR with normal renal blood flow in 13 lupus patients without significant renal disease. Two other controlled studies in lupus patients revealed a 16% reduction in GFR with ibuprofen or indomethacin consumption. Patients with active lupus glomerulonephritis were found to have larger changes in renal function compared to lupus patients without renal disease (25 ,26 ,27). Moreover, there have been reports of acute tubular necrosis in lupus patients with the use of ibuprofen, naproxen, and fenoprofen (28 ,29 ,30). A case-control study revealed a twofold increase in chronic renal disease with daily NSAID use. After risk factor adjustments, men older than 65 appear to be at an increased risk (31). In a Malaysian prospective study of 259 patients with a minimum total of 1-kg analgesic intake, renal papillary necrosis was noted in 29 patients by computed tomography (CT), ultrasound, or intravenous urography (32). Thus, careful review of the above collected data stresses the need for a risk/benefit decision in each individual case before the use of NSAIDS in considered in lupus patients with underlying or active renal disease.

Finally, there are no long-term observational studies evaluating the effects of NSAIDs in lupus patients. Renal papillary necrosis and chronic renal failure have been associated with prolonged NSAIDs intake in normal human subjects; however, the actual risks for these complications have not been established (7).

Gastrointestinal

Currently there are no published studies evaluating GI side effects associated with nonsteroidals in lupus patients. In the general population, it has been noted that dyspepsia develops in approximately 10 to 20% of patients taking nonsteroidals on a regular basis. Endoscopic evidence showing gastric mucosal damage secondary to aspirin use was first shown 60 years ago. Numerous studies have substantiated such damage, as well as both the direct toxicity on GI mucosa and indirect damage secondary to prostaglandin blockade (33).

Many therapies have been studied to prevent GI mucosal injury. The FDA has approved the use of misoprostol four times daily to prevent NSAID-induced ulcers. Although beneficial in certain cases, misoprostol use has been associated with diarrhea, bloating, and increased risk of spontaneous abortion (34). H2 blockade has been shown to prevent duodenal ulcers in two randomized studies but was not as effective for gastric ulcer prevention (35 ,36). Proton pump inhibitors are effective in preventing mucosal injury (37).

A lower incidence of ulceration has been associated with nonacetylated salicylates as they seem to inhibit prostaglandins to a lesser degree. Second-generation NSAIDs with an affinity for COX-2, such as etodolac and nabumetone have a lower GI risk as well (38).

COX-2 inhibitors have shown promise in the prevention of GI toxicity (4 ,5 ,39). However, the long-term GI safety of COX-2 inhibitors in SLE has yet to be determined. Reports have suggested the COX-2 enzyme may be associated with gastroduodenal ulcer healing. Not only has COX-2 been located at the rim of gastroduodenal ulcers in human models, but is also associated with the healing of gastric mucosal damage in mice (40 ,41). The mechanism of COX-2 inhibition prevention remains unclear. Theories suggest a possible interaction with other causal agents of gastrointestinal ulcers; more trials are needed to substantiate such positions.

Hepatic

To date, there are no reported cases documenting clear chronic hepatitic sequelae secondary to the use of NSAIDs in lupus patients. However, there have been several studies addressing the possible risks associated with salicylate usage. Mild elevations of serum transaminases without associated elevations of bilirubin or alkaline phosphatase have been noted in patients with lupus and juvenile rheumatoid arthritis (42). A dosage of 2 g/day is the current threshold that may carry a significant risk. Aspirin, secondary to a direct toxic effect associated with the orthoacetyl moiety, appears to be the most frequent offending agent (43). In 1975, Fries and Holman reported transaminase elevations in 48 of their 192 lupus patients taking aspirin. This was followed in 1978 by a report from Travers and Hughes, who reported similar elevations in seven of their 74 lupus patients using aspirin at recommended doses. Interestingly, four of these seven patients replaced aspirin with diflunisal for approximately 2 months; no corresponding transaminase elevations were noted.

Central Nervous System

NSAID-induced aseptic meningitis is a rare event, but reports indicate that it occurs more often in SLE. Forty-three reported cases have been noted in the literature thus far. Of those, many presented with a clinical scenario similar to

infectious meningitis. A headache, stiff neck, and fever were often the major complaints. It appears that a rechallenge with the same NSAID will cause the event to take place again; this is based on the author's anecdotal experience as fortunately such situations are even more rare. CSF analysis revealed an elevated white blood cell (WBC) count, elevated protein, and normal glucose level. No bacteria were isolated (44 ,45 ,46 ,47 ,48). Of these patients in the literature, 18 cases were associated with SLE. Ibuprofen was by far associated with the highest percentage of cases, followed by tolmetin, sulindac, naproxen, and diclofenac (29 ,49 ,50 ,51 ,52). The mechanism and etiology of such association is not well understood. Hypersensitivity reaction, a lack of suppressor cells in SLE patients leading to an exaggerated response, and a cell-mediated cross-reactivity with ibuprofen have all been postulated (32).

Dermatologic

When compared to the general public, SLE patients have higher rates of allergic reactions to all drugs, especially antibiotics and, to a lesser extent, NSAIDs (53 ,54). Most NSAIDs usage reactions are mostly associated with cutaneous reactions manifestations. Angioedema, urticaria, erythema multiforme, exanthema, and Stevens-Johnson syndrome have all been linked to NSAID use in SLE patients. Naproxen, aspirin, diflunisal, and sulindac have a 5% incidence of rash (55 ,56). A specific photosensitivity rash presenting within 3 days as a vesiculobullous eruption has been reported with piroxicam (57).

Sulfonamide-containing medications continue to present a problem for some SLE patients. In 1992, Petri and Allbritton (58) published a case-control study of 221 patients with SLE. Thirty-one percent of the SLE patients exposed to sulfonamides developed allergic reactions, such as rash, angioedema, bronchospasm, and fever, whereas only 12% in the control group experienced similar complications. Moreover, six patients had documented lupus flares in the sulfonamide group. A retrospective study published in 1993 compared drug hypersensitivity reactions in 340 lupus patients and 306 controls; 56% of the former group developed complications and only 14% in the latter group. Of these, sulfonamide antibiotics were the leading causative agents, followed by penicillin, cephalosporins, and NSAIDS (59). Once again, several lupus flares were noted in the SLE patients.

Celecoxib, a COX-2 inhibitor, is a derivative of bezenesulfonamide. This raises a concern as COX-2 therapy is often used first line in patients who may or may not even have lupus or before the disease is diagnosed. Structural differences among sulfonamide antibiotics and celecoxib exist. Notably, the lack of an arylamine group located at the N4 position is perhaps the most critical. Without this moiety, the incidence of cross-reactivity with resulting clinical adverse reactions is rarely seen (60 ,61). In a retrospective analysis of fifty SLE patients who were followed for 8 months while openly being treated with celecoxib for musculoskeletal pain, there were no differences in the incidence of allergic reactions in patients with a self-reported history of sulfa allergy compared to those without such complaints (11).

Potential Increased Cardiovascular Risk

Great debate has recently taken place regarding cardiovascular risks associated with NSAID usage. The largest risks appear to be associated with COX-2 selective inhibitors. In the mid-1990s, selective COX-2 inhibition was thought to favor thrombosis by mostly inhibiting the COX-2-derived vascular prostacyclin without affecting the COX-1 mediated generation of thromboxane A2 (62). The Vioxx Gastrointestinal Outcomes Research (VIGOR) trial suggested a fivefold increase in the risk of myocardial infarction with the use of rofecoxib versus naproxen (63 ,64). The lack of a placebo group, in addition to the possible antithrombotic effects of naproxen made the study difficult to interpret (65 ,66). Recently, results of three randomized, placebo-controlled case studies have suggested elevated cardiovascular risks associated with COX-2 inhibitors (67). The APC (Adenoma Prevention with Celecoxib) trial included patients with a history of colorectal cancer; these patients were randomized to celecoxib or placebo. A 2.3% cardiovascular endpoint of death secondary to cardiovascular causes, nonfatal myocardial infarction, nonfatal stroke, or nonfatal heart failure was achieved in patients taking 400 mg of celecoxib per day. A 3.4% cardiovascular endpoint was noted in patients receiving 800 mg of celecoxib per day. This is in contrast to the 1% composite cardiovascular endpoint noted in the placebo group (68). In early December 2004, the NIH subsequently suspended the trial because of its increased cardiovascular events. The APPROVe (Adenomatous Polyp Prevention on VIOXX) trial randomized patients with a history of colorectal adenomas to 18 months of rofecoxib or placebo. An increased risk of thrombotic complications (1.5 events/100 patient-years vs. 0.78 events/ 100 patient-years) was noted in the rofecoxib arm (69). Lastly, in a study of post-CABG patients, subjects were randomized to valdecoxib/parecoxib or placebo. This study also noted an increase in cardiovascular events associated with COX-2 inhibitor use (2.0 % vs. 0.5% for the placebo group.) In summation, recently published large scale studies have strongly suggested increased cardiovascular risks with COX-2 inhibitor use and emphasize the need for additional data collection and careful risk assessment when these agents are employed.

Drug Interactions

Adverse reactions may come from medication alone or in combination with other therapy. SLE patients often take multiple medications with the increased opportunity for potential interactions. NSAIDs have been shown to diminish the antihypertensive effects of thiazide and loop diuretics (70). Additionally, NSAID usage may increase prothrombin

time if taken with warfarin. Documented cases of bone marrow aplasia and renal failure have been published when methotrexate is used with aspirin, naproxen, or indomethacin (45 ,71).

One additional potential concern is the interference of aspirin, in anti-inflammatory doses, with systemic and renal clearance of methotrexate. This in turn, may lead to higher methotrexate levels and potential toxicity (72).

Pregnancy

Indomethacin (Indocin) is the most studied NSAID that is commonly used during pregnancy. Although generally considered safe until the third trimester, indomethacin has been associated with oligohydramnios, premature closure of the fetal ductus arteriosus with subsequent persistent pulmonary hypertension of the newborn, fetal nephrotoxicity, and periventricular hemorrhage (73). Other NSAIDs, such as ibuprofen, have been studied less often during pregnancy. However, an analysis of 50 pregnant patients who overdosed on ibuprofen revealed no evidence of fetal abnormalities (74).

Most recently, several population based cohort studies have shown an increased risk of miscarriages associated with exposure to NSAIDs (75 ,76). The highest risk of miscarriage was associated with NSAIDs use around the time of conception, which may be related to altered prostaglandin interactions during normal implantation. This risk may be further increased with the use of COX-2 inhibitors, as inducible prostaglandins are thought to be necessary for proper implantation. However, there is very limited information on the indications for NSAIDs use in these observational studies. The finding that filling a prescription for an NSAID was associated with an increased risk for miscarriage may simply reflect a higher proportion of women with rheumatic diseases in the exposed group. In a large randomized controlled trial, low-dose aspirin was not associated with an increased risk for miscarriage (77). Whether this association is caused directly by NSAIDs or by the indication for prescribing the drug is still not understood. It may be prudent for physicians caring for these patients and women who are planning to be pregnant to be aware of potential miscarriage risks and avoid using NSAIDs, especially COX-2 inhibitors, around the time of conception.

Monitoring Therapy

Most lupus patients take NSAIDs on a regular basis, even though they are at an increased risk for experiencing complications from the drug. In our practice, lupus patients on NSAIDs are seen at least quarterly, at which time a history is obtained, physical examination performed that screens for hypertension and edema, and blood tests are obtained, which include a complete blood count, hepatic and renal screen.

Conclusion

Current literature review reveals that NSAIDs can be used with caution in the management of non-organ threatening SLE inflammation such as arthralgia, myalgia, headache, serositis, fever, and synovitis. However, lupus patients, specifically those with active nephritis, are at increased risk for developing renal toxicity associated with NSAIDs and should have close regular monitoring in order to avoid toxicity.

Additionally, great controversy has recently surrounded the safety of COX-2 inhibitors. Several studies have linked their usage to increased cardiovascular and cerebrovascular events. In September 2004, Merck voluntarily withdrew rofecoxib from the market and valdecoxib in March 2005, because of their increased risk of myocardial infarction and stroke. The NIH soon followed by suspending the APC clinical trial because of the increased cardiovascular events associated with the use of celecoxib. Three days later, the NIH halted the ADAPT (Alzheimer's Disease Anti-inflammatory Prevention Trial), because this study revealed an increased risk of cardiovascular events in patients receiving naproxen, but not in those given celecoxib. Thus, it is alarmingly clear the benefits and risks must be heavily weighed and compared before employing the use of COX-2 inhibitors. Moreover, recent data suggests this risk/benefit ratio must be strongly considered in all NSAID use.

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Chapter 59
Antimalarial Therapies

Daniel J. Wallace

Antimalarials are effective nonsteroid drugs for some patients with systemic lupus. Unlike other disease-modifying therapeutic agents that are used to treat systemic lupus erythematosus (SLE), antimalarials do not suppress the bone marrow or increase the risk for opportunistic infections.

Historical Perspective

Antimalarials were first used therapeutically in 1630 as an antipyretic (1). They were first employed in the treatment of cutaneous lupus in 1894, when Payne (2) tried quinine. In 1928, Marstenstein (3) reported good results in 22 of 28 patients with discoid and subacute systemic lupus who were treated with pamaquine (Plasmachin), which is similar to quinine in that both are substituted 8-aminoquinolines. In 1938, Davidson and Birt (4) reported excellent results in 19 of 29 patients who were treated with quinine bisulfate. In 1941, Prokoptochouk (5 ,6) successfully treated 35 patients with discoid lupus erythematosus (LE) by giving daily doses of 300 mg of quinacrine (Atabrine, mepacrine), a compound first synthesized by the Germans during the 1920s but possibly first discovered by Paul Ehrlich a decade earlier (7). During World War II, quinine supplies were cut off by the Japanese, and the U.S. Surgeon General declared Atabrine to be the official drug to treat malaria (8 ,9). Between 1943 and 1946, 3 million Americans took the drug daily (10). Anecdotal evidence of its efficacy in treating some skin disorders among British soldiers prompted Page (11) to study the drug for use in the treatment of discoid lupus. Unaware of Prokoptochouk's work, in 1951, Page reported its benefits in an uncontrolled study of 18 patients with lupus and two with rheumatoid arthritis (RA), and the report received wide attention. These findings were soon confirmed at the Mayo Clinic by O'Leary et al. (12).

Subsequently, it was shown that other antimalarials also are effective. Chloroquine was patented in 1934 and hydroxychloroquine synthesized by the mid-1940s and shown to be less toxic than quinacrine. In 1953, Goldman et al. (13) reported that chloroquine was helpful in 21 patients, including 3 with subacute disseminated disease, and in 1954, Pillsbury and Jacobson (14) noted that 15 of 16 patients with SLE taking chloroquine improved. Synthesized by Surrey and Hammer in 1946, hydroxychloroquine (Plaquenil) was released in 1955 after it was found to be effective in SLE and RA, with fewer adverse reactions than chloroquine (15 ,16 ,17 ,18). Amodiaquin (Camoquin) also was efficacious in SLE, but it was taken off the market in the United States in the early 1970s because of its propensity to induce agranulocytosis (19 ,20 ,21). It is still available in other countries. Finally, an extremely potent antimalarial, Triquin (which contained chloroquine, hydroxychloroquine, and quinacrine) took advantage of the synergy between quinacrine and the chloroquines. It was released in 1959 after a report claimed that 44 of 45 patients with lupus, mostly antimalarial-resistant, at Boston City Hospital had dramatic responses (22). The preparation sold well until it was withdrawn in 1972 as part of a campaign against the use of combination drugs. Sanofi-Winthrop discontinued the production of Atabrine in 1992, but it is still available from 2,500 compounding pharmacists in the United States who can obtain quinacrine hydrochloride powder from chemical suppliers (23).

Pharmacology of the Antimalarials

Chloroquine

Chloroquine (7-chloro-4-{4-diethylamino-1-methylbutylamino}) (Fig. 59-1). Water soluble and almost completely absorbed by the gastrointestinal (GI) tract, it achieves a peak concentration in 8 hours and is only 10% is fecally excreted. Renal excretion (50%) is increased by acidification and decreased by alkalinization. The drug is bound by plasma proteins and largely deposited into tissues. High concentrations can be found in the liver, spleen, kidney, lung, and all blood elements (2 ,24 ,25 ,26), as well as in pigmented tissues. This can be 6,000 to 20,000 times the plasma level (15). Doses of 250 mg a day lead to plasma concentrations of 100 to 500 ng/mL after 2 months (27). Chloroquine is broken down into three N-dealkylated metabolites that are of toxologic and pharmacologic importance, including desethylchloroquine and bisdesethylchloroquine. Chloroquine and desethylchloroquine competitively inhibit CYP2DI/6-mediated reactions in vivo and in vitro. The R (-) chloroquine enantiomer may be more potent, has a longer half-life, and higher unbound plasma concentrations (28 ,29 ,30). The drug readily crosses the placenta and is excreted in small amounts in breast milk. A child receives less than 1% of the maternal dose (31 ,32).

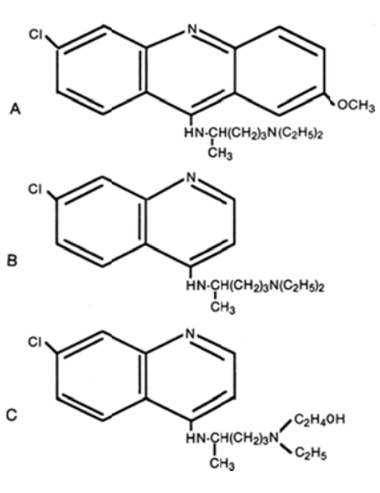

Figure 59-1. Structural formulas of commonly used antimalarial drugs. **A.** Quinacrine. **B.** Chloroquine. **C.** Hydroxychloroquine. (From

Wallace DJ. Antimalarial agents and lupus. *Rheum Dis Clin North Am* 1994;20:243–263, with permission.

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Chloroquine is slowly excreted with elimination half-lives of 20 to 60 days, but detectable amounts in the urine, red cells, and plasma for as long as 5 years after discontinuation (33). The half-life is governed by dose-dependent kinetics, increasing from 3 hours after a single 250-mg dose to 13 days after 1,000 mg. Chloroquine decreases creatinine clearance by a mean of 10 in 55% of its users, probably by increasing plasma aldosterone levels (34 ,35). Drug interactions have been reported with ampicillin and methotrexate (chloroquine decreases its bioavailability), but not with aspirin (36 ,37 ,38). It has in vitro synergy with cyclosporine and antagonism with d-penicillamine (39).

Hydroxychloroquine

Hydroxychloroquine sulfate (2-[[4-[(7-chloro-4-quinolinyl) amino]pentyl]ethylamin]ethanol sulfate; Plaquenil is a 4-aminoquinoline that differs from chloroquine by a hydroxyl group at the end of a side chain (Fig. 59-1). The two agents have similar pharmacokinetics. The 200-mg tablets contain 155 mg of hydroxychloroquine base, consisting of equal amounts of (-)-(R) and (+)-(S) enantiomers. The (+)-(S) form has greater bioavailability (40) but a shorter half-life (41). Hydroxychloroquine is broken down into three metabolites: desethylchloroquine, desethylhydroxychloroquine (to the greatest degree), and bidesethylchloroquine by CYP2D6, -2C8, -3A4, and -3A5 human p450 enzymes (42 ,43). Hydroxychloroquine is 75% to 100% absorbed in the GI tract, with 50% in 2 to 10 hours and 50% bound by serum proteins (44 ,45 ,46). Some is conjugated with glucuronide and excreted in the bile, but 30% to 60% is biotransformed in the liver. Excretion occurs in two stages: a rapid one, with a half-life of 3 days; and a slower one, with an overall half-life of 40 days. Forty-five percent is excreted by the kidney, 3% by the skin, and 20% fecally. It takes 6 months to reach a 96% steady state. Much of the drug is deposited into tissues, with the highest concentrations in the adrenal and pituitary glands. Other areas with high concentrations include melanin-containing tissues, liver, spleen, and leukocytes. It has been disputed whether inflammatory disease activity or toxicity correlates with drug blood concentrations (42 ,47). Hydroxychloroquine is approximately two thirds as effective as chloroquine and half as toxic (48).

The drug may slightly alter kidney function; 15 of 118 patients with RA had a mean 10% decrease in creatinine clearance (34). The dosage must be reduced in patients with renal failure. Dialysis does not help overdosage, because the agent is extensively sequestered (46).

Hydroxychloroquine can interact with digoxin and reduce its levels (a quinidine-like effect) (49). It increases the potency of adding methotrexate in combination (50). One five-center survey has suggested that hydroxychloroquine administration may decrease the frequency of liver enzyme abnormalities seen in patients with RA who are on methotrexate or salicylate therapy (51), and this has been confirmed by another report (52).

Quinacrine

Quinacrine (6-chloro-9-[-methyl-4-diethylamine]butylamine- 2-methoxyacridine; Atabrine, mepacrine, Atebrine, chinacrin, Erion, Acriquine, Acrichine, Palacrin, Metoquine, Italchin) differs from chloroquine only in having an acridine nucleus (i.e., an extra benzene ring) instead of a quinoline (Fig. 59-1). The drug is rapidly absorbed after oral administration. Plasma levels rise in 2 to 4 hours, reaching a peak in 8 to 12 hours (53). Plasma concentration increases rapidly during the first week, and 94% equilibrium is attained by the fourth week. The drug is distributed widely in tissue but is slowly liberated, with the highest concentrations in the liver and spleen and the lowest concentrations in the brain and heart. The liver concentration may be 20,000 times that in plasma. Skin deposits often are clearly visible. Quinacrine crosses the placenta and reaches the fetus. Spinal fluid levels are 1% to 5% of plasma levels. Eighty percent to 90% of the drug is bound to plasma proteins in therapeutic doses, and it has a half-life of 5 to 14 days. It is slowly excreted from the body; less than 11% is eliminated in the urine daily (54).

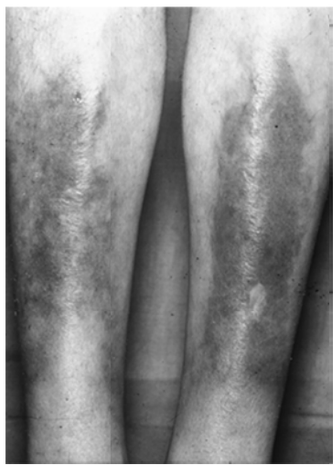

Figure 59-2. Blue-black pretibial pigmentation from prolongued antimalarial administration.

Quinacrine also can be administered intralesionally (55 ,56), for discoid lesions, intramuscularly, intravenously, rectally, transcervically, or delivered through a chest tube for malignant pleural effusions (54 ,57 ,58).

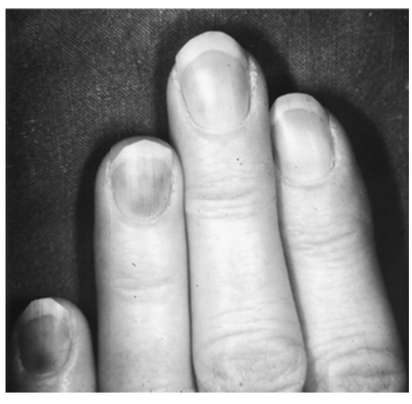

Figure 59-3. Nail-bed pigmentation resulting from antimalarial therapy.

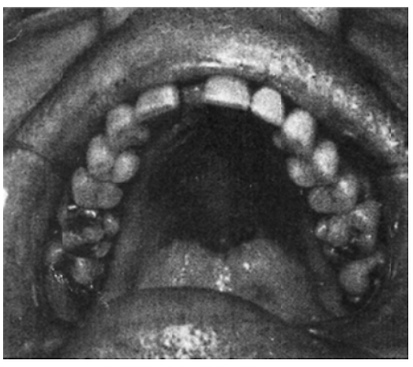

Figure 59-4. Blue-black pigmentation on the hard palate caused by antimalarial therapy.

Mechanisms of Action

Light Filtration

One of the major aggravating factors in patients with SLE is ultraviolet (UV) light exposure. More UV radiation is absorbed (Table 59-1) when skin concentrations of antimalarials are higher (45 ,46 ,47). Antimalarials deposited in the skin absorb UV light in a concentration-dependent manner (59 ,60 ,61). Hydroxychloroquine, but not methotrexate, methylprednisolone, or saline, blocked cutaneous reactions induced by UV light (62). Topical chloroquine protects against UVA- or UVB-induced erythema (63). Sontheimer's group (64) has shown that chloroquine modulates UV activation of the c-jun proto-oncogene, which protects against UV damage. Ironically, the chloroquines and quinacrine can also induce a photosensitivity reaction (65 ,66 ,67). An anti-inflammatory effect or downregulating action on keratinocytic function also might be important (68). Quinacrine can impede photodynamic actions, inhibit laser-induced photosensitivity, and increase UV light tolerance (11), perhaps by scavenging water radicals (69 ,70 ,71).

Table 59-1: Important Mechanisms of Action of Antimalarial Drugs

Immunologic actions
Blocks antigen processing by raising intracytoplasmic pH, which depletes cells of their receptor sites with consequent decrease in cytokine production (especially IL-1, IL-6, and TNF-α)
Inhibit natural killer activity, mitogenic stimulation
Impedes formation and helps dissolve immune complexes
Inhibits toll receptor activation
Antiinflammatory effects
Inhibits phospholipase A_2 and C
Prostaglandin antagonization
Stabilizes lysosomal membranes
Decreases fibronectin release by macrophages
Blocks superoxide release at multiple sites
UV light absorption
Hormonal actions
Decreased estrogen production
Hypoglycemic, impairs insulin release
Antiproliferative activities
Blocks graft versus host disease
Intercalates with DNA
DNA, RNA polymerase inhibition
Decreased tumor size and chemotherapy resistance
Inhibits platelet aggregation and adhesion
Antimicrobial effects, decrease antibiotic resistance
Anticholinesterase and sympatholytic actions
Quinidinelike cardiac actions, reduction of infarct size
Lowers cholesterol and LDL levels by 15% to 20%

TNF, tumor necrosis factor; IL, interleukin; LDL, low-density lipoprotein.

Immunologic Effects

The principal mechanism of action of hydroxychloroquine and chloroquine relates to their elevation of intracytoplasmic pH. Because antigen processing is an acidic, pH-dependent component, the chloroquines turn off this process by decreasing the number of autoantigenic peptides presenting on the cell surface (15 ,72 ,73). The tendency of chloroquine and hydroxychloroquine to target macrophage-mediated cytokines such as interleukin-1 (IL-1), IL-2, and tumor necrosis factor-α (TNF-α) to a greater extent than T-helper cell–mediated cytokines, such as IL-2, IL-4, and IL-5 suggests that its sites of action are preferential (74 ,75 ,76 ,77 ,78 ,79 ,80 ,81 ,82 ,83). Hydroxychloroquine can inhibit calcium signals in T cells (84). Chloroquine also inhibits interferon-γ production by CD4- and CD8-positive synovial T cell clones in patients with RA (85), suppresses natural killer cell activity (86 ,87), and suppresses IL-6 immune activity in a variety of mitogenic stimulation studies (86 ,87 ,88 ,89 ,90 ,91 ,92 ,93 ,94 ,95). Both chloroquines can enhance IL-10 activity (96), block tumor necrosis factor expression (97), and inhibit monocyte chemoattractant production (98).

Raising the pH of cytoplasm has a profound effect on the lysosomal membranes, which are stabilized (90 ,99 ,100 ,101 ,102 ,103 ,104). Chloroquine is concentrated in the nuclear and lysosomal fractions of white blood cells (100 ,105). Chloroquine becomes trapped in lysosomes and alters their pH, which results in an increase in lysosome number and volume. Inclusion bodies (i.e., myelin bodies) containing plasma membrane phospholipid accumulate, because of the reversible inhibition of membrane recycling; this remarkable action leads to decreased phagocytosis, chemotaxis, and cell functioning. More specifically, it substantially depletes the cell of its surface receptors (trapping approximately 50% of them), which alters the cell's responsiveness to mitogenic stimuli (45 ,69 ,101 ,102 ,106). Quinacrine probably has similar actions (69 ,107 ,108 ,109).

Chloroquine has opposing actions, in that it inhibits the formation of antigen-antibody complexes in vitro and enhances the association of such complexes in a dose-dependent fashion (110) or can split them (111). Chloroquine has been used to dissociate antigen-antibody complexes as part of a laboratory technique that is used in typing red blood cells with a positive direct antiglobulin test (112 ,113). Also, it strips human leukocyte antigens (HLAs) from lymphocyte and platelet membranes (114 ,115 ,116). Clinically, it has been shown to decrease circulating immune complex levels in patients with RA (117).

Chloroquine blocks the DNA–anti-DNA reaction by binding not to the anti-DNA antibody but to the DNA (118). Competition for binding sites on DNA among chloroquine, sodium ions, and anti-DNA antibodies occurs only under nonphysiologic conditions (119). Work from Dubois' and Kunkel's laboratories documented that the binding of quinacrine to nucleoproteins can block the LE cell factor (120 ,121 ,122).

Antiinflammatory Effects

Chloroquine is a strong prostaglandin antagonist (especially against phospholipase A2) and a weak agonist (123 ,124 ,125 ,126). The antagonist effect clearly is demonstrable at concentrations

that are reached in human plasma when the drug is used therapeutically (126). Chloroquine reduces prostaglandin synthesis through the inhibition of phospholipases A2 and C and blocks mitogen activated protein kinase signalling (124 ,127 ,128 ,129 ,130). Antiphospholipase A2 blockade decreases the actions of bradykinin in synovial fibroblasts, suppresses its algesic effects, and decreases histamine release from basophils (131 ,132 ,133 ,134 ,135). In vitro hexosamine depletion of intact articular cartilage by E prostaglandins is accomplished through the DNA-dependent RNA synthesis of cathepsin-like proteases (136). This can be inhibited by chloroquine through the inhibition of DNA primer.

Chloroquine and hydroxychloroquine also exhibit anti-inflammatory actions by decreasing IL-1–induced cartilage degeneration, perhaps through inhibiting elastase (137 ,138 ,139), fibronectin release by macrophages (140), and reactions that are dependent on sulfhydryl-disulfide interaction (141). Chloroquine inhibits angiogenesis in vitro, which results in anti-inflammatory effects (142 ,143).

Quinacrine also is a potent inhibitor of phospholipase A2, which results in decreased leukotriene and prostaglandin release (125 ,127 ,143 ,144 ,145 ,146 ,147 ,148 ,149 ,150 ,151 ,152 ,153). It is a nonselective antilipolytic agent that decreases prostaglandin E2 production in a dose-dependent fashion. Thromboxanes B2 and A2 are specifically suppressed. Quinacrine also stabilizes cell membranes as a result of its Na-K-adenosine triphosphatase (ATPase) inhibitory effects (150), and it inhibits lysosomal enzymes that are involved in phospholipid catabolism. Strongly concentrated in leukocytes and lysosomes, quinacrine has a stabilizing effect (145). Phagocytosis, chemotaxis, RNA synthesis, and hexose monophosphate shunt burst activity are inhibited by the drug (69 ,70 ,154 ,155 ,156). Chloroquine and quinacrine also are antipyrogens (157 ,158 ,159).

Neuroprotective and Antiprion Effect

Quinacrine can decrease glial injury and reaction through its inhibition of phospholipase A2 (160). It also acts as a noncompetitive inhibitor of prion formed channels (161 ,162) and is a candidate to manage some prion-associated disorders.

Hormonal Effects

Chloroquine may reduce estrogen production (163) and have an adrenal-stimulating effect (164). In patients with RA, SLE and sarcoidosis, it decreases 1,25-hydroxyvitamin D levels (165 ,166 ,167). A major area of interest concerns its applications in diabetes as a result of the agent's hypoglycemic action. This occurs secondary to chloroquine-induced decreased degradation of insulin (168 ,169), inhibiting lysosomal proteolysis (170), decreased gluconeogenesis (171), and, possibly, decreasing insulin-induced loss of receptors (172). Although hydroxychloroquine lowers blood sugars in patients with SLE by a mean 5 mg/dL (173), occasional reports of clinically evident, chloroquine-associated hypoglycemia have been published (174 ,175 ,176).

Quinacrine accumulates in peptide hormone-producing cells (177 ,178), and it can block prolactin (179 ,180) and insulin release. Conflicting reports about its effects on 17-ketosteroids have been published (11 ,134).

Antioxidant Effects

In high doses, chloroquine can inhibit polymorphonuclear oxidative bursts (181). Chloroquine, hydroxychloroquine, and quinacrine block superoxide release by actions at multiple sites on the metabolic pathway (150 ,155 ,182 ,183 ,184 ,185 ,186), which may have a beneficial effect on bronchial asthma (187).

Antiproliferative and Graft versus Host Effects

Chloroquine interferes with protein synthesis in vivo and in vitro (188 ,189). It blocks DNA and RNA biosynthesis and produces rapid degradation of ribosomes and dissimilation of ribosomal RNA. By intercalation, chloroquine inhibits DNA and RNA polymerase reactions in vitro and DNA replication and RNA transcription in susceptible cells (190). Chloroquine does not alter the ability to repair damage from UV light–induced DNA excisions (191). It impedes DNA synthesis stimulated by platelet-derived growth factor (192). Interest has focused on its anticarcinogenic properties. Chloroquines can inhibit the replication of Moloney leukemia virus and tumor development in newborn mice (193), be toxic to melanoma cells (194), block breast tumor growth (195), and inhibit pancreatic adenocarcinoma cell growth (196). Further, it can potentiate hyperthermia therapies with or without radiation (197 ,198 ,199), block Z-DNA formation (200), and enhance chemotherapy cytotoxicity in multiple drug-resistant human leukemic cells (201). Both chloroquine and hydroxychloroquine decreased alloreactivity in three separate studies as part of preventing graft-versus-host disease among transplant patients (202 ,203 ,204).

Quinacrine also binds to DNA by intercalation between adjacent base pairs (205 ,206 ,207). Quinacrine blocks radiation-induced DNA strand breaks, activates innate immunity, and potentiates the antiproliferative effects of radiation (71 ,207 ,208 ,209 ,210 ,211 ,212 ,213). It reduces the incidence of cancer in rats given nitrosourea (214), decreases the number of somatic mutations induced in murine leukemia cells (215), enhances carmustine therapy in rat gliomas (216), decreases tumor size in mice (199 ,217), and reverses resistance to vincristine (218). Lymphocytes in vitro treated with quinacrine show increased chromosomal aberrations (219).

Antiplatelet and Antithrombotic Effects

Hydroxychloroquine and chloroquine that accumulate in blood platelets can inhibit platelet aggregation and adhesion in a dose-dependent fashion (220 ,221 ,222 ,223 ,224). A desludging effect was demonstrated in the retinal veins of 20 patients with RA (225), and additional studies suggested that hydroxychloroquine reduces the size of thrombi and does not prolong bleeding

time (222 ,226 ,227). Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies (228), and reduces anti-β_2-glycoprotein-1 titers in mice (229). As a result, the drug has been used for thromboprophylaxis of postoperative pulmonary emboli in orthopedic patients (230). Quinacrine also can inhibit platelet aggregation, probably because of its antiphospholipase actions or its interaction with 38,58-cyclic guanosine monophosphate (cGMP) (231 ,232 ,233 ,234 ,235).

Antimicrobial Effects

Inhibition of DNA replication may be the mechanism of action for the antimicrobial effects of chloroquine. It does not impede the growth of viruses but does protect the cells against virus-induced cell damage (236). Chloroquine's discontinuation has been associated with flares of viral infections (237). Chloroquine and hydroxychloroquine inhibit replication of the human immunodeficiency virus in T cells and monocytes (238 ,239), which may account for the negative association between AIDS and SLE (240). Quinacrine has antiparasitic, antiprotozoan, antibacterial, antiviral, and antifungal actions (54 ,241). It can prevent resistance to various antibiotics and increase interferon production (242), and perhaps prevent pneumocystis infection (243).

Muscle and Nerve Effects

Chloroquine is a muscarinic receptor antagonist, which results in an atropine-like effect in humans (242 ,244). The agent also can block dopamine ß-hydroxylase (245). Quinacrine is a strong inhibitor of cholinesterase, because of its inhibition of cGMP (151 ,202 ,246 ,247). It can block ß-agonists, α-adrenergic actions, and norepinephrine (151 ,246 ,248 ,249 ,250). Quinacrine protects mice from lethal amounts of snake venom neurotoxins (251).

Cardiac Effects

Both chloroquine and quinacrine possess quinidine-like actions. Chloroquine increases heart rate, lowers blood pressure, and decreases premature ventricular contractions in humans (252 ,253 ,254). Patients taking chloroquine routinely show electrocardiographic flattening of T waves and a slight prolongation of the QT interval (255). Quinacrine's antiarrhythmic actions occur as a result of slowing inward current and decreasing the automaticity of Purkinje fibers; it can treat atrial fibrillation and prevent ventricular fibrillation (256 ,257). The antiphospholipase A2 actions of chloroquine and quinacrine resulted in studies demonstrating that both can decrease acute myocardial ischemic damage in dogs, rats, pigs, and cats (258 ,259 ,260 ,261 ,262 ,263 ,264 ,265 ,266 ,267 ,268).

Antihyperlipidemic Effects

Animal studies have shown that chloroquine decreases serum bile acid and cholesterol levels by 10% to 20% (269 ,270 ,271). Lysosomotropic agents reduce the proteolysis of many plasma membrane receptors, and chloroquine increases the number of low-density lipoprotein (LDL) receptors (272 ,273 ,274 ,275). Alternatively, inhibition of the hydrolysis of internalized cholesterol esters also may lead to increased LDL receptor levels (273 ,274) (discussed later).

Promotion of Apoptosis and Inhibition of Toll Receptor Activation

Hydroxychloroquine induces lysosome mediated apoptosis (276 ,277). Meig et al. (278) showed that hydroxychloroquine was able to induce apoptosis in lupus peripheral blood lymphocytes in a dose- and time-dependent manner, and Potvin et al. (279) were able to reproduce this with chloroquine in human endothelial cells. Krieg's group (280) suggested that this could be prevented in vitro when CpG dinucleotides activate NF-κB. Chloroquine is able to inhibit the production of proinflammatory cytokines induced by TLR9 (toll receptor 9) (280a).

Clinical Studies

Chloroquines: Antilupus Activity

As early as 1956, Ziff et al. (281) noted a favorable response in 11 of 12 patients with SLE who are given antimalarials and commented on the reduction of steroid requirements (Table 59-2). Dubois (282) claimed that 90% to 95% of patients had a favorable response. To evaluate the effectiveness of medication for the treatment of discoid LE, it is essential to know the incidence of spontaneous remissions. In Dubois' (283) studies, 10% of patients had a history of spontaneous improvement. In the series of Herrman et al. (284), 14% of patients healed spontaneously, compared with 85% or more who improved with antimalarials (285 ,286). Callen (287) treated 62 patients with discoid lupus at the University of Louisville over a 5-year period, and he reported on 34 who were given hydroxychloroquine. Of these, nine patients were said to have an excellent response, 15 were very good, 6 good, 3 fair, and 1 poor.

Rothfield et al. (288 ,289 ,290) studied the discontinuation of antimalarial therapy on SLE activity in 27 patients who had developed maculoretinopathy. Exacerbations during the 2 years before and 1 year after discontinuation were compared. On this basis, ten patients had more exacerbations during the year after antimalarials were stopped than during either of the 2 previous years while receiving these drugs. Of these, three had no increase in the number of exacerbations, and four had fewer exacerbations after the discontinuation of therapy. The required maintenance dose of prednisone was higher after the discontinuance of antimalarial treatment. The data suggested that chloroquine therapy is a factor in the suppression of disease in ten of 17 patients and is steroid-sparing. Hughes (291) found hydroxychloroquine to be particularly useful for anti-Ro/SSA–positive disease, confirmed its steroid-sparing properties, and advocated combined antimalarial therapy for resistant cases.

Table 59-2: Major Clinical Trials of Chloroquines in SLE

Source	Findings
Ziff et al. (281)	11 of 12 patients had favorable responses; steroid-sparing properties noted
Dubois (282,283)	90% with nonorgan-threatening disease improved with hydroxy chloroquine; over 300 patients treated
Rothfield et al. (288,289,290)	27 patients who stopped chloroquine, because of macular changes had more flares after 1 year than in either of the prior 2 years while taking it
Callen (287)	33 of 34 given hydroxychloroquine for cutaneous lupus responded (9, excellent; 15, very good; 6, good; 3, fair; 1 poor)
Esdaile et al. (293)	47 patients controlled with hydroxychloroquine were given continued therapy or placebo for 24 weeks; treated group had fewer disease flares and severe disease exacerbations
Williams et al. (295)	71 patients in controlled/placebo trial of articular complaints showed subjective joint pain assessment improvement with hydroxychloroquine
Meinao et al. (297)	24 patient, double-blind study in Brazil; chloroquine was steroid sparing and prevented disease flares
Molad et al. (301)	Slows the rate of SLICC/ACR Damage index among 151 patients
Harley & colleagues (300)	When given to those without but early lupus symptoms, delays onset of disease

The Canadian Hydroxychloroquine Study Group (292 ,293) studied 47 patients with SLE whose disease was controlled with hydroxychloroquine and randomized them to either continued hydroxychloroquine or placebo for 24 weeks. The hydroxychloroquine group had significantly fewer disease flares and a lower risk of severe disease exacerbation. A 3-year follow-up study by the group confirmed this. Hydroxychloroquine was felt to have a favorable impact on the morbidity and mortality of the Johns Hopkins Lupus Cohort (294).

Nine centers from the Cooperative Systemic Studies of the Rheumatic Diseases studied hydroxychloroquine versus placebo among 71 patients with mild SLE in a 48-week trial. Patient assessment of joint pain significantly improved, and only two patients withdrew because of adverse effects (295). Similarly, Littlejohn's group in Australia noted only 8% of patients with lupus who were given hydroxychloroquine discontinued the drug at 12 months, and only 24% at 24 months (296). A double-blind study of chloroquine in 44 steroid-dependent patients with SLE found it to be steroid-sparing and to prevent disease flares (297). Petri and Yadia (298) negatively associated hydroxychloroquine use with the development of proteinuria. Ruzicka et al. (299) compared acitretin, a retinoid with hydroxychloroquine in 58 patients with cutaneous lupus. Although the drugs were only given for 8 weeks, 50% in both groups cleared; however, the retinoid group had many more side effects. Hydroxychloroquine may delay the onset of SLE and decrease the rate of increase of the SLICC/ACR damage index (300 ,301 ,302).

Antilupus Actions: Quinacrine with or without Chloroquines

The results of the last quinacrine-alone clinical trial were published in 1961, but between 1940 and 1961, 20 reports on 771 patients were published (54) (Table 59-3). Remarkable for the similarity of their findings, 27% of patients had an excellent response, 46% improved, and 27% did not respond. Cutaneous and constitutional symptoms improved first, and the chloroquines were superior to quinacrine in treating synovitis.

Combinations of chloroquines with quinacrine have shown favorable results. Ten of 14 patients with chloroquine- or hydroxychloroquine-resistant cutaneous disease cleared their lesions when quinacrine was added in one survey (303), and 13 of 15 cleared their lesions in another (304). Toubi et al. (305) confirmed this in 13 patients by lowering Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores (306).

Adjunctive Useful Actions of Antimalarials in Patients with Lupus

Antithrombotic Effects

The actions of chloroquines on platelets were reviewed earlier. In 1985, I reported that the antiplatelet effects of hydroxychloroquine were associated with a statistically significant decrease in thromboembolic disease among 92 patients evaluated (307). A follow-up survey suggested that this trend was applicable to patients with anticardiolipin antibody (308 ,309). Petri et al.'s (310 ,311) 393-member lupus cohort has confirmed this in a prospective study, and McCarty and Hellman (312) noted it among 121 patients at the University of Indiana, as did the 384 member LUMINA cohort (313).

Antihyperlipidemic Effects

As discussed earlier, antimalarials have an antihyperlipidemic action. In 1990, our group showed that hydroxychloroquine induced a 15% to 20% decrease in serum

cholesterol, triglyceride, and LDL levels in 150 patients (314) (Table 59-4). These actions might decrease the hyperlipidemic and atherogenic effects of corticosteroids and suggest a greater adjunctive role for hydroxychloroquine. Hodis et al. (315) confirmed these findings, as did Petri et al.'s (316) longitudinal regression analysis among her 264 patient cohort and the results of other investigators (317 ,318 ,319 ,320 ,321 ,322 ,323 ,324), including in children (325). Quinacrine also lowered lipid levels in a double-blind, placebo-controlled trial of 16 patients with diabetes (326). See also Chapter 19 .

Table 59-3: Twenty-One Clinical Trials of Atabrine in Lupus

Investigator	Year	Patients (*n*)	Response (%)		
			Excellent	Improved	None or Doubtful
Prokoptchouk[a]	1940	(35)	?	?	?
Sorinson	1941	51	23	33	43
Page	1951	18	50	33	17
Somerville et al.	1952	23	17	66	17
Cramer and Lewis	1952	6	83	0	17
Wells	1952	12	25	50	25
Sawicky et al.	1952	30	20	50	30
Black	1953	60	17	38	45
O'Leary et al.	1953	40	40	36	25
Courville and Perry	1953	13	38	54	8
Kaminsky and Knallinsky	1953	61	16	62	21
Harvey and Cochrane	1953	62	37	23	40
Kierland et al.	1953	52	33	46	21
Rogers and Finn	1954	45	47	38	15
Helanen	1954	36	28	58	14
Christiansen and Nielson	1956	97	32	40	28
Dubois	1956	61	25	56	20
Nielsen	1956	12	17	75	8
Buchanan	1959	25	28	52	20
Winklemann et al.	1961	67	10	75	15
Lipsker	1995	15	53	27	20
Totals (*n*)		771	209	352	210
Totals (%)		100	27	46	27

[a]Excellent or improved response, 73% (5).
Adapted from Wallace DJ. The use of quinacrine (Atabrine) in rheumatic diseases: a reexamination. *Semin Arthritis Rheum* 1989;18:282–296, with permission.

Table 59-4: Effect of Antimalarials on Lipids[a]

Parameter[b]	Group A (*n* = 58)	Group B (*n* = 35)	Group C (*n* = 18)	Group D (*n* = 44)
Cholesterol	180.93 ± 49.44	212.71 ± 36.84	186.22 ± 36.70	204.64 ± 49.44
HDL	59.40 ± 20.30	56.59 ± 14.00	57.41 ± 15.88	53.90 ± 16.95
LDL	101.00 ± 29.62	120.26 ± 32.59	102.41 ± 28.97	127.27 ± 41.10
Triglyceride	106.45 ± 51.41	172.57 ± 99.95	145.05 ± 61.54	128.93 ± 68.48
Medications (ms)				
Mean dose HCQ	386	0	400	0
Mean dose steroids[c]	0	10.83	8	0

[a]All serum levels expressed as mg/dL ± 1 SD. A—HCQ alone; B—steroids alone; C—HCQ and steroids; D—neither HCQ nor steroids.
[b]Important significant comparisons; $p < .05$, group B versus C (cholesterol) and HCQ versus no HCQ (triglyceride): $p < .01$, group A versus D (cholesterol); $p < .001$, HCQ versus no HCQ (cholesterol), HCQ versus no HCQ (LDL), and steroids versus no steroids (triglyceride).
[c]Steroid dose expressed as daily equivalent dose of prednisone.
HCQ, hydroxychloroquine: HPL, high-density lipoprotein.
Adapted from Wallace DJ, Metzger AL, Stecher VJ, et al. Cholesterol-lowering effect of hydroxychloroquine in patients with rheumatic disease: reversal of deleterious effects of steroids on lipids. *Am J Med* 1990;89:322–326, with permission.

Additional Actions: Sjogren, Nodulosis, and Bone Density

Fox (327) found that hydroxychloroquine decreased antibody levels in patients with Sjögren syndrome, most of whom reported less dryness after 1 to 3 years of therapy. Other studies tend to support this (328 ,329). It may also inhibit glandular cholinesterase (330). Reports also have suggested that hydroxychloroquine or chloroquine ameliorate methotrexate-induced rheumatoid nodulosis or liver function abnormalities (51 ,52 ,331) and has analgesic actions (332). Hydroxychloroquine significantly improved autoimmune urticaria in 18 patients (333), and protects against low bone mineral density in SLE patients (334).

In conclusion, in spite of only a small number of well designed controlled studies, it seems to be generally agreed that antimalarials are effective for cutaneous manifestations, polyarthralgia, pleuritis, and low-grade pericardial inflammation associated with SLE. Additionally, some of the associated malaise and lethargy are ameliorated. Antimalarials are of no effect in seriously ill patients with central nervous system involvement, hematologic changes, or renal disease. They help in withdrawing steroid therapy once remission has been induced by steroids and other agents (290 ,335) and may be useful in diminishing the atheroembolic complications of SLE.

Dosage

The dosing schedule used in the treatment of discoid LE varies with extent of the skin lesions and the patient's tolerance of the drugs. Theoretically, it is advisable to begin with a larger initial dosage so that equilibrium can be reached sooner. From a practical standpoint, however, the treatment of discoid LE is not urgent. Although an initial loading dose is advisable for the moderately ill patient with SLE, larger starting doses produce a high incidence of side effects, such as nausea, vomiting, and diarrhea, and therefore discourage the patient from further trials with the drug (336). In our experience, some patients who note cutaneous or gastrointestinal side effects with generic hydroxychloroquine do not have the same problems with brand name Plaquenil (337 ,338).

Hydroxychloroquine usually should be initiated in a dosage of 400 mg daily (given once daily or in 200-mg divided doses). This should approximate 5 to 6.5 mg/kg/day ideal body weight (339). Responses usually begin in 2 to 3 months, but the drug does not reach its peak efficacy for 6 to 12 months. In more urgent situations, 600 mg daily may be given for 1 to 2 months. This is associated with a greater incidence of GI complications and retinotoxicity if used for more than a few months (340 ,341).

Smoking may decrease the effectiveness of hydroxychloroquine in cutaneous lupus (342 ,343 ,344).

Chloroquine usually is given in a dosage of 250 to 500 mg daily; this should approximate 4 mg/kg/day. Chloroquine works within 1 to 2 months but is associated with a 10% incidence of retinotoxicity, compared with less than 3% for hydroxychloroquine (345). Hence, the eyes should be checked at 3-month intervals for patients on chloroquine and at yearly or recommended intervals for those on hydroxychloroquine. Plasma levels do not correlate with efficacy (346). After a response is achieved in 1 to 3 months, maintenance chloroquine dosing should not succeed 250 mg a day.

If additional therapy is required, quinacrine can be added. It has an established synergy with the chloroquines (22 ,54 ,60 ,303 ,304 ,305 ,347). Usually, 100 mg are given daily (although up to 200 mg daily can be administered), but as little as 25 mg may be effective. Occasionally, therapy can be initiated with quinacrine as opposed to the chloroquines when ophthalmologic considerations contraindicate the latter's use. Also, quinacrine is a much greater cerebral cortical stimulant than the chloroquines and is used for patients in whom fatigue is overwhelming (348). Quinacrine is not retinotoxic. Its onset of action is 3 to 6 weeks.

In my experience, at least 95% of patients with skin lesions of discoid LE and SLE show moderate to significant benefit from treatment with antimalarials (349). The most common cause of failure is the physician's impatience in giving the drugs adequate time to work. After 1 to 2 years of therapy, antimalarials can be tapered. Hydroxychloroquine is decreased to 200 mg daily for 3 to 6 months, then reduced by eliminating days of the week (e.g., the next decrement from 200 mg daily would be 5 days a week for 3 months, then 3 days a week). One or two tablets per week may be all that is required to suppress skin lesions, and this helps to minimize toxicity. Chloroquine can be reduced to 1 to 2 tablets a week. The reader is referred to Bernstein (345) for a more detailed discussion of the issues reviewed in this section.

Pregnancy

See Chapter 51 .

Adverse Reactions

Table 59-5 lists the adverse reactions to antimalarials, which are discussed here.

Generalized and Gastrointestinal Reactions and Overdosage

Considering their remittive potential, antimalarial therapies generally are well tolerated when compared with other disease-modifying drugs. In one study, more than 90% of patients who were prescribed hydroxychloroquine for lupus were still taking the drug a year later (296). Approximately 10% of those receiving hydroxychloroquine and 20% receiving chloroquine complain of anorexia, abdominal distention and cramps, heartburn, nausea, vomiting, diarrhea, and/or weight loss. These symptoms are transient, decrease, or disappear with lower dosing or

changing to brand name Plaquenil, and they do not cause long-term sequelae. In Esdaile's group's (336) cohort, 20 of 156 (13%) patients followed for up to 15 years stopped antimalarials due to side effects, half of which were gastrointestinal. Quinacrine may create these symptoms in up to 30% of patients, and diarrhea may be particularly pronounced. It can be alleviated with lower doses or by taking a bismuth suspension (e.g., Pepto-Bismol) with quinacrine. The chloroquines are associated with musculoskeletal, flulike symptoms of aching and fatigue in 5% to 10% of patients, but symptoms resolve within 1 to 2 weeks even if therapy is continued.

Table 59-5: Non-Ocular Toxic Effects of Chloroquine and Hydroxychloroquine

1. Seen in 1%–20% given chloroquine and 1%–10% given hyroxychloroquine
   a. Skin—pruritus, urticaria, morbilliform rash, maculopapular rash, increased skin pigmentation
   b. Gastrointestinal—nausea, vomiting
   c. Nervous system—insomnia, nervousness
2. Seen in <5% given chloroquine and <1% given hydroxychloroquine
   a. Nervous system—neuropathy, hearing change, tinnitus, nervousness, confusion, psychosis, vestibular change, seizure
   b. Skin—dryness, desquamative rash, porphyria, hair loss, hair bleaching
   c. Blood—leukopenia, anemia
   d. Gastrointestinal—anorexia, abdominal distension, heartburn, diarrhea, weight loss, liver function abnormalities

Antimalarials rarely are hepatotoxic (54 ,329). One report of hepatic failure possibly associated with hydroxychloroquine has appeared (350).

As little as 1 g of chloroquine can be fatal to a child, and 3 g can be fatal to an adult. The pills taste bitter, which tends to discourage abuse. Overdoses are managed with mechanical ventilation, epinephrine, activated charcoal, and diazepam (351 ,352 ,353 ,354). Patients have survived up to 22G of hydroxychloroquine as part of an overdose (355 ,356).

Neuromuscular and Cardiac Effects

In 1948, Nelson and Fitzhugh (357) first reported that the chronic administration of chloroquine to rats induces necrosis of cardiac and voluntary muscle. The term chloroquine neuromyopathy has evolved over the years; it is clinically evident in fewer than 1% of those taking chloroquine and has been the subject of fewer than 20 case reports with hydroxychloroquine (358 ,359 ,360 ,361 ,362 ,363 ,364 ,365 ,366 ,367 ,368 ,369 ,370 ,371 ,372 ,373 ,374 ,375). Nord et al. recently reviewed the literature (376). Biopsy findings demonstrate a vacuolar myopathy, myeloid and curvilinear bodies. Patients complain of muscle weakness, numbness, and tingling, and they sometimes have myasthenic symptoms. Active inflammatory myositis, hypokalemia, and steroid myopathy must be considered in the differential diagnosis. A myasthenia gravis–like picture with ptosis occasionally appears, which is reversible with drug discontinuation (377 ,378). The patient may present with an acute polyneuropathy. Peripheral nerves may demonstrate segmental demyelination and cytoplasmic inclusions in Schwann cells (and in perineural and endothelial cells to a lesser extent). Histopathologic investigation of the muscles reveals a vacuolar myopathy, acid phosphatase–positive vacuoles in type I fibers, and lysosomal hyperreactivity with large secondary lysosomes. Electron microscopy reveals electron-dense curvilinear bodies and concentric and parallel lamellae within the muscle (379). Both skeletal and cardiac muscle can be involved. Muscle enzyme levels only occasionally are elevated. Electromyography reveals fibrillations, positive sharp waves, complex, repetitive discharges, and sometimes a myotonia pattern. Dramatic recovery is associated with discontinuation of the drug. Plasma chloroquine levels do not correlate with the clinical or pathologic picture (380). Muscle enzyme levels only occasionally are elevated. This syndrome does not occur with quinacrine.

Rarely, acute urinary retention, heart block, or a congestive cardiomyopathy has been associated with chloroquine, but not with hydroxychloroquine (381 ,382 ,383 ,384 ,385 ,386 ,387).

Cutaneous and Pigmentary Changes

Antimalarials can induce skin dryness, pruritus, pustulosis, porphyria, erythema annulare, urticaria, changes in pigment, rashes, psoriatic flares, and exfoliating lesions (388 ,389). Approximately 3% of patients have to discontinue the drug secondary to adverse cutaneous reactions (378). Psoriatic flares result from the ability of hydroxychloroquine to interfere with epidermal transglumatase activity, which stimulates epidermal proliferation (390). Quinacrine is associated with a lichen planus or eczema-like eruption that, if ignored, can be the first sign in a chain of events that ultimately leads to aplastic anemia. Any rash resulting from quinacrine requires its immediate cessation (54).

Pigment changes occur in 10% to 25% of those who are receiving long-term chloroquine therapy and in a smaller percentage of those taking hydroxychloroquine (391 ,392 ,393 ,394). These adverse effects rarely, if ever, require discontinuing treatment. Gum pigmentation is common (395), and grayness at the roots of scalp hair, eyelashes, eyebrows, and beard may be observed, along with gray streaks in the hair. A blue-black discoloration occasionally is noted on the skin (Fig. 59-2). Chloroquine binds with melanin in vivo and in vitro by the electrostatic attraction of positively charged drug molecules to negative groups of the melanin polymer; this probably is supplemented by van der Waals forces or charge transfer complexes. These changes are reversible when chloroquine therapy is stopped. Nail beds can be affected and appear to be diffusely pigmented (Fig. 59-3) or to display transverse bands.

Chloroquines frequently can cause light sensitivity (396 ,397) or, rarely, porphyria (398) in patients with SLE.

Quinacrine also binds to melanin. Membrane-bound intracellular quinacrine granules combined with large amounts of iron and sulfur produce asymptomatic black-and-blue marks, especially on the shins and hard palate (Fig. 59-4) (399 ,400). Quinacrine also can induce a yellow stain that, like the pigment, is dose related and resolves with cessation of therapy or lowering of the dose. The stain may be evident in up to 30% of patients; it sometimes looks like a suntan and may enhance the patient's appearance (54). All of these changes also are reversible.

Central Nervous System

Quinacrine and, to a lesser extent, the chloroquines, are cortical stimulants. Engel et al.'s (348) classic study documented electroencephalographic patterns that were compatible with pronounced psychic stimulation in a group of healthy volunteers given 200 to 1,200 mg of quinacrine daily for 10 days. Symptoms of fatigue and mental clouding may be ameliorated. On the other hand, excessive dosing can result in psychosis, seizures, and hyperexcitability (54 ,282 ,348 ,401 ,402 ,403 ,404). A 0.4% incidence of reversible, toxic psychosis was reported among 7,604 U.S. soldiers who were given 100 mg of quinacrine daily in World War II and in 28 patients among 30,000 who were treated for malaria (0.1%) (405 ,406). Central nervous system (CNS) complications of chloroquine, such as mania or insomnia, have infrequently been noted in SLE (407 ,408).

Hematologic Toxicity

Hydroxychloroquine has been associated with only one case of agranulocytosis, in a patient who was given 1,200 mg daily (409), which is three to six times the current recommended dose. Chloroquine, but not hydroxychloroquine, has been implicated in some reports with glucose-6-phosphatase deficiency hemolysis (410) and with agranulocytosis (411). Toxic granulation has been observed in the leukocytes of patients receiving long-term chloroquine, which represents large, membrane-bound myelin bodies in mature neutrophils and lymphocyte (412 ,413).

The prevalence of aplastic anemia among U.S. soldiers in the Pacific during World War II increased from 0.66 to 2.84 per 100,000 after quinacrine's introduction (414). This represented 58 patients, 48 of whom received quinacrine. Of these, 16 were associated with overdoses, and two received other marrow-suppressant drugs concurrently. Wallace's review analyzes these cases, reviews the literature, and suggests ways to prevent this from occurring (54 ,415 ,416).

Miscellaneous Effects

Antimalarials may inhibit gastric motility based on their parasympatholytic effects (417), and they may induce hypokalemia (418), and its quinine-like actions are responsible for sensineural hearing loss (419).

Ocular Toxicity

Corneal, Ciliary Body, and Lens Changes

Corneal deposits of chloroquine are observed in 18% to 95% of patients, appear within several weeks, and are symptomatic in 50% of patients (342 ,420 ,421 ,422 ,423 ,424 ,425). Keratopathy is limited to the corneal epithelium; the pattern can vary from punctate opacities to whirling lines. Visual acuity is not reduced, but patients may complain of halos around light sources and of photophobia. No residual damage occurs, and corneal deposits disappear with drug discontinuation and usually are not a reason to stop therapy. Corneal sensation may be decreased by 50% (426). Because recommended chloroquine doses have decreased over the last 30 years, corneal problems now occur less frequently (427). Two studies were unable to find any corneal changes among 164 patients who were given hydroxychloroquine for 3 to 7 years (428 ,429). Easterbrook (430 ,431) reported a 5% to 10% incidence of corneal infiltrates with hydroxychloroquine, but none were symptomatic. A decreased dosage is advised for these patients. Hydroxychloroquine crystals may be seen in the tear film by slit-lamp examination (420). In doses three to six times greater than those currently recommended, quinacrine can induce corneal edema rarely (432 ,433).

Alterations in accommodation and induction of cataracts rarely occur with long-term chloroquine therapy and have not been reported with hydroxychloroquine or quinacrine (424). Patients who discontinue hydroxychloroquine have resolution of keratopathy and the drug can be restarted upon its disappearance without recurrence.

Retinopathy

Since the first report appeared in 1957 (434), approximately 300 cases of chloroquine and hydroxychloroquine retinopathy have been reviewed in the literature. Retinopathy is an often-misunderstood problem that needlessly deters patients from initiating antimalarial therapy. Patients taking chloroquine have an approximate 10% risk for developing retinopathy using appropriate doses, which is usually but not always reversible (345). In 2003 and 2004, Mavrikakis et al. conducted a prospective study and literature review and concluded that “no documented case exists of hydroxychloroquine induced retinopathy in patients who were taking the drug in recommended doses for less than 6 years and had normal renal function” (435 ,436). It was noted in 2003 that 19 cases of confirmed hydroxychloroquine retinopathy had been reported in the literature since 1957. All had abnormal renal function, had taken the drug for more than 6 years, or were dosed using their actual body weight as opposed to their ideal body weight (339 ,340).

Clinical Presentation and Pathophysiology

SLE can induce retinal vascular lesions secondary to disease activity that are unrelated to any form of therapy, and macular degeneration is a common feature of the normal aging process. Thus, it is not always easy to implicate antimalarials as the cause of retinal dysfunction. The chloroquines usually take years to induce pathology, and early retinopathy is asymptomatic. Patients with lupus who are aware of the retinotoxicity of antimalarials often complain of visual symptoms weeks after starting therapy; this can be attributed to corticosteroid or nonsteroidal anti-inflammatory drug (NSAID) treatment, or to psychopathology.

The most common presenting symptoms are difficulty in reading, photophobia, blurred distance version, visual-field defects, and light flashes. Premaculopathy consists of fine pigmentary stippling of that area. Eventually, it becomes surrounded by a zone of depigmentation encircled by an area of pigment, giving a bull's-eye appearance (Fig. 59-5). Rods and cones (which compose the macula) are particularly sensitive to the chloroquines. With more extensive retinal damage, the arterioles show generalized attenuation and segmental constriction with disk pallor. In the periphery

of the fundus, a prominent choroidal pattern and fine granularity of the retina are seen. Many years later, gross pigment changes of hereditary retinal depigmentation may occur, and color vision and the foveolar reflex are lost (424 ,425 ,437 ,438 ,439 ,440).

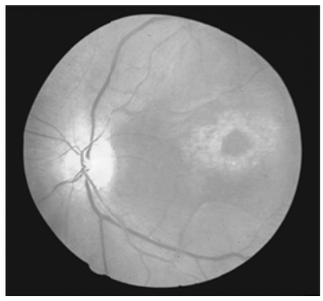

Figure 59-5. Bull's-eye macular pigmentation secondary to hydroxychloroquine (1,200 mg/dL for 15 months in a 90-lb woman).

Several theories account for chloroquine retinopathy. Retinal pigmented epithelial cells perform as macrophages and digest the discarded outer segments of photoreceptor cells as they are physiologically shed. Lysosomal accumulation (discussed earlier) results in an intracellular buildup of lamellar myelin bodies, which leads to scotoma (441 ,442). Alternatively, melanin deposits in the retina produce pigmentation of the rods and cones and of the pigmented cells in the outer nuclear and outer plexiform layers, with resulting pathology (443 ,444). Platelet-activating factor or chloroquine's effect on inhibiting protein synthesis may play a role (445 ,446). Cases of retinopathy have been reported, however, in the absence of pigment deposition (447). Chloroquine, but not hydroxychloroquine, breaks down the blood–retina barrier, as documented by vitreous fluorophotometry (447 ,448).

Important Clinical Studies in Lupus Erythematosus

The incidence of retinopathy has decreased over the last 30 years, because currently recommended doses of both chloroquine and hydroxychloroquine are approximately 50% of the formerly recommended doses. In 1992, Bernstein (449) reviewed all published cases of hydroxychloroquine retinopathy and those reported by the Food and Drug Administration (FDA) between 1960 and 1990. Only 20 patients fulfilled validated criteria. In 15 of the patients, recommended dosing levels were exceeded, and the remaining five patients took the drug for longer than 10 years.

The reader is referred to previous editions of this textbook for a review of 37 studies that review the prevalence and characteristics of retinal toxicity of the chloroquines since 1957 in thousands of patients which are listed here (437 ,438 ,439 ,440 ,441 ,442 ,443 ,444 ,445 ,446 ,447 ,448 ,449 ,450 ,451 ,452 ,453 ,454 ,455 ,456 ,457 ,458 ,459 ,460 ,461 ,462 ,463 ,464 ,465 ,466 ,467 ,468 ,469 ,470 ,471 ,472 ,473 ,474 ,475 ,476). They come to strikingly similar conclusions that are summarized in this section.

Only one case of questionable retinotoxicity has been reported with quinacrine (477). Zuehlke et al. (478) noted no eye toxicity from quinacrine in 26 patients followed at the University of Iowa over a 30-year period and reviewed the literature. None of the 200 patients treated with quinacrine by Dubois or those that I have treated have evolved retinopathic changes (54).

Once the lesion appears, no specific therapy other than cessation of the antimalarial is required. Although antimalarial excretion can be increased by acidification with ammonium chloride, ascorbic acid, or British antilewisite, nothing indicates that the ocular lesion is improved (437). The best and only treatment is discontinuation of the drug if even equivocal changes of retinopathy occur. Mackenzie and Szilagyi (479) suggested that sunglasses may prevent the retinal lesion. They showed that high-dose chloroquine concentrates in melanin in pigment epithelium, blocking the normal light-absorbing action of melanin, and thereby removing its protective mechanism. This has been supported by laboratory studies in rats (480), but it is premature to advocate the use of sunglasses in all patients who are taking antimalarials.

Retinal Testing and Clinical Correlates

Ophthalmologists have many techniques that purport to evaluate retinal and macular integrity and function, but most agree to disagree about the optimal sequence of testing. Helpful reviews of these methods for antimalarial monitoring have been published (424 ,481).

If color vision is abnormal, testing is inexpensive and reliable; some investigators believe it is the most sensitive method (482). The time it takes to recover macular function after illumination of the retina is known as the macular dazzle test. Although it is thought to be prolonged in patients with early antimalarial maculopathy (483), Easterbrook (484) found that it is abnormal in almost all patients who are on antimalarials and does not distinguish those with retinopathy from those without. Fluorescein angiography shows striking macular uptake. Its sensitivity and specificity ratings are disappointing (424), however, and it was less reliable than color vision testing in a controlled, comparative study (482).

Visual field testing, especially if augmented by Amsler grids, is a simple and inexpensive screen for paracentral scotomas (484). Amsler grids can be self-administered and are easily reproducible in cooperative patients, but not always reliable (485 ,486). Electo-oculography (EOG) reflects the metabolic integrity of the retinal pigment epithelium but correlates poorly with macular changes that are induced by chloroquines (487). Electroretinography (ERG) is another technically complex procedure that detects late changes, but it is difficult to interpret in early disease. It can show paracentral loss, foveal loss, peripheral loss, and generalized loss (488 ,489 ,490 ,491). Whether or not it can detect preclinical changes is controversial. Additionally, many nonspecific abnormal readings are noted in normal patients. Dark adaptation testing probably is of little value. One controlled study found contrast sensitivity testing to be superior to pattern visual-evoked potentials and EOG (492). In a fascinating report, a British survey found that rheumatologists could identify 52 of 65 minor retinal changes in patients on chloroquine and concluded that expensive, frequent eye examinations generally are unnecessary (493). Table 59-6 provides a summary of, and our group's recommendations for, eye toxicity in antimalarials.

When Is Retinal Testing Cost Effective?

Rynes et al. (494) estimated that $20 million is spent on retinal testing in the United States for patients taking hydroxychloroquine each year. Two groups have suggested that $126,000 to $200,000 is spent on biannual exams or $74,000 on annual exams to pick up each case of retinopathy (472 ,495).

Table 59-6: Eye Toxicity from Antimalarial Medications

Cornea	Symptoms of photophobia and halos around light sources, decrease in sensation
	Usually mild and can come and go; rarely requires drug to be stopped
	These changes are always reversible with drug cessation; no permanent damage reported
	Chloroquine: noted in 50%
	Hydroxychloroquine: found in 5%–10%
	Quinacrine: reported in 5%
Retina	Melanin deposits or lamellar myelin bodies in retinal pigmented epithelial macrophages
	Clinically presents as scotomas; especially targets rods and cones
	Must be differentiated from macular degeneration associated with aging
	Chloroquine: found in 10% after 10 years of continuous use; can be irreversible
	Hydroxychloroquine: found in <3% after 10 years of continuous use; always reversible if doses of <6.5 mg/kg/day used with lean body weight and patient not in kidney failure
	Quinacrine: not reported

Published Guidelines for Retinal Testing

The Physician's Desk Reference (PDR) recommends quarterly eye examinations for both chloroquine and hydroxychloroquine (496). The American Academy of Ophthalmology (AAO) assembled a task force to develop more realistic screening recommendations that would be in accordance with both modern knowledge and the economic realities of practice (497 ,498). Noting that there are extremely infrequent reports of toxicity in individuals taking more than 6.5 mg/kg/day of hydroxychloroquine or 3 mg/kg/day of chloroquine for less than 5 years, they advised dividing patients into low- and high-risk groups. The latter consists of those taking the drugs for more than 5 years, those older than 60 years of age, or individuals with a high body fat level, renal or hepatic disease. All patients should have a baseline ophthalmologic exam and visual field testing. Low-risk patients do not need to be re-evaluated for 5 years. Patients at higher risk should be evaluated annually. A Canadian Concensus Conference recommended similar testing for all patients on hydroxychloroquine at 12- to 18-month intervals (499). A United States consensus conference recommended annual eye examinations in low-risk individuals on hydroxychloroquine therapy who have been on the drug for less than 10 years in doses of 6.5 mg/kg/day or less (500).

Thus, the PDR guidelines, which were written in the 1950s and have not been revised since, do not reflect the experience of several million patients who have taken the drug, and this writer feels comfortable with more recent recommendations.

Summary

- Three antimalarials that are commercially available in the United States have documented efficacy in the treatment of lupus erythematosus: chloroquine, hydroxychloroquine, and quinacrine.
- These agents are recommended for patients with non–organ-threatening lupus who require more than sunscreens, steroid salves, or NSAIDs.
- Chloroquines are most effective for treatment of the following features of LE (in decreasing order): cutaneous lesions, arthritis-arthralgias, fatigue, and serositis. Chloroquine is more powerful than hydroxychloroquine. Quinacrine is most effective for the following (in decreasing order): cutaneous lesions, fatigue, arthritis-arthralgias, and serositis.
- Chloroquine and quinacrine are effective in 1 to 2 months; hydroxychloroquine often requires a 3- to 6-month wait. Chloroquines and quinacrine are synergistic and can be combined.
- Antimalarials work by turning off antigen processing by raising intracytoplasmic pH. They also work by blocking damaging UV light, suppressing immune reactivity, promoting apoptosis, inhibiting antibody formation, and blocking prostaglandin and leukotriene synthesis by the inhibition of phospholipase A2. They also inhibit platelet aggregation and adhesion, decrease membrane receptor sites because of lysosomal membrane accumulation, and are antimicrobial and antiproliferative.
- Steroid-sparing actions also have been documented.
- Higher dosing can cause a faster response but also greater toxicity.
- Generalized gastrointestinal and musculoskeletal complaints are reversible and usually minor. The only serious complication of the chloroquines is retinotoxicity, which is observed in 10% of patients on chloroquine and in less than 3% of those on hydroxychloroquine. This can be minimized by frequent eye examinations and the use of hydroxychloroquine. Irreversible retinal changes have never been reported in a patient with lupus who was taking hydroxychloroquine in recommended doses for up to 6 years with normal renal function and undergoing eye checks every 6 months (501). Quinacrine is not retinotoxic, but blood counts need to be monitored because it can cause aplastic anemia (rarely).

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Chapter 60

Systemic Glucocorticoid Therapy in Systemic Lupus Erythematosus

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Phillip Hench was the first to introduce glucocorticoids (GC) into clinical medicine when he successfully treated a patient with rheumatoid arthritis (RA) in 1949 (1). The long record of GC in the management of rheumatic diseases testifies to their clinical usefulness. However, GC benefits occur at a high cost of serious side effects. Therapeutic strategies aiming to decrease exposure to GC are, therefore, imperative. The treating physician, optimally a rheumatologist with experience in the management of SLE (2), should carefully evaluate the patient and attempt to distinguish inflammation because of disease flare from infection, thrombosis, and drug (including GC) adverse effects. Combination therapies of GC with other immunosuppressive or anti-inflammatory agents can help achieve disease control with less exposure to GC. Hopefully, future research on both SLE pathogenesis and mechanisms of GC action will add safer and more effective therapies to our armamentarium against SLE.

In this chapter we will briefly review the basic pharmacology of endogenous and synthetic glucocorticoids, the mechanisms of their action at the molecular level, and their anti-inflammatory and immunosuppressive effects. Next, the pharmacokinetics and drug interactions of GC will be discussed, and the authors' opinions regarding their use in SLE will be presented. Lastly, adverse effects of GC with relevance to SLE will be analyzed, and the glucocorticoid withdrawal syndrome will be briefly reviewed.

Endogenous and Synthetic Glucocorticoids

Steroidogenesis in the adrenal cortex produces endogenous glucocorticoids, mineralocorticoids (MC), and adrenal androgens (3 ,4). Cortisol (hydrocortisone) is the main human endogenous GC and is secreted primarily in response to adrenocorticotropic hormone (ACTH). Secretion follows a circadian rhythm that achieves maximum plasma concentration at 8 A.M (16 μg/dL) (3 ,4 ,5). However, in the context of stressful stimuli and hypothalamic-pituitary-adrenal (HPA) axis stimulation, these levels can increase to more than 60 μg/dL losing their diurnal variation (5 ,6). The ability of an organism to maintain appropriate GC levels before and during stress is quintessential for its survival (7 ,8).

Synthetic GC, more potent and with less mineralocorticoid effects than cortisol, have been developed. Figure 60-1 shows the biochemical structure of cortisol and synthetic GC; Table 60-1 compares their pharmacological properties. Regulatory mechanisms of synthetic GC with regard to binding to the corticosteroid-binding globulin (CBG), tissue specific metabolism, affinity for GC receptors (GR), and interaction with transcription factors may substantially differ from those of native GC (4 ,9 ,10).

The great need for improved synthetic GC with less adverse effects and intact anti-inflammatory/immunosuppressive action has led to the development of newer synthetic GC, by modifying their pharmacokinetic or pharmacodynamic properties. With regard to the first, GC with high topical activity, but low systemic bioavailability because of rapid first-pass liver inactivation such as budesonide, have been designed. Thus, by design, this agent is suitable only when topical anti-inflammatory action is desired such as in asthma (given by inhalation), and in active Crohn's disease (orally) (11 ,12). Of more interest to rheumatology is the development of liposomal glucocorticoids by encapsulating GC in polyethylene glycol (PEG)-coated liposomes. Since liposomes are preferentially phagocytosed by activated macrophages and PEGylation limits their removal by the mononuclear phagocyte system in liver and spleen, these GC are able to remain in the circulation for prolonged periods of time and target inflamed tissues efficiently. A single dose of liposomal GC strongly inhibited inflammation in collagen type II arthritis for 10 days with evidence of localization in the synovial lining of only the affected joints (13). With regard to pharmacodynamics, substantial progress has been made in the development of synthetic GC that dissociate their potent activator protein-1 (AP1) and nuclear factor κB (NF-κB) transrepression activities (anti-inflammatory) from their weak transactivation ones (see below) that are likely responsible for GC adverse effects (14 ,15 ,16). Another category are the nitric oxide (NO) glucocorticoids that take advantage of the synergistic (to the GC) anti-inflammatory effect of NO, as well as the fact that these agents induce tyrosine nitration of the GC receptor (GR), which further enhances their anti-inflammatory effects (17).

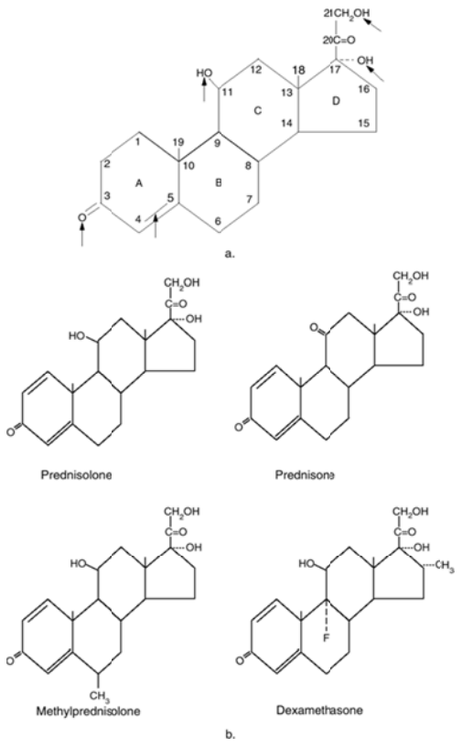


Figure 60-1. A. Structure of cortisol (hydrocortisone). All Δ4 double bond, 3-keto group, and 11ß-OH group are essential for glucocorticoid function and the first two are also required for mineralocorticoid activity. The hydroxyl group at C21 is required for mineralocorticoid activity and is present on all natural and synthetic GC. The 17α-hydroxyl group, present on cortisol and synthetic GC (but not on corticosterone), enhances GC potency. **B.** Structure of selected common synthetic GC. The addition of a Δ1 double bond on cortisol (as in all shown GC) selectively increases glucocorticoid activity and delays GC metabolism. The methyl group at position 6α (methylprednisolone) increases GC, over MC, activity even further. Notably, fluorination at the 9α position (fludrocortisone; not shown) greatly enhances GC and MC activity (the latter much more than the former). However, when modified with a Δ1 double bond and a methyl group substitution at C16α, fludrocortisone loses all MC activity and becomes dexamethasone.

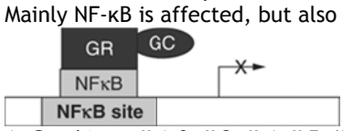
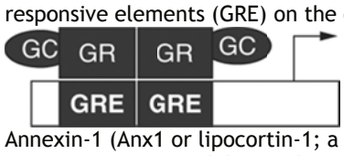
Molecular Mechanisms of Glucocorticoid Action (see Table 60-2)

Glucocorticoid effects are mainly mediated via specific GC receptors (GR). These are conspicuous cytoplasmic proteins and operate as hormone-activated transcriptional regulators (reviewed in (18 ,19 ,20)). Hydrocortisone and some other GC are also capable of binding mineralocorticoid receptors (MR) with higher affinity than they bind GR and mediating aldosterone-like effects (Table 60-1). GR specificity, at the relatively low baseline body cortisol levels, is maintained because of the action of 11-β-hydroxysteroid dehydrogenase2 (11-β-HSD2), a steroid metabolizing enzyme expressed at MC-sensitive tissues (i.e., the kidney). 11-β-HSD2 metabolizes hydrocortisone to its inactive 11-keto derivative (cortisone) (3 ,4 ,7).

Table 60-1: Relative Biologic Potency and Pharmacokinetics of Selected Glucocorticoids (GC)

GC	Genomic Anti-Inflammatory	Mineralocorticoid Activity	Half-Life (Minutes)	Biologic Half-Life (Hours)
Cortisol	20	1	60	8–12
Cortisone	25	0.8	60	8–12
Prednisone	5	0.8	180	12–36
Prednisolone	5	0.8	180	12–36
Methylprednisolone	4	0.5	180	12–36
Triamcinolone	4	0	180	12–36
Dexamethasone	0.75	0	220	36–72

Table 60-2: Mechanisms and Examples of Anti-Inflammatory and Immunosuppressive Actions of GC

A.	Transrepression through protein-protein cross-talk: activated glucocorticoid receptor (GR) monomers interact and inhibit proinflammatory transcription factors. Mainly NF-κB is affected, but also AP1, NFAT, and so on with end result the transcriptional inhibition of:  1. Cytokines: IL1-β, IL2, IL4, IL5, IL6, IL12, TNF, GMCSF, etc. 2. Chemokines: IL8, MCP-1, MIP1-α, eotaxin, etc. 3. Proinflammatory enzymes: iNOS, COX2, collagenase. 4. Adhesion molecules: ICAM1, E-selectin, etc.
B.	Transactivation of anti-inflammatory genes through binding of activated GR dimers to glucocorticoid responsive elements (GRE) on the corresponding gene promoter or enhancer regions.  Annexin-1 (Anx1 or lipocortin-1; a phospholipase A2 inhibitor), IκB (inhibitor of NFκB), IL1 receptor antagonist (IL1Ra; inhibitor of IL1-β), MAPK phosphatase-1 (MKP1), secretory leucocyte inhibitory protein (SLPI), neutral endopeptidase, IL10, etc.
C.	Post-transcriptional effects through inhibition of p38 MAPK. Probably mediated through induction of MKP1 and inhibition of p38 MAPK (see B) Decreased mRNA stability of cytokines, COX-2, iNOS.
D.	Rapid nongenomic effects through cytoplasmic GRE. Activation of enthothelial NOS (eNOS) and generation of NO through GR-mediated activation of phosphatidylinositol 3-kinase (PI-3K) and protein kinase Akt. Rapid phosphorylation of Anx1 and blockade of activation of cytoplasmic PLA2 by growth factor receptors.
E.	Rapid nongenomic effects through membrane GR.
F.	Rapid nonspecific effects through physicochemical interaction with cell membranes. These are thought to occur at pulse IV doses of GC. Disruption of calcium and sodium cycling with low intracellular calcium levels, as well as mitochondrial proton leak, probably contribute to rapid GC-mediated immunosuppression.

Anx1, annexin-1 (or lipocortin-1); AP1, activator protein-1; COX-2, cycloxygenase-2;GMCSF, granulocyte-macrophage colony-stimulating factor; ICAM1=intercellular cell adhesion molecule-1, IκB=inhibitor κB; IL, interleukin; iNOS, inducible nitric oxide synthase; MAPK, Mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; MIP1α, macrophage inflammatory protein-1a, NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; NO, nitric oxide; PLA2, phospholipase A2; TNF, tumor necrosis factor.

Glucocorticoid receptors, when inactive, are bound to several receptor associated proteins (RAP) including the heat shock proteins HSP90 (of critical role in GR ligation and activation) and HSP70, immunophilins (IP), and the Src kinase of the mitogen activated protein kinase (MAPK) signaling pathway (18 ,19 ,20). Upon GC binding, GR dissociate from these proteins and translocate to the nucleus. There they mediate their effects mainly via two mechanisms (see Table 60-2):

- They form homodimers and bind to glucocorticoid responsive elements (GRE) on gene promoters and enhancers with positive (i.e., annexin-1 [Anx1] (21), and IκB (22 ,23)) and negative gene transcription effects (i.e., osteocalcin; (24 ,25)). Notably, such GRE-mediated (transactivation) effects have also been implicated in neoglycogenesis, by means of inducing the transcription of phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase, and therefore, in diabetogenesis. Moreover, transactivation of phosphatases can indirectly lead to decreased mRNA stability of cytokines and inducible enzymes (26 ,27 ,28 ,29) (Table 60-2).
- Alternatively, activated GR monomers are able to transrepress the function of other transcriptional factors like NF-κB (15 ,30), AP1 (31 ,32 ,33), and signal transducers and activators of transcription (STATs) (34) through direct protein-protein "cross-talk."

The recent development of transgenic mice that express a homodimerization deficient GR (GRdim/dim) has helped address the hypothesis that anti-inflammatory effects of GC are primarily mediated by transrepression, whereas adverse effects by transactivation (35). In these mice GR cannot form dimers and thus cannot have GRE-mediated effects. However, the same GR in their monomeric form can transrepress NF-κB and AP1. Indeed topical dexamethasone (DEX) inhibited PMA-induced skin inflammation and endogenous GC terminated LPS-induced systemic inflammation in these mice as well as it did in wild type mice. Additionally, inflammatory cytokines were strongly inhibited in macrophages and T cells from these mice in vitro and NFκB was inhibited without a parallel IκB induction. A more recent study described the use of ZK 216348, a nonsteroidal selective GR agonist (SEGRA) with dissociated activity for transrepression (potent) and transactivation (weak) in a mouse model of skin inflammation (16). The compound showed comparable anti-inflammatory activity to prednisolone, but markedly less increases in blood glucose levels and somewhat less skin atrophy. However, ACTH suppression was similar with the two agents.

Besides having GR-mediated transcriptional genomic effects that depend on new protein synthesis and therefore have a delayed onset (at least 30 minutes), GC may have more rapid (seconds or minutes) nongenomic effects (36 ,37 ,38 ,39) (Table 60-2). These GC effects usually occur at relatively large pharmacologic or pulse GC doses and can be either cell surface receptor-mediated and therefore specific, or initiated by physicochemical interactions (mainly with pulse GC) with cellular membranes and nonspecific (36 ,37). Membranous GR-mediated effects have been described in amphibian brains (40), and these receptors have been recently identified on human peripheral blood mononuclear cells (PBMC) by an ultrasensitive flow cytometry immunofluorescence technique (41). On the other hand, inhibition of cation cycling and respiration in concanavalin A-stimulated rat thymocytes constitute nonspecific GC effects (36 ,37). Interestingly, DEX and methylprednisolone (MP) are 5 and 3.3 times, respectively, more potent than prednisolone as far as these high-dose nonspecific nongenomic effects are concerned (42 ,43).

Anti-Inflammatory and Immunosuppressive Effects

The biologic effects of GC are multiple, affect all tissues, and are essential for body homeostasis during normal or stress conditions. Although, in clinical medicine, GC are used to suppress inflammation and pathologic immune responses, a growing number of studies "paradoxically" attributes immune enhancing effects to these agents (7 ,9 ,10 ,42 ,44 ,45 ,46). It seems that endogenous GC have an important overall regulatory role in modulating immune responses that develop to such stressors as infections. For example, GC on one hand act permissively to help immune responses develop adequately and in a timely fashion in order to fight the invading organisms and on the other, act suppressively to restrain a potentially deleterious "overshoot" of those same responses (7). Parameters that determine the direction of GC immune effects include primarily the serum levels and timing of GC exposure relative to the initiation of stress. Higher (pharmacologic) levels such as those occurring after the initiation of stress are in general immunosuppressive, whereas lower physiologic levels of GC present before stress initiation may enhance immune responses. Notably, acute stresses (or short exposure to GC) enhance immune responses, whereas chronic exposure to stress or GC has the opposite effect (44). Endogenous GC (cortisol) appear to mediate both types of effects. DEX, however, a synthetic GC, mediates predominantly immunosuppressive effects, a phenomenon that may be explained by DEX's inefficient binding to CBG, its longer half life, its higher affinity for GR and absence of MC effects (Table 60-1) (9 ,10 ,44). The immune-enhancing GC effects may be mediated through MR. Augmentation of T-cell proliferation to antigenic stimuli (46), of lymphocyte trafficking to skin and regional lymph nodes (during a DTH response) (44), of cytokine production (including macrophage migration inhibitory factor or MIF) (45 ,47), as well as synergy with different cytokines (such as with IL6 in the induction of acute phase responses) and upregulation of cytokine receptors (7 ,9 ,10) and CD40L (48) are some of these effects.

The anti-inflammatory effects of GC (Tables 60-2 and 60-3) are complex, mediated via their GR and correlate with dose and duration of GC treatment. At the level of blood vessels, GC inhibit vasodilatation and vascular permeability, limiting therefore erythema, plasma exudation, and swelling.

The inhibitory GC effects on the upregulation of the inducible nitric oxide synthase (iNOS), and therefore NO synthesis, by TNF, IL1-ß, and IFN-γ (49) may contribute to this effect. Neutrophils are affected primarily in their ability to migrate to inflammatory sites. This effect is probably due to inhibition by GC of chemokine synthesis (i.e., IL8) (50) and adhesion molecule expression (ICAM1, E-selectin) (51) and induction of annexin I (Anx1 or lipocortin-1) (52 ,53). In patients with RA, inhibition of neutrophil ingress into inflamed joints was seen as early as 90 minutes after pulse GC therapy (54), and was associated with rapid modulation of adhesion molecules and inflammatory mediators in the joints (54 ,55). Inhibition of synthesis and secretion of inflammatory mediators by the different cells involved in the inflammatory reaction (such as monocytes-macrophages, fibroblasts, and endothelial cells) is central in the action of GC. Eicosanoid generation is inhibited by GC through induction of transcription and/or rapid phosphorylation of Anx1, which is a potent inhibitor of phospholipase A2 activity (21 ,38 ,56), and inhibition of IL1-ß (or LPS)-mediated cyclooxygenase-2 (COX-2) induction (57). Additionally, destructive tissue enzymes such as collagenase are downregulated by GC (32 ,33). Inhibition of cytokine generation constitutes another important anti-inflammatory effect. Specifically, synthesis (and sometimes action) of IL1ß, IL2, IL3, IL4, IL5, IL6, IL8, IL12, TNF, GMCSF, IFN-γ, RANTES, and MCP1, etc. is blocked (15 ,27 ,30 ,50 ,58 ,59). On the other hand, synthesis of IL10 and TGF-ß cytokines considered to have anti-inflammatory properties, is either not affected or induced (60 ,61 ,62). The differential effects of various GC therapeutic regimens on cytokine production have been elegantly shown in human arteritis-severe combined immune deficiency (SCID) mice chimeras by Brack et al. (61). SCID mice engrafted with human temporal arteries were studied with regard to the effects of in vivo intraperitoneal GC therapy on inflammation of the temporal arteries. Doses of 0.04 and 0.4 mg DEX/kg for 7 days induced IκB transcription, inhibited nuclear NF-κB protein expression, and decreased transcription of the NF-κB-regulated genes iNOS, IL1ß, IL2, and IL6. Inhibition of IFN-γ (AP1-dependent) could only be achieved by high doses of DEX (4 mg/kg for 7 days) or chronic therapy (0.4 mg/kg for 28 days), whereas TGF-ß transcription was not inhibited by any DEX regimen. Interestingly, in a follow-up study, aspirin was much more potent than GC in inhibiting AP1 and IFN-γ (63).

Table 60-3: Important Anti-Inflammatory and Immunosuppressive Effects of Glucocorticoids

A. Anti-Inflammatory Effects
   1. Inhibition of blood vessel dilatation and permeability (i.e., because of downregulation of NO synthesis).
   2. Inhibition of neutrophil and monocyte migration to periphery (probably via inhibition of chemokine synthesis and adhesion molecule expression in leukocytes and endothelial cells).
   3. Inhibition of synthesis of inflammatory mediators such as eicosanoids by downregulating phospholipase A2 (via Anx1 induction) and COX-2.
   4. Downregulation of destructive enzymes (i.e., collagenase).
   5. Alteration of cytokine balance in favor of anti-inflammatory cytokines (i.e., IL10, TGF-ß) whereas proinflammatory cytokines (i.e., TNF, IL1-ß, GMC-SF) are suppressed.

B. Immunosuppressive effects*
   1. Lymphopenia** (T cells affected more than B cells and CD4 T cells more than CD8 T cells). Probably because of lymphocyte redistribution (mainly to bone marrow and spleen) and perhaps apoptosis.
   2. Inhibition of signal transduction events critical for T-cell activation (i.e., early tyrosine phosphorylation, CaM-kinase II activation, calcineurin-dependent transactivating pathways).
   3. Inhibition of IL2 synthesis and signaling.
   4. Downregulation of cell surface molecules (i.e., LFA1, CD2) important for full T-cell activation and function.
   5. Inhibition of antigen-presenting cell function (by blocking cell surface expression of MHC class II and CD80 molecules). Depletion of plasmacytoid dendritic cells and production of interferon-alpha.
   6. Deviation of immune responses towards a Th2-type cytokine formation (by preferentially inhibiting synthesis of IL12 over that of IL4 and IL10).
   7. Induction of T-cell apoptosis (of questionable contribution to GC-mediated immunosuppression).

CaM-kinase II, Ca^{2+}/calmodulin–dependent protein kinase II.

*Immunosuppression concerns primarily the cellular and less so the humoral immunity and is more evident with intermediate to high doses of glucocorticoids.

**When lymphopenia is associated with lymphocyte migration towards the regional nodes of inflammation, it represents immune enhancement rather than immunosuppression (see text for discussion).

Although GC induce peripheral neutrophilia, by reducing neutrophil migration to tissues, the peripheral numbers of eosinophils, basophils, monocytes, and lymphocytes decrease upon even a brief exposure to GC (18 ,19 ,20). Lymphopenia has been attributed to redirection of lymphocytes to bone marrow and spleen (64 ,65 ,66), but also to the

skin and regional lymph nodes of inflammation sites (44). T-cell numbers decrease more than B cells and CD4 T cells more than CD8 T cells (66). GC immunosuppression is mediated by inhibition of several stages of T-cell activation, including early tyrosine phosphorylation events (67), activation of $Ca2^{+}$/calmodulin-dependent protein kinase II (CaM-kinase II) (68), and calcineurin-dependent transactivating pathways (69 ,70). As already mentioned, GC inhibit IL2 synthesis, which is critical for T-cell proliferative responses (28 ,71), and additionally inhibits signaling of this cytokine (67 ,72). Downregulation of LFA-1, CD2, c-myc, and induction of cAMP (by inhibiting phosphodiesterase activity) further contribute to T cell dysfunction (73). Glucocorticoids also affect T-cell function indirectly by inhibiting the expression of MHC class II and CD80 molecules on antigen-presenting cells and by decreasing the number and IFN-α synthetic capacity of plasmacytoid dendritic cells (19 ,74). With regard to IFN-α, we and others have also noted a profound downregulation of IFN-α–inducible gene mRNA in PBMC from SLE patients after their treatment with PGC (75 ,76). Notably, B-cell function and immunoglobulin synthesis are relatively resistant to the immunosuppressive GC effects. Glucocorticoids have also important effects in shaping the developing immune responses as they favor deviation to Th2-type cytokine formation by preferentially inhibiting IL-12 synthesis and sparing IL-4 and IL-10 (62 ,77 ,78 ,79). This effect might suggest that these agents are more potent in the treatment of diseases characterized by Th1-type cytokine predominance, like RA. They also promote IgE synthesis in the presence of IL4 through induction of CD40 ligand (CD40L) (48).

The ability of GC to induce apoptosis of double-positive thymocytes (77) and activated mature T cells (80) correlates with low cellular levels of bcl-2 and appears to have important implications for maintenance of central and possibly peripheral tolerance as well (77). Interestingly, TCR-mediated activation-induced cell death (AICD) and GC-mediated apoptosis are mutually antagonistic, an effect that may be in part a result of downregulation of Fas ligand by GC (77 ,81). The degree to which GC-mediated apoptosis contributes to immunosuppression is not known, however. Seki et al. correlated clinical resistance to GC in SLE patients with decreased in vitro apoptosis of anti-CD3 activated PBMC (82). In another study, γ/δ T cells, that may be implicated in SLE pathogenesis, showed increased in vitro susceptibility to GC-mediated apoptosis (without requiring previous activation), and their in vivo downregulation by GC therapy correlated with disease control in SLE patients (83). Tables 60-2 and 60-3 summarize anti-inflammatory and immunosuppressive effects of GC.

Glucocorticoid Resistance

Pharmacokinetic causes of resistance to GC may include impaired oral bioavailability because of decreased GC absorption (i.e., by cholestyramine), or increased GC metabolism (i.e., by hyperthyroidism or drugs, such as barbiturates) (84). Additionally, decreased reactivation of cortisone to cortisol, because of reduced activity of 11-β-HSD1 in RA synovial cells, has been reported and the defect was hypothesized to be a result of the loss of sympathetic nerve fibers in the synovial tissue (85).

With regard to pharmacodynamic causes, resistance to endogenous GC can rarely occur in the generalized inherited glucocorticoid resistance (GIGR) syndrome characterized by GR abnormalities, high plasma cortisol levels, and the absence of Cushing syndrome symptoms (86 ,87 ,88). The opposite picture, increased primary GC sensitivity, has also been noted (86). Additionally, an acquired GR abnormality has been observed in a subset of AIDS patients that present with Addisonian symptoms and hypercortisolism (89). The acquired, tissue-specific GC resistance, which is clinically more important, has been studied best in steroid-resistant bronchial asthma, where the lack of GC benefit on airway inflammation contrasts with a high incidence of GC adverse effects from other organs (90). Cytokines, secreted in the context of such diseases as bronchial asthma, rheumatoid arthritis, SLE, and depression, are thought to play an important role in mediating tissue-specific GC resistance by inhibiting GR function. One way by which cytokines do that is perhaps the augmentation of the overall cellular activation level. For example, T cells costimulated by IL2 or CD28, in contrast to those only triggered through their antigen receptor, are resistant to GC-mediated inhibition of proliferation (91). This phenomenon was though to be a result of the potent induction of c-Fos (which together with c-jun form AP1) by the two costimuli. Notably, AP-1 has been reported by others to be mutually antagonistic with GR for transactivation effects (32 ,33). Additional proposed mechanisms of tissue-specific GC resistance include IL2-mediated induction of p-glycoprotein (P-gp) (92), inhibition of GR translocation, and induction of the beta isoform (GRß) of GR via alternative splicing of the GR pre-mRNA (90 ,93). GRß has been proposed as an important inhibitor of the functionally active GRα (94).

In SLE, increased catabolism of hydrocortisone by lymphocytes (95) and resistance to GC-mediated apoptosis of $CD8^{+}$ T cells have been proposed as mechanisms of GC resistance (82). More recently, high levels of P-gp (the product of the multidrug resistance-1 [MDR1] gene) were noted on lymphocytes of patients with active SLE who were resistant to high doses of prednisolone (96). Steroid-exclusion analysis revealed low intracellular DEX levels in lymphocytes form these patients. GC resistance was reversed after intensive immunosuppressive therapy, and in one case only by cyclosporine-A, which functions as a competitive inhibitor of P-gp.

Pharmacokinetics and Drug Interactions

Oral absorption of GC is excellent. Prednisone (PDN) is 80% to 90% absorbed when orally administered, whether on an empty or full stomach. Once systemically absorbed, a large fraction of GC (90% for hydrocortisone) binds to serum proteins and only their free fraction is biologically active (3 ,4). Of the two GC binding proteins, transcortin, or cortisol-binding protein (CBG), binds to GC with high affinity and low capacity, whereas albumin binds with low affinity and high capacity. Since hydrocortisone and prednisolone bind to both CBG and albumin their protein binding is concentration-dependent and varies from 90% at lower doses (i.e., with standard oral doses) to 60% at higher doses. In contrast, MP and DEX bind almost exclusively (99%) to the high-capacity albumin, and, therefore, have concentration-independent protein-bound fractions (. The difference in the plasma free concentrations of DEX and prednisolone at standard oral doses may explain the better CSF penetration and better efficacy of the former in the preventive therapy of meningeal leukemia (97). The 11-keto GC derivatives such as PDN and cortisone are inactive unless reduced by 11-ß-HSD1 in the liver to their 11-OH analogs prednisolone and hydrocortisone (Fig. 60-1) (3 ,4). Inactivation of GC occurs predominantly in the liver and involves the sequential reduction of the Δ4 double bond (the rate-limiting step in cortisol metabolism), and the 3-keto group (Fig. 60-1). Glucuronidation and sulfation follow, which confer water solubility and allow for urine excretion. Additionally, 6ß-hydroxylation by the cytochrome P450 microsomal enzyme, CYP3A4, also enhances water solubility and urinary excretion of GC. Serum half-lives of different GC differ and vary from 60 to 300 minutes. However, biologic half-lives of GC are dependent on their tissue levels and are much longer than their serum half-lives (Table 60-1).

Since, in liver failure, decreased conversion of PDN to its active form (prednisolone) is overcompensated by reduced clearance of unbound prednisolone (resulting in higher prednisolone concentrations after oral PDN administration), there is no reason to substitute PDN with another inherently active GC agent in these patients. Additionally, low serum albumin does not seem to decrease the unbound concentration of prednisolone, and therefore, (mild) dose adjustments of GC should be based only on the lower clearance of these agents in liver failure and not on the albumin levels per se (reviewed in (98)).

In addition to the above mentioned inhibition of enteric GC absorption by cholestyramine, other important drug interactions also exist. Drugs that induce hepatic microsomal enzymes (especially CYP3A4), such as phenobarbital, phenytoin, rifampin, and carbamazepine, increase GC elimination. In contrast, ketokonazole, erythromycin, ethynylestradiol, and norethindrone inhibit CYP3A4 and can increase GC activity. Mifepristone (RU 486), an antiprogestin drug marketed as an abortifacient outside the United States, has potent antiglucocorticoid properties (at the level of GR) as well (99). Conversely, GC can also reduce the serum level of salicylates (100) and CYP3A4 substrates. Additionally, pulse GC have been recently shown to significantly increase INR, 2 to 6 days after their administration, in patients on oral anticoagulation therapy (101).

Pharmacodynamic interactions include the GC sparing effect of theophylline, and long-acting ß-receptor agonists in asthma (102). More relevant to lupus, Kammal et al. recently used phytohemagglutinin-treated T cells from SLE patients to study the interactions of prednisolone and other commonly used immunosuppressive agents (103). They noted additive effects of dehydroepiandrosterone (DHEA), synergy with tamoxifen, and slight antagonism with mycophenolic acid. Other studies have shown synergy with cyclosporine-A and sirolimus (104). Again, aspirin at anti-inflammatory doses, but not indomethacin, can complement the anti-NF-κB GC activity with anti-AP1 activity in a mouse model of temporal arteritis (63).

General Principles of Glucocorticoid Therapy

Uncontrolled disease activity in SLE can be both debilitating and life-threatening and thus demands rapid and effective intervention. The value of therapy with high doses of GC (i.e., more than 0.6–1 mg PDN/kg/day) in dramatically improving SLE survival has been established (already by data from the predialysis era) in patients with diffuse proliferative glomerulonephritis (DPGN) (105 ,106). Subsequent studies focused on evaluating different strategies of GC use in order to maintain high effectiveness and yet reduce the risk of the increasingly apparent grave adverse effects. Although parenteral corticotrophin (ACTH) has been used clinically as a substitute to GC (i.e., acute gout), there are no obvious advantages of such practice and no studies in SLE. With regard to dosage, ample evidence exists that high GC doses (especially when time intervals between doses are small) for prolonged periods of time are invariably toxic. In fact, Sergent et al. have shown increased infection-related mortality in patients with severe neuropsychiatric SLE (NPSLE) when treated with PDN doses of more than 100 mg/day, for 37 days on average (range, 8–68 days) (107). On the other hand, low-dose GC (LDGC) therapy (dose equivalent to ≤7.5 mg/day of PDN) appears to be better tolerated but not risk-free, since complications such as growth suppression, osteoporosis, and cataract formation can still occur. Therefore, the ultimate goal of therapy should always be complete cessation of GC, if possible.

Alternate day GC therapy (ADGC) also has a better safety profile compared to that of daily GC regimens. The incidence of skeletal growth inhibition, HPA-axis suppression, hypertension (HTN), Cushing syndrome, hypokalemia, overcatabolism, myopathy, and infection have been noted to be reduced (108). However, ADGC effectiveness is less and, excluding perhaps membranous nephritis with nephrotic syndrome, its use should be restricted to GC-tapering or maintenance regimens.

Definitions of GC therapy with regard to dosage. In addition to ADGC, we have previously classified the various dose GC regimens in: (1) Pulse GC (PGC: 15–30 mg MP/kg/day or 1 g MP/m^2 body surface area IV × 1–3 days); (2) very high-dose GC (VHDGC: >1–2 mg PDN/kg/day); (3) high-dose GC (HDGC: 0.6–1 mg PDN/kg/day); (4) medium-dose GC (MDGC: 0.125–0.5 mg PDN/kg/d); and e) low dose GC (LDGC: <0.125 mg PDN/kg/d), based on

GC usage in SLE trials or clinical practice (109). Particularly, HDGC were separated from VHDGC, based on two facts. First, HDGC therapy, for up to 6 to 8 weeks, has been considered as one of the standard regimens for the treatment of lupus nephritis (110), perhaps the most important and best studied SLE manifestation, and second the fact that for very severe disease manifestations, larger doses (VHDGC) are usually required. Additionally, the latter regimen is probably more toxic, especially if followed for more than a few weeks (107) without appropriate dose tapering. Since then, the First European Workshop on Glucocorticoid Therapy has proposed a similar terminology for GC doses (43), taking also into account the percent saturation of GR at different doses: 42%, 63%, and almost 100% at oral doses of 7.5, 15, and more than 100 mg (respectively) of PDN. It was also noted that nongenomic effects become increasingly important with VHDGC and PGC. These definitions have been adopted here (Table 60-4).

Table 60-4: Usual Regimens of Systemic Glucocorticoid Therapy in SLE*

GC Regimen	Representative Indications	Common Adverse Effects (AE)
Pulse GC (PGC): ≥250 mg PDNeq/d × 1–5 days. Typically 0.5–1 g MP/d IV × 1–3 d, monthly as indicated. Usually with oral GC (30–60 mg PDNeq/d).	Life or organ-threatening complications (i.e., RPGN, myelopathy, severe acute confusional state, alveolar hemorrhage, vasculitis, optic neuritis)** HDGC-refractory Disease DPGN or severe FPGN	Same as with HDGC (see below), but overall incidence of AE may be lower, partly because they allow more rapid taper of oral GC doses. Special considerations because of large dose and route of administration: fluid overload, hypertension, neuropsychiatric symptoms. Rarely: cardiac arrhythmias-sudden death, myalgias/arthralgias, seizures, intractable hiccups, GC-anaphylaxis
Very High Dose GC (VHDGC): >100 mg PDNeq/d, IV/PO (Start with divided doses)	Life or organ-threatening complications (as for PGC)**	Same but more severe than with HDGC Psychosis Risk of severe-fatal infection may be particularly high (avoid use for more than 1–2 weeks)
High Dose GC (HDGC): >30 mg and >100 mg PDNeq/d, IV/PO	DPGN or severe FPGN (for less than 6–8 weeks)† Thrombocytopenia/hemolytic anemia Acute lupus pneumonitis; “Lupus crisis”‡	Same AE for both HDGC and MDGC but incidence and severity are at lower levels with the latter.
Moderate Dose GC (MDGC): >7.5 mg and ≤30 mg PDNeq/d, IV or PO	Moderate SLE flares (i.e., myositis, severe pleurisy, ophthalmoplegia [except optic neuritis], thrombocytopenia) With PGC, or CY/AZA for severe disease	HPA-axis suppression, Cushing syndrome, hypertension, hypokalemia, hyperglycemia, hyperlipidemia, atherosclerosis, OP, ON, risk of infection, skeletal growth retardation, glaucoma, cataracts, skin fragility, acne, insomnia, steroid psychosis, mood swings, etc.
Low Dose GC (LDGC): ≤7.5 mg PDNeq/d, PO	Arthritis, mild constitutional symptoms (unresponsive to analgesics/NSAID/AM). Generalized LN Maintenance therapy	Least toxic daily regimen. Cataracts, GC-withdrawal symptoms (upon tapering to or below LDGC), skeletal growth retardation can occur. Probably minimal OP, ON, HPA-axis suppression.
Alternate Day GC (ADGC)	Membranous nephritis with nephrotic syndrome (120 mg PDNeq) During tapering GC dose. Maintenance therapy (i.e., 15 mg PDNeq for GN)	Decreased adverse effects (i.e., HPA-axis suppression, skeletal growth retardation, infection, Cushing syndrome) compared to daily regimens OP can occur.

AZA, azathioprine; CY, cyclophosphamide, LN, lymphadenopathy; MP, methylprednisolone; ON, osteonecrosis, OP, osteoporosis; PDNeq, prednisone equivalent; RPGN, rapidly progressive glomerulonephritis: FPGN, Focal proliferative glomerulonephritis; AM, Antimalarial.

*All PDNeq doses assume a 60 Kg patient; adjustments should be done for different weights.
**Cyclophosphamide therapy, usually I.V. (IVCY), is often needed as well.
†In combination with IVCY.
‡Lupus crisis refers to the acutely and severely ill patient with high fever, extreme prostration, and other symptoms of active SLE (i.e., pleurisy, arthritis, vasculitic rash), who requires large doses of GC for disease control. Infection has of course been excluded as the cause of the symptoms.

The most effective approach to initiating HDGC or VHDGC for severe SLE disease, and especially when constitutional symptoms (i.e., high fever and prostration) are present, is to administer it in 2 to 4 doses per day (19).

A notable exception is the management of severe focal or diffuse proliferative glomerulonephritis (FPGN or DPGN) where once-a-day regimens are adequate (110). Should the condition prove GC-unresponsive, use of pulse GC and/or additional immunosuppressive agents is necessary. Most disease complications will respond in less than 1 to 2 weeks. However, markers of lupus nephritis (especially proteinuria) may take more than 2 to 6 weeks to improve.

Within 1 to 2 weeks from initiation of therapy, whether a satisfactory response has occurred or a cytotoxic agent has been added to the regimen for refractory disease, tapering of GC therapy should be initiated (19). The first step is to consolidate the GC regimen into a once-a-day morning dose. The daily dose can then be decreased by 5 mg (or 5%–10%) per week until a dose of 0.25 to 0.5 mg/kg/day is reached, and more slowly thereafter, aiming for either a complete withdrawal or, if that is not possible, for LDGC. It may be preferable to follow an ADGC tapering regimen during which the second day's dose is usually first gradually decreased to zero, before further dose decreases are made. Caution should be applied during tapering, as too fast or too slow dose decrements can lead to disease flare/withdrawal symptoms and increased GC toxicity respectively. In the event of a flare during the tapering, the dose is increased to the immediate previous effective level for a few weeks, before the next, perhaps slower, tapering attempt. Less severe SLE manifestations are managed with LDGC or MDGC accordingly (19 ,110). Table 60-4 provides an overview of the suggested GC use in SLE. Finally, some studies have argued for the use of prophylactic GC in SLE patients with serologic flare as defined by increases in anti-dsDNA titers or decreases in complement levels (111 ,112). In those studies clinical relapses were prevented without increased cumulative GC doses.

The importance of other immunosuppressive agents (such as cyclophosphamide, azathioprine, etc.) in helping control the disease while allowing safe tapering of GC (steroid-sparing activity) cannot be overemphasized. DPGN is the best studied SLE complication and randomized controlled clinical studies have documented the superiority of IV cyclophosphamide (IVCY)-containing regimens over those with GC (113 ,114 ,115 ,116) or IVCY alone (117). Moreover, with combinations therapies a more effective GC tapering scheme can be achieved. Additionally, many observational studies, case series, and case reports favor the use of cyclophosphamide (mainly IV) in other life/organ-threatening SLE complications that may be refractory to GC alone (at appropriately high doses or pulse therapy) (118 ,119 ,120 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129). Severe NPSLE of nonthrombotic etiology (especially acute confusional state, myelopathy, optic neuritis) (118 ,119 ,120 ,121 ,122 ,123 ,124), pulmonary hemorrhage (126), interstitial pneumonitis (127), acute cardiomyopathy (128), and severe vasculitis of other systems such as the GI (129) are such examples. In such grave cases, patients might benefit from simultaneous administration of GC and other immunosuppressives (mainly IVCY) from the outset of the disease. For less severe disease manifestations such as arthritis, serositis and mild constitutional symptoms, agents such as hydroxychloroquine, NSAIDs, analgesics, and local GC (i.e., intraarticular) should be given priority, and systemic GC used only if necessary and at the lowest effective dose. Dehydroepiandrosterone (DHEA; or prasterone), an adrenal androgen with immunomodulatory properties whose serum levels are low in SLE and further decrease with GC therapy, was recently shown to have a steroid sparing effect in a double-blind, randomized, placebo control trial (130). However, the effect was seen only in a subgroup analysis of active at baseline SLE patients (SLEDAI of >2) who received the high dose of DHEA (200 mg daily).

The above approach to GC use in SLE is based on the assumption that alternative noninflammatory or nonautoimmune diagnoses have been carefully excluded, before a patient is committed to prolonged immunosuppressive therapy. Infections hold the first priority and they can closely mimic many lupus complications, including acute confusional states, aseptic meningitis, lupus nephritis, lupus pneumonitis, arthritis, and GI vasculitis. Presentations of SLE patients with acute abdomen (AA) are a particularly challenging problem in management. For example, Medina et al. reported that although vasculitis correlated with overall SLE disease activity (53% of 36 patients with active vs. none of 15 with inactive lupus patients), common surgical diagnoses and primary abscesses (in immunosuppressed patients) were more common (131). Interestingly three cases in the active group had abdominal thrombosis and high anticardiolipin titers. Only one patient with hepatic artery thrombosis survived after thrombectomy and 100 mg/day PDN therapy. In the same study, a delay in surgery for more than 48 hours (excluding the cases that had a complete response to HDGC) was associated with much higher mortality, especially in the active group. Once infection was ruled out, however, vasculitis responded to PGC with or without IVCY (131).

Generally, arterial or venous thrombosis without concomitant SLE activity (i.e., CVA secondary to the antiphospholipid syndrome) require anticoagulation alone and increases of GC dosage should be avoided (19 ,110). If TTP is diagnosed, plasma exchanges may be useful (110). Late complications of SLE (132), such as advanced atherosclerosis-CAD, scarring nephritis (with high chronicity and low activity indices on kidney biopsy) osteonecrosis, shrinking lung syndrome and chronic dementia should discourage heroic interventions (107 ,110). Seizures or acute confusional states may not be secondary to SLE and in fact may result from hypertension or metabolic/electrolyte abnormalities, whereas psychosis might result from GC therapy itself (107). The probability of these alternative diagnoses substantially increases when SLE activity in other systems is low. In such cases of isolated seizures or psychosis, conservative management (that may include anticonvulsant and psychotropic agents) along with careful monitoring is all that is usually required (107).

When managing certain SLE complications with GC, it is often prudent to aim for “reasonable” but not complete resolution of disease activity, since often the latter translates

into higher and more toxic GC dosages. For example, asymptomatic hemolytic anemia or ITP with a hematocrit and number of platelets of more than 30% and 20,000 to 50,000 (and no other coagulopathy) respectively do not, per se, warrant increases of GC therapy (110). In more severe cases that invoke long-term HDGC therapy for adequate control, splenectomy and or cytotoxic medicines should be considered (110).

Pulse Glucocorticoid Therapy

Pulse glucocorticoid (PGC) therapy, traditionally 1 g (or 1 g/m^2 of body surface area) of MP IV per day for 3 days that may be repeated at monthly intervals, was first used in SLE to treat DPGN (110 ,113 ,116 ,133 ,134). PGC are also effective for pneumonitis (110), serositis (135), vasculitis (136 ,137), and thrombocytopenia (110 ,138). Many published series showed a role of PGC in moderate-severe NPSLE (110 ,119 ,120 ,122 ,123 ,124 ,138), although a recent randomized controlled trial that compared PGC with IVCY clearly demonstrated the superiority of the second (139). It is generally felt that for very severe DPGN (or rapidly progressive glomerulonephritis; RPGN) PGC work faster than standard oral HDGC and probably permit both use of MDGC (0.5 mg/kg/day) at therapy initiation and a faster taper of GC dose (116). However, two randomized controlled trials showed that PGC therapy (monthly for 6 months or for at least 1 year, respectively) was not as effective as an IVCY-containing regimen (monthly for 6 months and then quarterly) for proliferative lupus nephritis (113 ,116). The second study and especially another more recent NIH trial (117) that included 5 years of protocol therapy with IVCY, PGC, or both and an extended median follow-up of 124 months, have both suggested that the combination can lead to a better renal outcome than therapy with either agent alone. It appears that concurrent use of both agents offers a therapeutic advantage for severe SLE in general (120 ,126 ,140), possibly because of a synergistic effect between the two agents. PGC appear to have additional nongenomic immediate effects (36) that may allow for a faster and more effective action than conventional HDGC. On the other hand IVCY has better long-term effects on the scarring consequences of inflammation (141) and a very potent ability to suppress humoral immunity (140). Advocates of PGC therapy argue that this therapy may have less adverse effects than oral GC alone, partly because it allows a more rapid tapering of the latter. A small case control study of the authors suggested that use of PGC was not associated with more osteoporosis/osteopenia than use of oral GC alone despite significantly larger cumulative (pulse and oral) GC doses in the first group (142). A more recent 12-month randomized prospective controlled study of RA patients also reported that PGC did not cause bone loss, in contrast to oral GC that did (143). The cosmetogenic and diabetogenic effects of PGC may be less severe as well (133). However, complications, such as glucocorticoid-induced osteonecrosis (GION) (116 ,144), major infections (116 ,133), and mood disorders/psychosis (145) can still occur. Seizures (146), myalgias/arthralgias, and dangerous cardiac arrhythmias attributed to potassium deficits (147 ,148), and anaphylaxis (116) have been rarely reported, as well, with this therapy.

Badsha et al. have recently published the results of a small retrospective study of 55 very active SLE that examined the safety and effectiveness of two PGC regimens for 6 months after therapy (149 ,150). Patients who received 500 mg of MP IV daily X3 days (low dose) had less serious infections (in 7 out of 26 patients) and the same therapeutic response as those who received the high dose (1 g MP IV daily × 3 days; infections in 17/29 patients). Most infections were a result of Gram-negative bacteria and occurred within 1 month of PGC. Hypoalbuminemia, was a risk factor and the authors recommended “low” over “high” PGC, especially for those patients with low serum albumin.

Although MP appears to be the most frequently used GC agent for PGC therapy, megadoses of DEX have also been very effective for the same indications, including severe NPSLE (151). Becuase there are no head to head studies comparing pulse DEX and pulse MP, it is not clear whether there is an advantage to either therapy at equivalent doses. With regard to NPSLE management, both agents appear to be comparable in their capacity to penetrate CSF, based on their protein binding characteristics (see pharmacokinetics section). In cases of severe brain edema, where agents with low MC activities might be preferable, although both MP and DEX are suitable, the latter might offer a slight advantage (Table 60-1).

Use of Depot Glucocorticoid Agents

Depot preparations of GC are designed to have long-lasting effects (3–4 weeks) after a single intra-articular (IA) or IM injection. Examples include MP acetate and triamcinolone hexacetonide. Intramuscular injections are used for their potent systemic effects and IA injections for their local action in the affected joint. However, even in the latter case, some systemic absorption and GC toxicity can occur (152). Use of IM depot GC can be considered for the treatment of acute minor flares of the disease. Interestingly, Dasgupta et al., in a controlled trial for polymyalgia rheumatica, used regular 3-weekly IM depot MP acetate injections in comparison with standard oral GC therapy. Adverse effects, especially fractures and weight gain, were less with the depot GC therapy, probably a result of lower cumulative GC dose in that arm of the study (153). A brief (<2 weeks) suppression of the HPA has been reported, even after one IA dose (154).

Glucocorticoid Use During Pregnancy and Lactation

Use of GC therapy during pregnancy is indicated primarily to treat active SLE in the mother and probably for incomplete heart block of neonatal lupus in the fetus. Because only fluorinated GC (i.e., DEX, betamethasone) are able to

enter the fetal circulation in significant amounts, as they are only partially metabolized by the placental 11-β-HSD2 (155), nonfluorinated GC (usually PDN) are used for the first and fluorinated GC for the latter indication. Saleeb et al. (156), in a retrospective study, have found that fluorinated GC given to mothers with anti-Ro or anti-La antibodies at the time of echocardiographic detection of fetal CHB, were able to at least prevent progression of second-degree blocks and reverse hydropic fetal changes. However, and of some concern, oligohydramnios were seen more frequently in the treatment than in the placebo group.

When treating the mother for active SLE, the lowest effective GC dose should be employed. Although development of cleft palate has been reported in animal studies with use of GC (155 ,157), this fact probably carries no clinical significance and should not restrict use of GC when otherwise indicated (155). Other GC adverse effects on pregnancy outcomes include a high incidence of preterm deliveries, which are mainly a result of premature ruptured membranes (158 ,159 ,160 ,161) and possibly fetal growth restriction (155). Gestational hypertension or diabetes mellitus (158 ,160 ,161) are not uncommon. Additionally, osteoporosis (155 ,162) and, probably less commonly, maternal cataracts (160), or GION can also occur (155). With regard to osteoporosis, the combination of GC and heparin may be particularly toxic to the bones and vertebral fractures have been reported (162). Mothers treated with GC during pregnancy may need stress GC doses in the peripartum period, especially when there is prolonged labor/delivery or a C-section is required (155). Careful monitoring of the neonates born to these mothers for development of adrenal insufficiency is also recommended (155). In order to avoid disease flares that can lead to both disease and treatment complications, patients that are already on hydroxychloroquine should probably be kept on this (relatively safe) therapy during pregnancy (163 ,164 ,165). Additional immunosuppressive medications that may be safe during pregnancy, such as azathioprine, cyclosporin A, and IVIG, should be considered for moderate-severe SLE disease activity (155) and might help decrease GC doses.

Because of their unfavorable adverse effect profile, GC have lost ground to the combination of heparin and aspirin in treating the pregnancy loss of the antiphospholipid syndrome (APLS) (158 ,159 ,161). Additionally, Laskin et al. found the combination of PDN and aspirin of no benefit to the treatment of women with autoantibodies (including antiphospholipid antibodies) and recurrent fetal loss (160). However, GC may still have a place in the management of women with APLS secondary to SLE to treat thrombocytopenia or a concomitant SLE flare (161).

The use of PDN at doses below 20 to 30 mg/day in breastfeeding mothers is probably safe as less than 10% of the active drug enters breast milk (155). It is prudent to wait 4 hours after GC intake before breastfeeding, especially when higher doses are necessary (155).

Use of Glucocorticoids During Stress

Because of the high risk of HPA-axis suppression (see below), patients on chronic GC therapy should be given supplemental GC during the stress of surgery or moderate-severe illness. Traditional recommendations called for 100 mg of a water-soluble form of hydrocortisone (i.e., sodium succinate) IV every 8 hours, tapered as the patient recovers. However, more recent data have shown that individuals with intact adrenal function rarely generate more than 150 to 200 mg of cortisol even during major surgery (5 ,166). Based on this information, more recent recommendations argue that 25 mg of hydrocortisone (or its equivalent) should be given daily for 1 to 3 days during minor stress, whereas for moderate and severe stress 50 to 75 mg and 100 to 150 mg daily for 1 to 3 days should be given respectively (5 ,167). Another argument in favor of this approach may be that unnecessarily high GC doses that might interfere with wound healing and increase the risk of infection are avoided (5 ,167). Glucocorticoid agents with less mineralocorticoid activity than hydrocortisone would be preferable when dealing with fluid-overload states (i.e., in a lupus nephritis patient).

Adverse Effects of Glucocorticoids

Both clinicians and patients should be fully aware that adverse effects (AE) of GC therapy are not uncommon and can in fact be very serious. Although brief courses of high dose GC therapy (i.e., for bronchial asthma) are probably well tolerated and AE (i.e., Cushing syndrome, glucose intolerance, HPA-axis suppression), if they occur, are rapidly reversible (84), more prolonged therapy, as it is usually the case with SLE patients, invariably leads to complications. Of note, some AE (i.e., skeletal growth inhibition, HPA-axis suppression, GION, cataracts) occur even with LDGC, others usually require larger doses of GC before they occur (i.e., infection, psychosis, myopathy, hyperlipidemia). Because susceptibility to GC AE may vary from person to person, according to individual pharmacokinetic differences, Sarna et al., in order to minimize AE, have suggested the use of specific GC exposure indices (instead of the absolute or cumulative GC doses) in guiding GC dosing of children with transplants (168). Comorbid conditions and risk factors that may predispose to more severe GC AE (i.e., hyperlipidemia, hypertension, hyperglycemia-DM, hypokalemia, osteoporosis, personal or family history of cataract or glaucoma, prior exposure to tuberculosis) should be identified prior to institution of GC therapy. Patients and their families should be educated to recognize and promptly report symptoms of such complications as infection (i.e., fever), diabetes, psychosis, and osteonecrosis (joint pain). In parallel, careful clinical and laboratory monitoring for the development of osteoporosis, DM, hyperlipidemia, hypertension, glaucoma, and so on, should not be neglected (2). Interventions known to prevent or

ameliorate GC AE (summarized in Table 60-5) should be undertaken (2). This is particularly true for GIOP, infection susceptibility, myopathy, and atherosclerosis (see below). Reversal of some AE (i.e., HPA-axis suppression, Cushing syndrome, and psychosis) can be achieved with cessation of GC therapy, or at least modification into the safer ADGC or LDGC regimens. Unfortunately some AE, like cataract formation, GION, osteoporotic fractures, growth retardation in children, and atherosclerotic vascular events, are irreversible (169).

Table 60-5: Suggested Monitoring and Preventive-Therapeutic Interventions for Selected GC Adverse Effects (AE)*

AE	Monitoring for	Intervention
HPA-axis suppression	Symptoms of adrenal insufficiency during significant stress (illness, or surgery)	Choose low-risk GC regimens (LDGC, ADGC) when possible. Medical alert bracelet. Stress-doses of GC perioperatively or during severe illness.
Osteoporosis	BMD at baseline and q12 months thereafter if bisphosphonates are not given. (for high risk patients more aggressive monitoring is probably necessary)	Smoking/ETOH cessation; exercise; low salt diet; calcium (1,500 mg/d) and vitamin D (400-800 IU/d). Bisphosphonates** for patients initiating GC therapy with ≥5 mg PDNeq/d for ≥3 months, or patients on long-term GC ≥5 mg PDNeq/d and low BMD (T-score <-1) Gonadal hormone replacement in hypogonadic patients on long-term GC therapy.† Calcitonin if bisphosphonates cannot be used.
Osteonecrosis	Unexplained joint/bone pain. (Perform MRI to detect early GION if plain radiography not revealing).	Avoidance of weight bearing and joint-salvage surgery (core decompression, osteotomy, non vascularized or vascularized bone grafting) may arrest progression to bone collapse.
Cardio-vascular‡ Lipidemia Hypertension Hyperglycemia Obesity	Baseline fasting serum lipid profile (TC, HDL-C, LDL-C, TG) and then yearly TC. Symptoms of polyuria, polydipsia; after baseline serum glucose, urinary glucose tests q3–6 months; baseline serum potassium. BP at baseline and every visit. Edema, shortness of breath	Diet (to control one or all of hyperlipidemia, hyperglycemia, and hypertension, accordingly) Aerobic exercise Antimalarial therapy (cholesterol-lowering and anticoagulant properties) Lipid-lowering and/or antihypertensive agents, if previous measures not adequate. Folate, and vitamins B6, B12, for elevated homocysteine levels
Infection	Fever; PPD skin testing (+anergy panel); severe lymphopenia.§ Maintain high index of suspicion for OIs	Vaccinations for influenza, pneumococcus, tetanus. Antituberculosis therapy, if PPD+, or evidence for TB exposure. PCP prophylaxis¶ avoid contact with children vaccinated with OPV¶
Muscle	Proximal muscle weakness	Muscle strengthening exercises
Psychiatric	Symptoms of depression, psychosis	Antipsychotics as an adjunct therapy to GC dose lowering or cessation
Eye	Visual changes,	Decrease/cessation of GC dose (for glaucoma).
PUD	Other PUD risk factors (NSAIDs, co-morbidities)	Gastroprotection (H_2-blockers, proton-pump inhibitors, misoprostol)

BMD, bone mineral density; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; BP, blood pressure; PPD, purified protein derivative; TB, tuberculosis; OI, opportunistic infection; PCP, pneumocystis carinii pneumonia; OPV, oral polio virus; PUD, peptic ulcer disease.

*Generally, if GC cessation is not feasible, tapering to LDGC or modification to an ADGC regimen, is the safest intervention for most GC adverse effects.
**Caution in premenopausal women.
†Caution with estrogen in the presence of prothrombotic risk factors, atherosclerosis, or active lupus.
‡APLA, homocysteine, and SLE activity are GC-unrelated but significant cardiovascular risk factors in SLE patients.
§Usually when lymphocytes <350/mm3, but PCP can occur with higher numbers.
¶For severely immunosuppressed SLE patients (i.e., on combination therapy with moderate-high GC and other immunosuppressive agents and/or significant lymphopenia).

Iatrogenic Cushing syndrome (with the typical centripetal body fat redistribution), a characteristic feature of GC excess can occur in a period of less than 1 month during HDGC therapy (4). It differs from the native Cushing syndrome in that there is less androgen excess (androgens are suppressed by GC excess) and less hypertension (4). On the other hand, the prevalence of GION, posterior subcapsular cataracts, glaucoma, pseudotumor cerebri, pancreatitis are more commonly seen.

Recent studies have shown that damage in SLE, as measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index, is clearly associated with GC use (170 ,171). One of these studies described an inception cohort of 73, mostly Caucasian patients, and noted that although disease-activity related damage occurred early, GC-associated damage accumulated over time to constitute most of the damage at 15 years. (170). This was especially true for musculoskeletal damage (55% of patients at 15 years) mainly because of osteonecrosis and deforming arthritis, and ocular damage (32%) as a result of cataracts.

Chronic Suppression of the Hypothalamic-Pituitary-Adrenal Axis

Suppression of the HPA-axis after chronic exposure to moderate-high doses of GC begins from suppression of corticotropin-releasing hormone (CRH) and ACTH and eventually leads to atrophy of adrenal zones fasciculate and reticularis, the principal cortisol-producing structures (secondary adrenal failure). Exposure to GC for periods of less than 2 to 3 weeks or for longer periods to low dose GC (<10 mg/day of PDN, as long as is not taken as a single bedtime dose) are considered safe and do not lead to clinically significant adrenal insufficiency (4 ,84). On the other hand, patients exposed to any doses of more than 20 mg/day for more than 3 weeks should be considered as having HPA-axis suppression (4 ,84). These patients need proper GC supplementation during surgical procedures or illnesses. Recovery from suppression varies depending on the intensity of GC exposure but usually lasts from 6 to 9 months and is gradual. CRH secretion recovers first, followed by ACTH, and lastly by cortisol secretion. Additionally, adrenal responsiveness to stress normalizes much later than the baseline adrenal cortisol secretion. Patients should be informed about the potential dangers of this important problem, advised not to abruptly discontinue their GC unless authorized by their physician, and wear medical alert bracelets (4 ,84). GC withdrawal should start by tapering GC dose to physiologic levels (20 mg of hydrocortisone or 5 mg of PDN in a single morning dose). Serum AM cortisol levels are measured and only when they are more than 10 μg/dL can maintenance dose be discontinued. However, these patients may still have abnormal cortisol responses to stress and coverage should be given when necessary. Next, an acute ACTH test is performed. If 30- or 60-minute cortisol serum levels, after subcutaneous injection of 250 mg of cosyntropin (synthetic ACTH1–24), are more than 20 μg/mL, adrenal insufficiency is unlikely and stress GC supplementation is no more necessary. However, exceptions can occur and if there is any clinical suspicion of adrenal insufficiency (i.e., hypotension after surgery), GC coverage should be given (4 ,84).

Bone Toxicity

Glucocorticoid-induced osteoporosis (GIOP) and osteonecrosis (GION) are frequent adverse effects of GC and contribute substantially to the morbidity associated with these agents. Recent advances in bone biology have shown that osteoblasts and osteoclasts are both derived from bone marrow precursors and that osteoblastogenesis is a prerequisite for osteoclastogenesis (172). The link is provided by osteoprotegerin ligand (OPGL, or ODF, or TRANCE or RANK ligand), a member of the TNF family of proteins, which is expressed on committed preosteoblastic cells and mediates osteoclast differentiation and activation (172 ,173). The main histologic findings in GIOP are decreased bone formation rate, decreased wall thickness of trabeculae, a strong indication of decreased osteoblastic work output and in situ death of portions of bone (172). Weinstein et al., by studying a mouse model of GIOP, found that there was an early (7 days after exposure to GC) increase in osteoclast perimeter and bone resorption (174), an effect that might have been because of OPGL induction by GC (175). On the other hand chronic GC exposure (27 days) led to decreases in bone turnover and bone formation, probably explained by inhibited osteoclastogenesis and osteoblastogenesis respectively. Osteoblast function was further compromised as these cells underwent increased GC-induced apoptosis (174). Additionally, the augmented osteocyte apoptosis observed was proposed by the authors as a potential mechanism for GC-induced osteonecrosis as osteocytes are critical in sensing microdamage and regulate bone remodeling accordingly (172). Dysregulation of calcium balance (decreased intestinal absorption and increased urinary excretion), gonadal hormone repression, myopathy, and in SLE downmodulation of DHEA are other proposed mechanisms of GIOP (176 ,177 ,178 ,179).

Glucocorticoid-induced osteoporosis predominantly affects cancelous bone and the axial skeleton. During the first 3 to 6 months of GC therapy there is a rapid bone loss (up to 12%) which slows down thereafter to 2% to 5% annually (172). SLE patients may have additional risk factors for osteoporosis such as use of sunscreens (inadequate vitamin D formation) inability to exercise due to musculoskeletal inflammation or fatigue, hormonal changes (including premature ovarian failure due to cyclophosphamide therapy), kidney damage, or medications known to induce osteoporosis (heparin, anticonvulsants, cyclosporin) (177). Additionally, the disease itself could theoretically cause bone loss given the recent evidence that activated T cells express functionally active OPGL (180).

Careful evaluation of SLE patients before and after initiation of GC therapy should be performed to identify other potentially modifiable OP risk factors and guide further management. Specifically, baseline and every 6 to 12 months thereafter, evaluation of BMD is essential to assess bone loss. Markers of bone formation and bone resorption might offer additional help in assessing the effectiveness of current OP-preventive measures. Serum levels of 25-hydroxyvitamin D, luteinizing hormone (LH; for females) and gonadal hormones can be measured if indicated to help identify potential targets of intervention. General measures for OP prevention including a well-balanced, low salt diet, avoidance of alcohol and smoking and weight-bearing muscle-strengthening exercises should be encouraged. A short-acting GC at the lowest effective dose should be preferentially used, although doses as low as 6 mg PDN per day, on average, may lead to spinal bone loss (181). Unfortunately, alternate-day therapy does not seem to protect from GIOP (177). As a first step to GIOP prevention, calcium and vitamin D intake should be optimized and 1,500 mg/day (by diet or supplements) and 400 to 800 IU/day should be taken respectively. Use of pharmacologic doses of vitamin D are discouraged except in the case of documented vitamin D deficiency (182). Active vitamin D metabolites, like calcitriol, have been shown to preserve bone during GC therapy (183) but the relatively high risk for hypercalcemia/hypercalciuria and its potential association with soft tissue calcifications in SLE patients with lupus nephritis (184) make their use less desirable. Thiazide diuretics (with potassium supplementation) might be useful in the case of hypercalciuria (177). Hormone replacement therapy (HRT) should be considered in all postmenopausal women, and documented hypogonadism in men and premenopausal women, if there are no contraindications (176 ,185 ,186). In SLE patients, there is concern that estrogen therapy might induce flares of the disease. In fact the Safety of Estrogens in Lupus Erythematosus-National Assessment (SELENA) trial has shown only a small risk for mild/moderate lupus flares in postmenopausal (without prothrombotic risk factors) patients that received HRT compared to placebo-treated women (187). Nevertheless, because of the recent negative data regarding the cardiovascular effects of HRT, this therapy has lost ground in the treatment of OP (188). The best evidence regarding effective prevention and treatment of GIOP exist for bisphosphonates, especially during initiation of GC therapy when bone loss is worse (189 ,190 ,191). Although there is some concern with prolonged use of these agents in young individuals (because of their long-term retention in bones) (176), this should not discourage physicians from using them when clearly indicated in young SLE patients (i.e., those on HDGC at high risk for, or already with, OP). However, bisphosphonates should not be used in pregnancy or moderate-severe renal failure and their discontinuation considered when GC doses have been substantially tapered with stabilization of BMD. Calcitonin has been recommended for GIOP, but it does not seem as effective (176 ,182 ,192). If the above measures fail, bone anabolic agents such as low doses of slow release sodium fluoride could be cautiously used (178). Other promising therapies for GIOP have, in the meantime, emerged. Specifically, daily subcutaneous injections of human parathyroid hormone 1–34 (hPTH [1–34]) in postmenopausal women, that also received HRT and calcium supplementation, reversed GIOP in the lumbar spine, whereas control therapy with HRT and calcium alone did not (193).

The pathogenesis of GION remains largely unknown. Proposed mechanisms include blood vessel occlusion by either external compression (fat accumulation in the bone marrow), or intravascular flow obstruction by thrombosis or fat embolism (194 ,195). Apoptotic death of osteocytes, which are considered as mechanosensors and initiators of repair in bone, has been recently proposed as a mechanism of femoral head collapse in the context of GC therapy (172). Pathologically, GION is characterized by empty lacunae in trabeculae, bone marrow necrosis, and later by repair processes such as vascular granulation tissue and appositional (on the dead bone) new bone formation (194). Although pure medullary infarcts are asymptomatic, corticocancellous osteonecrosis can lead to pain and joint destruction depending on the extent and location of lesions (194 ,195). Symptoms usually take a few months to a few years to develop after GC exposure (194 ,196 ,197). Typical sites of involvement include the subchondral regions of convex bone ends such as the proximal and distal femur, and the proximal humerus. With regard to time of onset, it has been suggested that the process of ON occurs within 1 to 2 months of exposure leading to early asymptomatic MRI lesions at 40 to 100 days after HDGC (58 mg PDNeq/day on avaerage) (198). In that study, 44% patient developed such lesions in hips and knees and the lack of progression to symptoms after 12 months was attributed to the brief followup time. The incidence of clinically occult ON was only 12% in another study perhaps because of lower mean GC doses and also no progression was seen after 1 year of follow-up (199). Notably, even short-term PGC doses (200), adrenal insufficiency replacement doses (201), and intraarticular injections (202) have been implicated in GION. Predicting which patients will develop GION is very difficult. In SLE patients, besides the magnitude of GC exposure (203 ,204 ,205 ,206 ,207), development of Cushing syndrome has been implicated in many studies (204 ,205 ,206), but there is no agreement with regard to the role of antiphospholipid antibodies (204 ,205 ,208). Patients should be informed about this potentially very serious GC complication that often occurs when SLE disease activity is under control; educated how to recognize GION symptoms (i.e., groin pain upon weight-bearing); and instructed to avoid high-impact activities (194 ,195 ,207). When hip GION is at an early stage, relief of weight bearing is recommended (179 ,180 ,191). For this purpose, Simkin and Gardner have suggested the use of crutches with a four-point walking

pattern to avoid excessive load on the opposite (at risk) hip (180). Operative management of precollapse lesions include core decompression, osteotomy, and nonvascularized and vascularized bone grafts (194 ,195 ,207). Advanced disease (stages III–IV by plain radiography according to the Ficat staging system) often requires total joint replacement therapy (194 ,195 ,207). Interference with potential key mechanisms of GION such as fat accumulation (i.e., by statins), intraosseous hypertension (i.e., vasodilators), coagulation (i.e., stanozolol) or osteocyte apoptosis (i.e., PTH) may offer additional hope in managing this disease in the near future (172 ,207 ,209).

Effects on Intermediary Metabolism: Abnormalities in the Metabolism of Glucose, Lipids, and Protein

Glucocorticoids induce insulin synthesis, but oppose its effects on glucose metabolism predisposing to or aggravating (if already present) diabetes mellitus (DM). These effects of GC are mediated mainly through decreased peripheral utilization of glucose and induction of gluconeogenesis in the liver. The latter is in part a result of the increased substrate availability for gluconeogenesis as a result of the GC enhancing effects on lipolysis and protein catabolism (3 ,4). The hyperglycemic effects of GC may be of particular importance in SLE, a disease with an increased prevalence of DM (210 ,211 ,212) and perhaps an increased frequency of the prediabetic insulin resistance syndrome (213).

Dyslipoproteinemias with elevated serum levels of triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), and decreased levels of HDL-C and apoprotein A-I can occur in active SLE even before GC treatment (214 ,215). Nephrosis, when present, is an additional aggravating factor for hyperlipidemia. GC have an important impact on lipid metabolism themselves as they act permissively to enhance the lipolytic effect of catecholamines and growth hormone (GH) and induce a centripetal body fat redistribution (4). GC can also induce cholesteryl ester synthesis by macrophages, in vitro, an effect that could be blocked by progesterone (216). Ettinger et al. have shown that administration of 0.35 mg PDN/kg/day for 14 days in healthy men increased their levels of VLDL-C, HDL-C, and triglycerides (TG) (217). In SLE, increases of TC, VLDL-C, LDL-C (215 ,218), and TG (215 ,219) have been noted and only PDN doses of more than 10 mg/day had significant hyperlipidemic effects (212 ,219). Moreover, Petri et al. have found that an increase in the PDN dose by 10 mg daily was associated with a 7.5 mg/dL increase in cholesterol levels (220). Interestingly, antimalarial therapy can counteract this GC effect and decrease cholesterol (220 ,221).

Despite inducing weight gain as a result of their appetite enhancing effects (220), GC favor protein breakdown and lead to steroid myopathy and poor wound healing (see below).

Cardiovascular Effects

Hypertension because of GC occurs at high doses. This is partly a result of the permissive effects of GC on the action of vasoactive substances (angiotensin II [ATII], catecholamines) on the vessel wall and myocardium that result in increased systemic vascular resistance (SVR), and increased cardiac contractility (3 ,4). Inhibition of PGE2 and kallikrein synthesis by GC also contributes to their adverse effects on blood pressure (BP). When 11-β-HSD2 functions normally, GC mineralocorticoid effects on BP are unlikely, except perhaps in very high doses of cortisol. In the Hopkins Lupus Cohort increases of PDN doses by 10 mg were associated with BP increases by 1.1 mm Hg (220).

Urowitz et al., in 1976, reported the occurrence of accelerated atherosclerosis in young premenopausal females with SLE (222). The prevalence of clinical atherosclerosis manifesting with angina, myocardial infarction, or peripheral vascular disease has been estimated to be 7% to 10% (211 ,212 ,223 ,224). Subclinical atherosclerosis is even higher (according to some studies (225 ,226). Disease activity of SLE itself is considered at least in part responsible for hyperlipidemia (214 ,215), and atherosclerosis (226) a process that is now considered inflammatory with evidence of immune activation (227). Experimental evidence that immune complexes (in association with high-cholesterol diet) can induce atherogenesis in rabbits (228) and that lupus sera are capable of accelerating cholesterol uptake by human aortic smooth muscle cells (229) may offer additional support to a pathogenetic association of SLE and atherosclerosis. Along these lines, Rahman et al., in a comparison study of patients with accelerated atherosclerosis with and without SLE, found one less traditional risk factor in patients with SLE, which led the authors to suggest that SLE itself be considered a cardiovascular risk factor, similarly to DM (230). Importantly, duration of GC therapy was found to be an independent risk factor for development of CAD in SLE (211 ,212 ,231), along with older age at diagnosis of SLE, hypercholesterolemia (210 ,211 ,212), and other cardiovascular risk factors including hypertension, obesity (212 ,226), elevated homocysteine (231) and even antiphospholipid antibodies (214 ,217 ,231) and CRP (226). Notably, some more recent studies failed to demonstrate a role for GC in development of subclinical atherosclerosis (232 ,233). Nevertheless, the potential of GC to aggravate SLE-atherosclerosis, based at least on their adverse effects on serum lipids and BP, cannot be overemphasized and every attempt should be made to keep their doses at levels below the equivalent of 10 mg of PDN daily. Moreover, activity of the disease should be adequately controlled, and other cardiovascular risk factors (including homocysteine levels) identified and properly addressed. If dietary measures and antimalarial therapy are not enough to control hyperlipidemia, lipid-lowering agents should be added to the therapeutic regimen.

Neuropsychiatric Adverse Effects

CNS GC effects are relatively common. Mood changes are probably the commonest occurring in half the patients (4). Depression more commonly, or euphoria can occur. Additionally, cognitive dysfunction and decreased duration of REM sleep can be seen (4). A recent cohort study reported a 5% incidence of psychiatric events after a mean of 3 weeks of initiation of GC therapy (234). Three of 92 SLE patients developed psychosis and another three mania. All episodes resolved with reduction of GC dose from 40 mg to 18 mg of PDN daily. Hypoalbuminemia was an independent risk factor for psychiatric events (234). When manic behavior, psychosis, or seizures supervene during therapy, they require differentiation from primary NPSLE (107 ,145 ,235). The distinction can be difficult but the temporal relationship to increases in GC dose, along with lack of focal neurologic signs or CSF abnormalities suggest the correct diagnosis. Discontinuation or reduction of GC therapy along with phenothiazine suffices to reverse GC-induced psychosis (235). Benign intracranial hypertension (pseudotumor cerebri) occurs only rarely.

Infection

Glucocorticoids predispose to infection and at the same time may mask clinical clues of infection as a result of their immunosuppressive and anti-inflammatory effects. On the other hand, reduction of inflammation is probably responsible for the improved outcomes observed when *Pneumocystis carinii* pneumonia and *Haemophilus influenza* meningitis are treated with adjunctive high-dose GC therapy (236 ,237). The incidence of infection after GC therapy was examined in a metaanalysis by Stuck et al., and was found to be 12.7% (1.6-fold that of the control population). In the same study, patients treated with less than 10 mg of PDN daily, or a cumulative dose of less than 700 mg were spared from a higher risk to infection (238). Besides increasing the incidence of bacterial infections, GC can reactivate latent tuberculosis (TB) or histoplasmosis and predispose to an accelerated form of herpetic keratitis that culminates in blindness (4). Active SLE, by itself, increases the risk of bacterial (239 ,240 ,241), and, more rarely, opportunistic infections (242), probably as a result of several immune system perturbations (reviewed in (243)). Therapy with GC further augments the susceptibility of SLE patients to infection (169 ,239 ,241 ,242 ,244). Interestingly, in one study treatment with GC and cyclophosphamide improved survival but at the same time was associated with sepsis as a cause of death (244). Sepsis has been frequently cited as the first cause of death in SLE patients being responsible for 19% to 54% of all deaths (169 ,222 ,239 ,241 ,243 ,244).

With regard to opportunistic infections, GC at relatively high doses (more than 40 mg of PDN daily) can increase the risk of PCP, especially when combined with other immunosuppressive agents and/or coexistent peripheral lymphopenia (242 ,245 ,246). Prophylaxis for PCP should be considered in such patients. Since use of trimethoprim-sulfamethoxazole may be of concern in SLE, alternative prophylaxis regimens should be tried (i.e., dapsone 100 mg/day) (247). Additionally, purified protein derivative (PPD) testing will help identify patients exposed to TB who will be candidates for antituberculosis prophylaxis. Notably, for patients on GC doses equivalent to more than or equal to 15 mg/day of PDN for 1 month or more, a PPD skin reaction more than or equal to 5 mm of induration is considered positive and warrants preventive therapy (248). Immunizations with *H. influenza* type B, tetanus toxoid, and pneumococcal vaccines were recently found safe and able to induce protective antibody titers in SLE patients (249). However, a trend towards lower protective antibody titers was noted for patients on immunosuppressive agents, perhaps suggesting that immunizations should best be done before initiation of such therapy. Vaccinations for influenza, *Pneumococcus,* (killed vaccines), and tetanus (component protein or peptide vaccine) are therefore highly recommended for SLE patients. In contrast, vaccinations with live attenuated viruses such as oral polio, varicella, and MMR should be avoided in immunosuppressed patients as they may lead to disease (247). Notably even contact with children vaccinated with OPV (but not MMR) is risky (247).

Steroid Myopathy

Glucocorticoids have permissive actions necessary for normal function of skeletal muscle. However, exposure to fluorinated GC or large daily doses (>30 mg of PDN) (250) of nonfluorinated GC administered for more than a few weeks (and in one study of cancer patients, within 15 days) (251) can cause a proximal myopathy characterized primarily by atrophy of type IIB muscle fibers (84 ,250 ,251 ,252 ,253). Usually the pelvic girdle muscles (252) are more severely affected and sometimes respiratory muscle weakness can occur (253). Although muscle enzymes are usually normal, urine creatine (252) and serum LDH (in SLE) might be elevated (253). Exercise (both resistance and endurance) is effective in attenuating GC-induced muscle atrophy (254).

The rare syndrome of acute myopathy of intensive care can occur in critically ill patients treated with high dose IV GC and neuromuscular junction-blocking agents (255). These patients develop a severe quadriplegia characterized by impaired muscle membrane excitability and a necrotizing myopathy with loss of thick filaments on muscle histopathology (255 ,256 ,257).

Skeletal Growth Retardation

Children treated with GC, especially during their prepubertal years, display delayed skeletal maturation and growth (258 ,259 ,260). Even regimens considered to be relatively safe, such as LDGC, ADGC, and inhaled or intranasal therapy have been implicated in growth retardation (260 ,261 ,262), which, of interest, has been observed in one

study without concomitant HPA-axis suppression (as assessed by AM serum cortisol levels and standard cosyntropin testing) (262). Among children treated with ADGC for cystic fibrosis, persistent growth impairment was observed only in boys (but not girls) after cessation of therapy (260). Mechanisms of GC AE on skeletal growth may include inhibition of chondrocyte IGF-I production, GH- and IGF-I-receptor expression, GH secretion, collagen type I synthesis, and upregulation of collagenase-3 expression (263 ,264). GC-treated children respond to GH therapy with increased growth velocity but responsiveness is not optimal and correlates negatively with GC dose (259 ,263).

Other AE

Effects of GC on protein catabolism, fibroblast function, and collagen metabolism are probably responsible for suppression of wound healing processes and skin atrophy-purpura. Notably, chronic steroid therapy in SLE patients has been associated with tendon ruptures (265).

Posterior subcapsular cataract formation is not uncommon with systemic, topical or inhaled GC use and children may be more susceptible to this complication. Open-angle glaucoma may occur rapidly with topical ocular administration, but may take years before it occurs with systemic GC therapy. Glaucoma, in contrast to cataracts, often resolves with GC discontinuation. Regular ophthalmologic follow-up for both potential AE is required (266).

There is probably no association between GC use and development of peptic ulcer disease (PUD) or its complications (267). However concomitant use of NSAIDs confers a higher risk of PUD (268) and the same is probably true when other comorbid conditions (i.e., CHF, renal failure, old age) are present (169).

Hypersensitivity reactions and severe anaphylaxis to GC can rarely occur. Intravenous, intraarticular, soft tissue, and intradermal injections have been usually implicated, but association with topical and oral GC use has also been reported (269 ,270 ,271). Asthmatics with atopy may be more susceptible to this complication (269). It has been suggested that injectable epinephrine and diphenhydramine be readily available in rheumatology offices, in case anaphylaxis to GC injections occurs (271).

Glucocorticoid Withdrawal Syndrome

Symptoms upon withdrawal of GC can be a result of either SLE reactivation or because of adrenal insufficiency. The latter usually occur with attempts to taper GC below physiologic levels (i.e., 5–7.5 mg of PDN) and consist of anorexia, nausea, weight loss, arthralgias, myalgias, lethargy, weakness and mild orthostatic hypotension-tachycardia. These can rarely occur even with normal adrenal function, as defined by normal morning cortisol levels and a normal ACTH stimulation test, and in that case may be due to GC resistance from prolonged LDGC use (4 ,84).

Future Directions

After half a century of continuous GC use in clinical practice, important advances have been made regarding both mechanisms of GC action and the rational usage of these agents for the benefit of our patients. However, much more research and progress is still needed in this field. First, developing new and safer GC agents is of utmost importance. In this direction some progress has already been made with the emergence of the “dissociated” GC and their encouraging results in animal models of inflammation. Hopefully, their dissociation of anti-inflammatory transrepression effects from transactivation effects that underlie at least some of GC adverse effects will hold also in human clinical trials. Second, understanding the pathogenesis of GC-induced adverse effects will help develop more effective interventions for their prevention or treatment. Along these lines, for example, the osteocyte antiapoptotic activity of PTH might explain its promising effects in GIOP (and perhaps GION). Blocking OPGL might be another approach to the management of GIOP. Finally, successful research into the mechanisms for GC resistance might help predict effective GC doses before therapy initiation and at the same time allow for design of new therapeutic interventions that will aim to decrease GC resistance. Use of inhibitors of proinflammatory transcription factors such as AP-1 and/or NFκB that are mutually antagonistic with GR might be one such approach. The design of these therapies for SLE patients should also, however, take into account parallel advances in our understanding of the pathogenesis of this disease.

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Chapter 61

Immunosuppressive Drug Therapy

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Immunosuppressive agents are widely used in the treatment of serious manifestations of systemic lupus erythematosus (SLE) to minimize irreversible injury characterized by fibrosis and organ dysfunction and to reduce toxicity from corticosteroids. Recent research efforts have focused on minimizing cyclophosphamide use, including evaluation of sequential therapies utilizing intravenous bolus or daily oral cyclophosphamide for induction of remission, followed by azathioprine or mycophenolate mofetil for maintenance of remission, and de novo therapy of severe lupus with mycophenolate mofetil rather than cyclophosphamide. In the near future, addition of new biologic agents to conventional immunosuppressives will have the potential to further reduce our reliance on alkylating agents.

Most studies of immunosuppressive agents in lupus have been performed in patients with nephritis. The availability of histology and relatively accurate tests of renal function allow a fairly accurate estimation of the response to therapy; however, the duration of such trials, usually 1 to 5 years, has been much less than the anticipated survival of most patients with lupus. Especially in patients who do not have fulminate disease, long-term trials are required, unless sensitive markers for treatment failure are employed (e.g., doubling of serum creatinine instead of progression to renal failure), prolonged treatment of patients randomized to suboptimal regimens may not be practical.

A meta-analysis that included 440 patients with lupus nephritis in 19 clinical trials concluded that the use of immunosuppressive drugs (azathioprine and/or cyclophosphamide) in addition to prednisone and the use of prednisone alone significantly reduced the risks of end-stage renal disease (ESRD) or death by 12.9% and 13.2%, respectively (1). These findings were consistent with those of Felson and Anderson (2), who in 1984, combined eight clinical trials employing prednisone alone versus prednisone plus azathioprine and/or cyclophosphamide for the treatment of lupus nephritis. They concluded that progression to both renal failure and death was delayed by immunosuppression, particularly in patients with diffuse proliferative nephritis, although statistical significance was lost when the azathioprine- or cyclophosphamide-treated groups were analyzed separately. A more recent meta-analysis from 2004 examined randomized controlled trials of therapeutic options for diffuse proliferative lupus nephritis and found 25 papers meeting criteria (3). This analysis concluded that cyclophosphamide plus corticosteroids remains the first choice for preservation of renal function, but that every effort should be made to limit the exposure to cyclophosphamide to the extent possible because of associated gonadal toxicity. There was no evidence from the analysis that azathioprine therapy had an effect on renal outcomes, and there was at that time insufficient evidence to draw conclusions about therapy with mycophenolate mofetil.

This chapter focuses on widely used immunosuppressive agents, emphasizing controlled trials, most of which have enrolled patients with lupus nephritis, and reviews the use of the alkylating agents, azathioprine, cyclosporine, methotrexate, and mycophenolate mofetil (MMF) and their roles in induction as well as sequential therapies following treatment with cyclophosphamide.

Alkylating Agents

Of the more than a dozen alkylating agents that are currently in use, cyclophosphamide (CYC), chlorambucil and nitrogen mustard (HN3) have been applied sufficiently in the treatment of SLE to warrant discussion. Although there are inherent similarities in mechanisms of action, the clinical effects, both therapeutic and toxic, of the individual alkylating agents differ from each other and may vary depending on the dose, route of administration, duration of administration, and cumulative dose of these agents. Larger cumulative doses of alkylating agents are associated with escalating risk of toxicity, particularly gonadal failure, and secondary malignancies. Because alkylators are the most effective agents studied in long-term trials for management of lupus nephritis, familiarity with strategies to minimize toxicity is important in order to use these drugs with maximal benefit.

The earliest use of alkylating agents, reported by Osborne et al. (4) in 1947, was topical application of nitrogen mustard in cutaneous lupus, followed in 1949 by the description by Chasis of the efficacy of nitrogen mustard in glomerulonephritis (5). Despite its toxicity and difficulty of administration, nitrogen mustard has proven to be a potent and rapidly acting immunosuppressive agent that is clearly effective both in lupus nephritis and rheumatoid arthritis (RA). However, CYC has effectively replaced nitrogen mustard in routine clinical practice. Chlorambucil, despite its efficacy and lack of bladder toxicity, is unacceptably toxic to the bone marrow and oncogenic in our opinion; its use is

reserved for the rare patients with CYC-induced bladder toxicity who still require an alkylating agent.

Cyclophosphamide

Cyclophosphamide is a mechlorethamine derivative that is inactive as administered. It is metabolized by mitochondrial cytochrome P-450 enzymes in the liver to a variety of active metabolites, an increasing number of which have been shown to have both therapeutic and toxic actions. It has been proposed that various genetic polymorphisms of the P-450 enzymes are associated with both the toxicity of CYC, as well as the clinical response to the drug in patients with lupus nephritis (6). In a retrospective study, 62 patients with proliferative lupus nephritis treated with CYC were genotyped for common variant alleles of the P-450 enzyme. Homozygosity or heterozygosity for a particular variant allele (CYP2C19*2) predicted not only lower rates of ovarian toxicity, but a worse clinical response, including higher risk of ESRD and doubling of serum creatinine, suggesting that efficacy and toxicity were both related to the effective dose given.

Active metabolites of CYC include 4-hydroxycyclophosphamide, phosphoramide mustard, and acrolein, all of which have differing rates of synthesis, half-lives, immunologic effects, and toxicities. Serum levels of these metabolites are not routinely measured; hence, dose adjustment in patients with renal or hepatic failure is largely empiric. Dosage should be reduced approximately 30% in patients with a creatinine clearance of less than 30 mL/minute. Furthermore, CYC is incompletely cleared by dialysis, and therefore, the dose should be lowered for these patients as well. The effect of hepatic insufficiency on CYC toxicity is incompletely understood, in part because the liver is responsible for both the production of active metabolites and their degradation. CYC is metabolized not only in the liver, but also in lymphocytes and transitional epithelial cells in the bladder, which may result in local toxicity and/or immunosuppression. CYC may have toxic and/or therapeutic effects in cells that are not actively dividing as well as in dividing cells.

CYC is well absorbed orally, and the oral and intravenous doses are equivalent. Large boluses of CYC can be given orally, achieving comparable serum levels versus IV administration. Approximately 20% is excreted by the kidney, and 80% is processed by the liver.

The immunologic effects of CYC have been described (7 ,8). Direct effects of CYC on DNA result in cell death. These effects may occur at any stage during the cell cycle. Direct immunomodulatory effects also may occur and may be responsible for the relatively rapid onset of therapeutic efficacy of CYC (i.e., within 2 to 4 days) that is seen in some patients at a time when attrition of immunocompetent cells, because of the inhibition of cell division, would not be expected. Putative mechanisms of action include alteration of macrophage function, increased production of prostaglandin E2, alteration of gene transcription, and direct functional effects on lymphocytes (7 ,8). IVC induces suppression of T cell activation by a combination of monoclonal antibodies to the CD2 antigen (9 ,10); however, modulation of T cell function has not been convincingly shown to play an important role in the treatment of lupus.

Cyclophosphamide produces dose-related lymphopenia, which is probably therapeutically important. However, a direct relationship between the degree of lymphopenia and therapeutic efficacy of CYC has not been established. Therapeutic doses of CYC produce dose-dependent reduction of both $CD4^+$ and $CD8^+$ T cells (9 ,10 ,11). IVC reduces the population of $CD4^+$ and $CD8^+$ lymphocytes and B cells, with a more marked reduction of $CD4^+$ lymphocytes and B cells during monthly therapy. Following cessation of monthly therapy, B cell populations rapidly return to baseline (9), but $CD4^+$ populations remain relatively suppressed during less intensive IVC therapy, resulting in prolonged reduction of the $CD4^+/CD8^+$ ratio (11). Persistent reduction of the percentage of $CD19^+$ lymphocytes and of the $CD4^+/CD8^+$ ratio 6 months after completion of 6 months of therapy has been reported (12). Other studies have suggested specific reduction of B cell function (13). Reduction of autoantibody production has been demonstrated in patients with SLE who are treated with both oral and IVC and in patients with rheumatoid arthritis who are treated with oral CYC (9 ,14). Despite reduction of pathogenic autoantibody production, reduction of overall levels of immunoglobulin (Ig) classes IgG, IgA, and IgM, and IgG subclasses has not been observed in our patient population. This suggests that specific suppression of autoantibody production is a function of CYC when used in therapeutic doses and may underlie its beneficial action in SLE.

Low doses of CYC in both animals and humans can heighten immune responses. This has been noted in both antibody-mediated and cell-mediated immunity, and it has been theorized that low doses of CYC could enhance antitumor immunity in humans (15). Low doses of CYC accelerate the production of diabetes in the nonobese diabetic (NOD) mouse (16). The mechanism of action of CYC in these situations is unclear, but it may represent functional alterations as well as depletion of lymphocyte subsets. These observations suggest that tapering the dose of CYC may produce unexpected effects. As a practical matter, however, there are no clinical data to support the hypothesis that during tapering of immunosuppressive drugs, particularly CYC, immunosuppression is supplanted by immunostimulation.

Daily oral CYC is usually initiated at 1 to 2 mg/kg/day. Dosing is adjusted downward in the presence of renal failure, edema, obesity, or inadequate bone marrow reserve. The use of a standard maximum dosage of 2mg/kg/day, with dose reduction in the presence of leukopenia (WBC <3,500) or neutropenia (WBC <1,000), is a common practice. Another treatment strategy that has been employed is gradually increasing the dose of CYC with the goal of producing mild leukopenia. Although these approaches have not been directly compared in a single trial, it is likely that avoidance of leukopenia, coupled with prophylaxis against *P. carinii,* may significantly reduce morbidity and mortality from infection during CYC therapy. Monitoring for toxicity

includes complete blood counts weekly initially and advanced to monthly when stable, urinalyses to detect hemorrhagic cystitis, and annual urine cytology.

Monthly bolus CYC (IVC) usually begins with a dose of 500 to 750 mg/square meter body surface area administered intravenously over 1 hour in normal saline. For each subsequent monthly treatment, the dose may be increased 10% to 25% with a goal of achieving a nadir of the white blood count to 2,000 to 3,000 cells/mm^3. Dose reduction should occur if the nadir of the CBC is less than 2,000 WBC/mm^3 or 1,000 granulocytes/mm^3. Many physicians limit the maximum CYC dose to 1 g/square meter, with dose adjustment downward in renal failure. To avoid bladder toxicity, it is important to instruct patients to drink several liters of water during the 24 hours prior to CYC treatment. They are then hydrated vigorously with half-normal saline during the infusion, followed by oral or IV hydration to maintain urine output more than 100 mL/hour continued for 24 hours. Additionally, sodium 2-mercaptoethane sulfonate (mesna) totaling 80% of the CYC dose is administered in divided doses over 12 hours. Patients unable to completely empty their bladder, such as those with neurogenic bladders, may require catheter drainage or irrigation during treatment. In our institution, two patients with decreased urine output who received IVC without bladder irrigation developed severe posttreatment hemorrhagic cystitis. Antiemetics, such as granisetron or ondansetron, are also routinely administered; initial administration of dexamethasone 5 to 20 mg, diphenhydramine 25 to 50 mg and/or lorazepam 1 mg may also be used. Potential toxicities of cyclophosphamide are described below.

Hemorrhagic Cystitis and Carcinomas of the Bladder

Acrolein is a CYC metabolite, which is directly toxic to the bladder. Exposure to acrolein in the bladder has been associated with hemorrhagic cystitis, a premalignant lesion identified in 50% of patients receiving CYC who eventually develop transitional cell carcinoma of the urinary tract, particularly the bladder. The risk of hemorrhagic cystitis (as well as bladder fibrosis) is increased with daily oral administration of CYC (reported in 5% to 34% patients) (17 ,18 ,19 ,20). Monthly urinalyses are therefore advised during treatment. The finding of hematuria warrants CYC discontinuation pending urologic evaluation. Hemorrhagic cystitis may present either with microscopic or gross hematuria, which may be life-threatening, mandating immediate discontinuation of CYC (18). If hemorrhagic cystitis occurs, therapy with CYC should not be reintroduced even after symptoms resolve. Furthermore, these patients should have annual evaluation by a urologist, including cystoscopy and urine cytologic examination (14). The risk of bladder carcinoma associated with CYC therapy is dose dependent and is markedly increased after a total dose of 30 g (19 ,20). In a study of RA patients, 9 of 119 CYC-treated patients developed bladder carcinomas after 20 years, of whom seven had received more than 80 g of CYC (21).

In comparison to oral administration, monthly IVC is rarely complicated by bladder injury if proper hydration and bladder emptying occur. However, it has been reported in patients who did not receive IV hydration and in cancer chemotherapy patients receiving high doses. IVC is not safe after bladder complications of daily CYC, however. As mentioned above, the use of mesna has been advocated to reduce the concentration of acrolein and perhaps other toxic metabolites in the bladder. Vigorous hydration and mesna used together may provide the best protection, although there is no established standard of care.

Other Malignancies

Development of malignancies following CYC administration is well described in patients with rheumatic diseases, particularly rheumatoid arthritis (RA) and Wegener granulomatosis (17). Nonurinary tract neoplastic complications of CYC include skin cancers and hematologic malignancies, as well as cervical atypia, which can be seen even in patients who have received cumulative doses of CYC less than 20 g. In those patients who have received 80 to 120 g cumulative CYC doses, myelodysplastic syndromes are observed, characterized by monozomy-5 and/or monozomy-7 (7 ,22). Long-term follow-up studies by Baltus et al. (23) and Baker et al. (18) of RA patients treated with oral CYC have established an approximately 10% additional incidence of malignancy compared with age-matched controls after a total dose of 30 g. Doses of less than 10 g are almost certainly safer; doses of 100 g or more are even more likely to produce malignancy. The overall incidence of hematologic malignancies in patients receiving more than 30 g of CYC may approach 5%. Radis et al. (21) reported a 20-year follow-up of the original study by Baker et al. and showed continued occurrence of CYC-induced malignancies, including bladder cancer throughout the 20-year follow-up period. At the completion of this study, only 40% of the original patient population that was treated with CYC remained cancer-free.

Although there is little data, there is no reason to believe that comparable doses of other alkylating agents, such as chlorambucil or nitrogen mustard are safer than CYC. An increased incidence of cutaneous malignancies, including melanomas, squamous cell carcinomas, and aggressive basal cell carcinomas, has been observed (24). Additionally, there is an increased risk of cervical dysplasia and carcinomas of the cervix and vulva. This risk is also a problem for transplant patients receiving various immunosuppressive drugs (24 ,25). IVC therapy of patients with lupus has not been associated with a statistically significant increase in the incidence of fully developed cancers, probably because of the lower cumulative doses and the use of IV hydration to protect the urinary tract. This information may be deceptive because of the relative shorter lengths of follow-up obtained thus far.

Hematologic Toxicity

Compared with other alkylating agents such as nitrogen mustard, the acute effects of CYC are relatively benign. Stem cells appear to be quite resistant to CYC. After pulse therapy, the nadir of the lymphocyte count occurs on approximately day 7 to 10, and that of the granulocyte count on approximately day 10 to 149. There usually is a prompt recovery from granulocytopenia after 21 to 28 days. In some patients, the recovery period may be prolonged, necessitating longer dose intervals. Prior use of alkylating agents may be associated with delayed recovery. Immunologically mediated cytopenias often improve after treatment with appropriate doses of IVC, whereas they are more likely to worsen after azathioprine administration. Daily oral CYC can result in the progressive development of macrocytosis and cytopenias. After prolonged administration, with cumulative doses in the range of 60 to 120 g, the risk of hematologic malignancies, particularly myelodysplastic syndromes, is increased. Thrombocytopenia, which rarely occurs as a result of treatment except after prolonged therapy, may signify the onset of a myelodysplastic syndrome.

Gastrointestinal Toxicity

Intravenous CYC can be associated with short term nausea and gastrointestinal dysmotility during treatment. Occasio-nally, significant hepatic toxicity may occur with the doses used for autoimmune diseases. With oral CYC treatment, both anorexia and nausea may occur, particularly with high doses.

Pulmonary Toxicity

Pulmonary toxicity is an infrequent complication during therapy with CYC. The most frequently encountered pulmonary involvement of CYC therapy is acute interstitial pneumonitis. Pulmonary injury due to alkylating should be suspected in patients treated with CYC during the past 6 months prior to presentation who have bilateral reticular or nodular diffuse opacities on chest radiograph, or peripheral ground-glass opacities in the upper lung fields on chest CT. Additionally, a late-onset pneumonitis associated with fibrosis may present insidiously after months to years of CYC therapy, even with relatively low doses (26). These late conditions are minimally responsive to corticosteroids, irreversible, and usually result in terminal respiratory failure or lung transplantation.

Gonadal Toxicity and Teratogenicity

CYC is toxic to the granulosa cell and, as a consequence, reduces serum estradiol levels and progesterone production, inhibits the maturation of oocytes, and reduces the number of ovarian follicles, ultimately resulting in ovarian failure. Studies in patients with breast cancer receiving CYC show that in women in their 40s, 30s, or 20s, the respective cumulative doses of CYC required to produce ovarian failure were 5, 9, or 20 g (7). Amenorrhea or premature ovarian failure is less likely to occur in patients who receive short-term (approximately 6 months) monthly IVC.

Several approaches to preserve fertility in women undergoing treatment with CYC have been proposed. These include preservation of oocytes, embryos, or ovarian tissue (a topic recently reviewed in the New England Journal of Medicine) (27), and the use of gonadotropin hormone-releasing analogs (depot-GnRH-a) to suppress the metabolism of the ovaries during cytotoxic therapy with CYC (28 ,29 ,30). We recently reported the results of an open trial of depot-leuprolide acetate (a synthetic GnRH-a) administration for ovarian protection during monthly IVC therapy (31). In this study, premature ovarian failure occurred in only 1 of 20 (5%) GnRH-a treated patients versus 6 of 20 (30%) of controls matched for age and cumulative CYC dose (p <0.05, McNemar test). Furthermore, time-to-event analysis demonstrated improved cumulative ovarian protection over time in the GnRH-a treated group (p = 0.04) (31). These data, in addition to other data from nonrandomized trials in the field of chemotherapy and reproductive preservation, suggest that this GnRH-a protocol for ovarian protection in women receiving CYC is both safe and effective. Should it prove to be effective in further trials, ovarian protection with leuprolide has the potential advantages of being noninvasive in severely ill patients, less expensive, well tolerated, and the additional advantage of not only preserving fertility but also avoiding premature menopause with its attendant consequences.

Patients who are receiving CYC may also develop transient amenorrhea resulting from their illness (i.e., hypothalamic amenorrhea) or true ovarian failure as described above. The risk of osteoporosis is increased by amenorrhea regardless of its cause.

Azoospermia frequently occurs in men following treatment with CYC, and therefore sperm banking should be considered prior to therapy (32). It has also been reported that testosterone supplementation offers protection of testicular function in men during CYC therapy, as evidenced by one report in which recovery of normal sperm counts was higher among men with azoospermia treated with testosterone vs. no testosterone during CYC therapy (33).

CYC is a potent teratogen that can cause severe birth defects after administration of as little as 200 mg during early pregnancy (34 ,35 ,36 ,37 ,38). Reported abnormalities included absent thumbs, absence of the great toes or all toes, palatal abnormalities, and a single coronary artery. Because fertility is preserved in most lupus patients, highly effective contraceptive techniques (e.g., oral contraceptives or injected progestins in appropriately selected patients) should be strongly considered. Use of CYC in life-threatening lupus during late pregnancy is controversial, but may be appropriate in special circumstances because fetal loss is extremely likely when severe maternal flares are uncontrolled. Major CYC-induced toxicities are felt to occur during the first half of pregnancy (39 ,40).

Infections

The risk of bacterial infections and herpes zoster is increased with CYC therapy (17 ,41 ,42), as is the risk of *P. carinii* pneumonia (PCP) (42). This risk is further increased when more than 20 mg/day of prednisone or bolus corticosteroids are administered concomitantly. In CYC-treated patients with lupus, increased risk of infection has been associated with higher daily doses of prednisone and depression of the $CD4^+/CD8^+$ ratio in circulating lymphocytes (43). Prophylaxis against PCP, now routine in the treatment of Wegener, has lagged in lupus treatment, perhaps because of reluctance to administer trimethoprim-sulfamethoxazole (TMP-SMX) to lupus patients. We have used TMP-SMX three times weekly in lupus patients known to tolerate this drug, and otherwise have used dapsone (100 mg/day) in patients without glucose-6-phosphate dehydrogenase deficiency or sulfa allergy. Hypogammaglobulinemia should be considered in patients who develop infections (44). Granulocyte colony-stimulating factor (GCSF) can also help decrease the morbidity and mortality associated with drug-induced leukopenia during CYC therapy.

Table 61-1: Controlled Trials of Cyclophosphamide and/or Azathioprine in the Treatment of Lupus Nephritis

Study	Patients (*n*)	Results
Fries et al. (52)	14	P > CTX alone
Garancis and Piering (53)	22	P + CTX > P + AZA
Donadio et al. (54,55,56)	26	More recurrences with P; P vs. P + CTX = survival, and on dialysis
Ginzler et al. (117)	14	P + AZA = P + CTX
Balow et al. (47)	111	P + IVC > P + AZA + CTX > P + AZA > P
Boumpas et al. (58)	65	IVC for 30 months >IVCX for 6 months >MP
Sesso et al. (60)	29	IVC or MP both unsuccessful
Gourley et al. (59)	80	IVC > MP; trend for IVC + MP > IVC

AZA, azathioprine; POC, oral cyclophosphamide; IVC, intravenous, intermittent cyclophosphamide; MP, bolus methylprednisolone; P, prednisone.

Finally, the syndrome of inappropriate antidiuretic hormone (SIADH) can also occur following IVC administration.

Clinical Trials Employing Cyclophosphamide for Lupus Nephritis

The literature regarding the use of CYC in SLE, particularly lupus nephritis and central nervous system (CNS) lupus, continues to increase at an exponential rate precluding a detailed review of all studies here. Tables 61-1 and 61-3 summarize results of controlled trials. Table 61-4 summarizes results of controlled trials of sequential therapy using CYC for induction of remission. In this discussion, trials of monthly bolus CYC will be emphasized, but many modified regimens have been proposed, such as weekly or biweekly bolus CYC given intravenously and boluses of CYC given orally (45 ,46). These regimens have been reported to be safe and effective in small series. More complicated regimens, including bolus CYC synchronized with plasmapheresis, high-dose CYC, and sequential regimens using CYC followed by azathioprine or mycophenolate mofetil will be discussed below.

A 20-year clinical trial comparing most of the regimens that have been widely used to treat lupus nephritis was performed at the National Institutes of Health (NIH) including patients with mostly proliferative nephritis, all of whom were treated with daily oral glucocorticosteroids at the initiation of immunosuppressive therapy (47 ,48 ,49 ,50). Patients were given (a) no additional therapy, (b) oral azathioprine, (c) oral azathioprine plus oral CYC, (d) oral CYC, or (e) IVC. The duration of administration of these agents differed, ranging from approximately 2 to 4 years. The IVC treated patients had a variable number of monthly pulses (often three) before being assigned to once-every-3-months CYC therapy (Fig. 61-1). There were several key findings:

- Differences in outcome were not apparent until more than 5 years had elapsed. Until that time, prednisone-treated patients had the same rate of renal failure and death as immunosuppressed patients. This may relate in part to the relatively mild degree of renal compromise in some patients at entry and the use of renal failure or death as endpoints. After 10 years, however, there were marked differences in renal survival, favoring any regimen that included CYC over the administration of prednisone alone.
- There was a trend for patients treated with either prednisone alone or with oral CYC to have higher death rates than patients who were treated with IVC or azathioprine plus CYC. This probably reflects the toxicity of oral CYC and ineffectiveness and toxicity of prednisone alone.
- The combination regimen of oral azathioprine plus CYC appeared to work as well as IVC in terms of progression to renal failure or death.
- Retrospective analysis suggested that the presence of chronic change on initial biopsy was a poor prognostic factor, predicting a poor outcome unless immunosuppressives were used.

Table 61-2: Parameters 40 Days after Nitrogen Mustard Administration

	Incidence		Disappeared at 40 Days		
Parameter	*n*	**%**	*n*	**%**	**Mean Duration of Response (mo)**
Hematuria	45/56	80	14/45	31	46
Hyaline casts	28/58	48	11/28	39	33
Granular casts	32/56	57	11/32	34	63
Oval fat bodies	19/56	34	2/19	11	—

	Decreased			
Parameter	*n*	**%**	**Criteria for Decrease**	**Mean Decrease**
Serum creatinine level	13/33	39	≥0.1 mg/dL	0.63 mg/dL
Serum cholesterol level	19/27	70	≥1 mg/dL	99 mg/dL
24-hour urine protein level	31/38	82	100 mg	3,595 mg
Prednisone dosage	26/43	60	≥1 mg	19 mg
Weight	39/57	68	≥0.1 kg	2 kg

From Wallace DJ, Podell TE, Weiner JM, et al. Lupus nephritis. Experience with 230 patients in a private practice from 1950 to 1980. *Am J Med* 1982;72(2):209–220, with permission.

Table 61-3: Controlled Trials Including Bolus Methylprednisolone, Cyclosporin, or IV Immunoglobulin in SLE

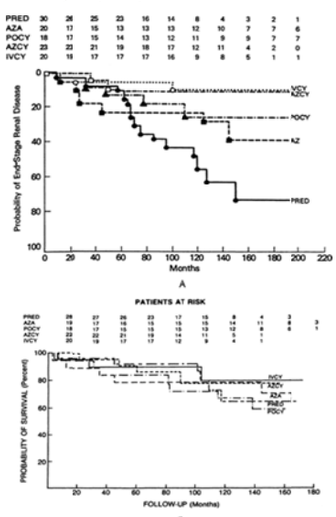
Author	Therapeutic Arms	Result
Boumpas (58) 1992	IVC short-term	Long-term IVC > short-term
	IVC long-term	Bolus MP < either IVC
	Bolus MP	
Sesso (60) 1994	IVC	Equivalent outcome
	Bolus MP	38% renal failure/15 months
Gourley (59) 1996	IVC	IVC > Bolus MP
	Bolus MP	Trend for IVC + Bolus MP > IVC
	IVC + Bolus MP	
Fu (57) 1998	CS without P	Similar renal outcome
	POC + P	38% renal failure/15 months
Boletus (61) 1999	IVC	Equivalent short-term results
	IVIG	

CS, cyclosporine; IVC, intravenous cyclophosphamide; IVIG, IV immunoglobulin; MP, methylprednisolone; POC, oral cyclophosphamide; P, prednisone.

Table 61-4: Controlled Trials Using Sequential Therapy with Azathioprine or Mycophenolate Mofetil Following Induction with Cyclophosphamide in the Treatment of Lupus Nephritis

Study	Patients (n)	Results
Chan et al.	42	MMF > AZA + CYC
Houssiau et al.	90	Low dose IVC + AZA = high dose IVC + AZA
Contreres et al.	59	MMF/AZA > = and less toxic than quarterly IVC + MP
Yee et al.	32	IVC + MP followed by AZA = and less toxic than POC + MP followed by AZA

AZA, azathioprine; IVC, intravenous, intermittent cyclophosphamide; MP; methylprednisolone; POC, oral cyclophosphamide.



- In the subset of patients who underwent serial biopsies, progression of chronic change occurred initially in all patients. Immunosuppressive-treated patients appeared to stabilize after an initial period of scarring; prednisone-treated patients had further scarring.

Oral Cyclophosphamide

Most studies of CYC have compared CYC and prednisone versus prednisone alone. An interesting study by Fries et al. (51) in 1973, however, compared oral CYC alone versus prednisone alone for a mean of 9 weeks in 14 patients with lupus, 10 of whom had nephritis. CYC failed to control either minor or major manifestations, despite development of leukopenia, and significant additional toxicity in many patients. Patients who were changed to prednisone from CYC did better. These results suggest that CYC and prednisone may act synergistically.

In a randomized trial, Garancis and Piering (52) treated 22 patients with biopsy-proven membranoproliferative glomerulonephritis using low-dose prednisone (approximately 10 mg/day) and 2 mg/kg/day of either CYC or azathioprine. After 6 to 36 months, the CYC-treated patients had better renal function and fewer deaths (0 vs. 4).

In another randomized trial, Donadio (53 ,54 ,55) assigned 50 patients with diffuse proliferative glomerulonephritis to treatment either with 6 months of oral CYC plus prednisone or with prednisone alone, retreating patients who subsequently flared with 6 additional months of oral CYC. The CYC-treated group had fewer flares of renal disease after 2 years and was felt to have a more favorable clinical course. CYC appeared to have a steroid-sparing effect. Patients with advanced renal insufficiency (i.e., creatinine clearance <30 mL/min) appeared not to benefit from this regimen. After 4 years, however, there was no difference in progression to death or renal failure between the two groups.

Fu et al. (56) compared daily oral CYC with cyclosporine in children with nephritis and found no difference in efficacy. The details of this somewhat atypical protocol are reviewed below (see Cyclosporine).

Recently there has been renewed interest in use of daily oral cyclophosphamide in lupus nephritis for remission induction followed with azathioprine (AZA) (57 ,58). Chan et al. studied 42 patients with moderately active diffuse proliferative glomerulonephritis who were randomized to daily CYC for 6 months followed by AZA for 6 months with high-dose MMF for 6 months followed by low-dose MMF for 6 months. At long-term follow-up, the results of this study reveal that in the MMF group, 81% experienced a complete remission, and 14% experienced partial remission. In the group randomized to CYC and AZA, 76% experienced a complete remission and 14% experienced a partial remission. In a second study, long-term outcomes in a cohort of lupus patients with diffuse proliferative nephritis who received sequential therapy with oral CYC and prednisolone for induction followed by AZA for maintenance therapy were studied (58). Sixty-six patients were included, of whom 82.4% achieved complete remission, and 39.1 of whom

relapsed during the follow-up period of 91.7 +/-36.7 months. There was no end-stage renal failure or death among study patients, although three (4.4%) patients had doubling of baseline creatinine.

Bolus Cyclophosphamide in Lupus Nephritis

Monthly bolus cyclophosphamide (IVC), first described as a treatment for lupus in 1984 by Sessoms and Kovarsky (59), is now the best studied immunosuppressive regimen for severe lupus. Early experience indicated that 6 months was too short a duration of treatment for patients with lupus nephritis, approximately 50% of whom experienced flares after discontinuation.

These observations were confirmed by Boumpas et al. (60), who compared three regimens in a relatively sick group of patients with lupus nephritis: seven monthly pulses of prednisone, seven monthly pulses of IVC, or seven monthly pulses of IVC followed by maintenance pulses of IVC every 3 months. All groups received oral prednisone. End points, including doubling of serum creatinine, were relatively sensitive, and differences between the treatment groups were evident after a few years. In this study, seven monthly pulses of IVC followed by an every-3-month maintenance regimen resulted in fewer flares and fewer doublings of serum creatinine compared with seven monthly pulses of CYC without maintenance pulses every 3 months or seven monthly pulses of methylprednisolone (Fig. 61-2). The longer IVC regimen resulted in more toxicity, however, particularly ovarian failure. Additionally, bolus methylprednisolone was not as effective as long-term CYC.

A related study compared bolus IVC versus bolus methylprednisolone versus both regimens as treatment for lupus nephritis (61). As in the previously described study, CYC was clearly superior to bolus methylprednisolone. Interestingly, although statistical significance was not achieved, there was a strong suggestion that addition of bolus methylprednisolone to bolus CYC increased the efficacy of the latter. Patients who received methylprednisolone pulses had more osteonecrosis than those who did not. During an extended follow-up with a median of 11 years (62), an intention-to-treat survival analysis of the patients in the above protocol revealed the likelihood of treatment failure to be significantly lower in the groups who received CYC (*P* = 0.04) and combination therapy (*P* = 0.002) than in group who received methylprednisolone alone. Furthermore, not only were there no more adverse events in the group treated with combination therapy versus the group treated with CYC alone, but the proportion of patients who had doubling of serum creatinine concentration was significantly lower in the combination group than in the CYC group. This study is noteworthy because it confirms the utility and relative lack of additional risk with combination pulse CYC and methylprednisolone therapy for lupus nephritis over methylprednisolone or CYC alone.

An opposite conclusion was reached by Sesso et al. (63). They compared short-term IVC versus bolus methylprednisolone in 29 patients with severe lupus nephritis. Patients received eight pulses (roughly comparable with the short-term arm on the previously described study by Boumpas et al. (60) in terms of total dose) over a 13-month period without maintenance IVC. This study found no difference in outcome. Survival without renal failure was a disappointing 62% over a mean 15 months of follow-up; 5 of 29 patients (17%) died. The high rate of initial treatment failures in the cyclophosphamide group and shorter-term follow-up could explain the lack of differences in outcome compared with those of other studies.

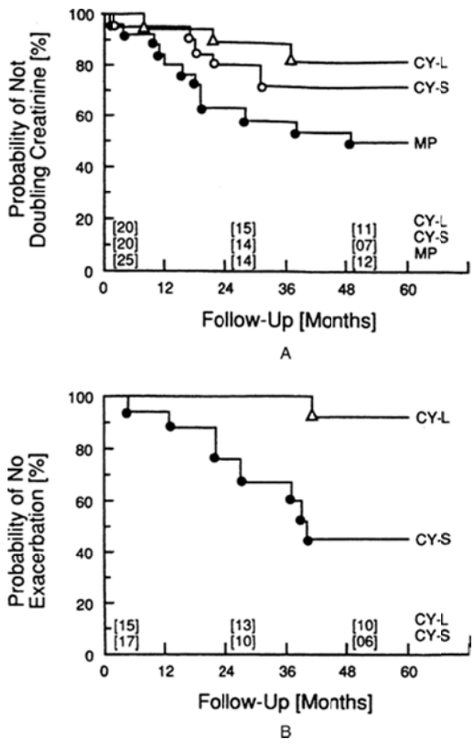


Figure 61-2. Treatment of severe lupus nephritis. **A,** Probability of not doubling serum creatinine levels in 65 patients with severe active lupus nephritis randomly assigned to receive: IV methylprednisolone (MP), 1.0 g/m^2 monthly for 6 months; IV cyclophosphamide (CY-S), 0.5 to 1.0 g/m^2 monthly for 6 months; or IV cyclophosphamide (CY-L), 0.5 to 1.0 g/m^2 monthly for 6 months, followed by quarterly infusions for 24 months (Gehan test comparing CY-L with MP, p = 0.037). **B,** Probability of no exacerbation of lupus activity on completion of monthly pulses in groups randomly assigned to receive CY-L and CY-S (Gehan test, p = 0.006). Numbers of patients that remain at risk at various times are shown along the abscissa. (From Boumpas DT, Austin HA, Vaughn EM, et al. Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* 1992;340:741–745, with permission.)

Boletis et al. (64) compared IVC with ten immunoglobulin infusions and found equivalent results over an 18-month period. Proteinuria actually increased slightly in the IVC group.

The possibility that brief administration of CYC might be effective in inducing remission was suggested by the

Euro-Lupus Nephritis Trial (65). In this study, 90 patients with proliferative glomerulonephritis were randomized to either “high dose” CYC (6 monthly and 2 quarterly pulses increased according to their WBC nadir) or “low dose” CYC (6 doses of 500 mg CYC every 2 weeks). Maintenance therapy was with azathioprine. Renal remission was achieved in 71% of low dose versus 54% of high dose patients, and renal flares were observed in 27% of low dose and 29% of the high dose group.

Sequential Therapy for Lupus Nephritis

Studies in both lupus and other systemic inflammatory diseases, particularly Wegener granulomatosis, have begun to focus on minimizing exposure to CYC by substituting less toxic immunosuppressive therapies after disease control with IVC has been achieved. In a 2004 study by Contreras et al., 59 patients with lupus nephritis WHO class III (14), IV (46) or Vb1 were treated with NIH IVC protocol for

7 doses (66). Patients were randomized to maintenance dosing for 1 to 3 years following initial therapy consisting of either quarterly IVC, MMF, or azathioprine (AZA). During maintenance therapy, there were four deaths in the CYC group and one death in the MMF group, and the incidence of chronic renal failure was three in the CYC group, one in the MMF group, and one in the AZA group. The 72-month event-free survival (defined as no death or progression to hemodialysis) was higher in groups treated with MMF ($p < .05$) and AZA ($p < 01$) versus CYC. The relapse-free survival was also higher in MMF versus CYC ($p < .02$) groups (see Fig. 61-3).

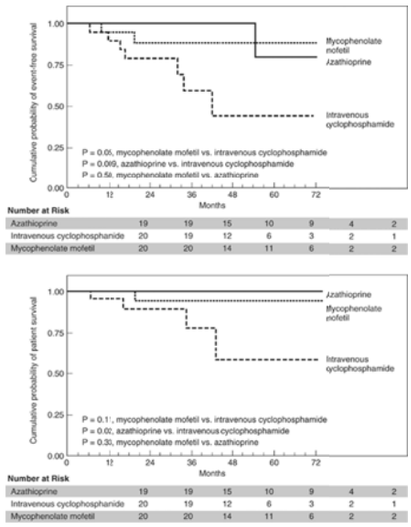


Figure 61-3. Mycophenolate for lupus nephritis.

Several clinical trials have employed daily oral CYC followed by AZA (57 ,58). For example, Chan et al. studied 42 patients with somewhat active diffuse proliferative glomerulonephritis who were randomized to daily CYC for 6 months followed by AZA for versus high-dose MMF for 12 months followed by low-dose MMF at 6 months. At long-term follow-up, the results of this study reveal that in the MMF group, 81% experienced a complete remission and 14% experienced partial remission. In the group randomized to CYC and AZA, 76% experienced a complete remission, and 14% experienced a partial remission. The study design does not afford comparison of this regimen with IVC, but oral CYC did appear to be more toxic than MMF.

As noted above, The EURO Lupus study compared “high dose” versus “low dose” CYC in lupus patients with proliferative nephritis who were then switched to maintenance therapy with AZA65.

Another study that employed sequential therapy, “The EULAR randomized controlled trial of pulse cyclophosphamide and methylprednisolone versus continuous cyclophosphamide and prednisolone followed by azathioprine and prednisolone in lupus nephritis” (67), suggested that there were no significant differences between these regimens; however, enrollment was difficult and only 32 patients were treated. The authors encountered cytopenias in the 2 mg/kg oral CYC group and concluded that, “the initial dose of 2 mg/kg oral cyclophosphamide was felt by the investigators to be too toxic to persist with. The intermittent intravenous pulse regimen appears to be better tolerated than oral continuous treatment, with less severe adverse effects.”

These studies provide examples of regimens utilizing sequential therapy, but because the regimens used for induction differed and the treatments were not compared with traditional treatment with monthly followed by quarterly CYC, it is not possible to compare these maintenance regimens with each other or with quarterly IVC.

For a summary of these studies involving sequential therapy for lupus nephritis, please refer to Table 61-4 .

Intravenous Cyclophosphamide in Nonrenal Lupus

In general, severe nonrenal manifestations of lupus that result from immune complex disease respond more rapidly and completely to IVC plus corticosteroids than to corticosteroids alone. The required treatment duration and size of individual IVC boluses varies with different disease manifestations. For example, severe transverse myelitis may respond to a shorter course of treatment than severe nephritis, and immune-mediated thrombocytopenia may respond to lower than usual doses. During treatment of severe lupus with IVC, improvement of minor manifestations, including constitutional symptoms, fevers, arthralgias, rash, pleurisy, and serologic abnormalities, and reduced prednisone requirements usually occur within 3 months.

Neuropsychiatric Lupus

No clear guidelines exist regarding therapy for neuropsychiatric lupus (NPSLE) with various modalities, including corticosteroids, CYC, and/or anticoagulation. Distinction between various primary pathogenic mechanisms, such as immune complex–mediated vasculitis, antibody-mediated cerebral injury, microangiopathy, thrombosis, or secondary causes such as atherosclerosis or infection, is notoriously difficult, and is further complicated by the multifactorial etiology of many events. In many cases, skilled physicians must make a “seat-of-the-pants” decision regarding the use of immunosuppression, anticoagulation, or both based on clinical, serologic, or magnetic resonance imaging evidence, unless there is biopsy evidence of tissue inflammation or cerebrospinal fluid pleocytosis. In many series, treatment decisions have been made (apparently appropriately) on the basis of clinical judgment rather than on specific inclusion criteria.

In our hands, active, steroid-refractory cerebral lupus that is adjudged to be secondary to immunologically mediated injury has responded well to IVC with or without bolus methylprednisolone in most cases. Anticoagulation has been used simultaneously when it has been impossible to distinguish thrombotic from inflammatory disease or to rule out the possibility that vascular inflammation is contributing to the development of thrombosis. Neither the presence of antiphospholipid antibodies nor involvement of one or more large vessels rules out use of immunosuppression as opposed to anticoagulation. Boumpas et al. (68) treated nine patients with monthly IVC, three of whom had transverse myelitis and five of whom had focal neurologic findings, seizures, or both. The duration of symptoms ranged from 3 to 45 days. All nine patients had findings suggesting an inflammatory process, including anti-DNA antibodies, and five had cerebrospinal fluid pleocytosis. Five of these patients concomitantly had antiphospholipid antibodies. All patients recovered either partially or completely. These observations suggest that in selected patients who have antiphospholipid antibodies that may not be the major cause of their events, IVC administration is associated with clinical improvement.

Other series of IVC in NPSLE report favorable results. Neuwelt et al. (69) retrospectively reviewed 31 patients with neuropsychiatric lupus who were treated with IVC and who had failed a variety of prior therapies, including corticosteroids, Coumadin, chlorambucil, and azathioprine.

Indications included organic brain syndrome in 55% of patients, strokes in 35%, peripheral mononeuropathies in 32%, seizures in 29%, and transverse myelitis in 16%. Patients with anticardiolipin antibodies were treated with warfarin. Treatment regimens varied from low to high doses of IV cyclophosphamide, and plasmapheresis was added to some patients when they appeared not to improve after IVC. Overall, 61% of patients were reported to improve, of whom 26% were not initially improved after 9 months of therapy and appeared to respond to addition of plasmapheresis. The failure rate for patients with organic brain syndrome was 83%, compared with 37% for other indications. Malaviya et al. (70) treated 14 patients with a variety of focal and diffuse neurologic deficits. All patients except the two with seizures stabilized or improved.

Numerous studies have demonstrated improvement of transverse myelitis with IVC, with or without bolus corticosteroids (71 ,72). Because of the catastrophic nature of transverse myelitis and the importance of prompt therapeutic intervention, it may be appropriate to have a very low threshold for prompt institution of IVC when this syndrome appears suddenly, with or without concomitant high-dose (e.g., 1 g/m^2) methylprednisolone. In our institution, prompt use of bolus CYC for transverse myelitis has been associated with preservation of the ability to ambulate in most patients, although many have continued to have neurogenic bladders.

Ten patients with bilateral corticosteroid-refractory optic neuritis and severe visual compromise were treated with bolus IVC for 6 months (73). Ten of 20 eyes recovered completely, six partially, and four did not.

Baca (74) treated seven children with NPSLE (including seizures, focal neurologic deficits, transverse myelitis, and organic brain syndromes) with monthly bolus CYC combined with three initial boluses of 30 mg/kg methylprednisolone. Three patients had anticardiolipin antibodies but were not anticoagulated. Six patients recovered completely and one had a minor residual deficit.

Other Disease Manifestations

Numerous corticosteroid-refractory manifestations of lupus have been reported to benefit from pulse CYC in case reports and uncontrolled series, including systemic vasculitis, gastrointestinal vasculitis and pneumatosis intestinalis (75). Hematologic conditions reported to respond include aplastic anemia (76 ,77), acquired factor VIII deficiency (78), and acquired von Willebrand disease (79), as well as lupus-induced cytopenias, particularly thrombocytopenia. Thrombocytopenia in active lupus possibly may respond to lower pulses of CYC than are necessary to control other disease manifestations. Roach and Hutchinson (80) successfully treated steroid-refractory thrombocytopenia on two occasions in one patient with only 400 mg of IVC. Boumpas et al. (81) found overall improvement of thrombocytopenia in patients who were treated according to the NIH protocol. Although IVC should not, in our opinion, be substituted for plasmapheresis and plasma exchange in thrombotic thrombocytopenic purpura (TTP) in patients with lupus, it has been added to plasma exchange for this indication (82 ,83). Bolus CYC alone may be ineffective for lupus-related TTP; two of our patients developed TTP during monthly bolus CYC therapy for nephritis, and, despite prompt addition of plasma exchange, one patient died and the other progressed to renal failure.

Several studies suggest that lupus-related interstitial lung disease and bronchiolitis obliterans may respond to monthly CYC (84 ,85). Fukada et al. (86) also noted a response of pulmonary hemorrhage to IVC. In our experience, IVC is associated with control of pulmonary hemorrhage in patients with lupus who appear to be steroid refractory in the majority of cases. Although cases of idiopathic inflammatory myositis have not uniformly responded well to IVC in the published literature, three patients with SLE were reported by Kono et al. (87) to have remission of refractory polymyositis with the addition of IVC.

In summary, these nonrenal manifestations of lupus appear to respond to IVC in most cases when steroid therapy had apparently failed. These results do not establish the superiority of IV over oral CYC for these indications, however.

Bolus Cyclophosphamide in Children

IVC has been used successfully in children of all ages, including infants (88). Studies by Lehman and Onel (89) of treatment with IVC for 36 months have shown good disease control and arrest of progression of the chronicity index. This group has also added intravenous methotrexate to IVC in refractory patients with benefit (90). It is our opinion that daily oral CYC is clearly less desirable in children with lupus, as it is in adults, because of its toxicity, and that it should not be used as first-line therapy instead of bolus CYC.

Combination therapy for severe lupus with synchronized plasmapheresis and CYC has been proposed by Euler et al. (91 ,92). The rationale for this mode of therapy is that the depletion of circulating autoantibodies may stimulate increased activity of lymphocytes and plasma cells that are responsible for immunoglobulin formation, resulting in increased susceptibility to CYC. Two regimens have been proposed, one of which involves performing a single plasmapheresis before each of a series of monthly pulses of CYC. A controlled trial did not show significant benefit from adding plasmapheresis to IVC. The second, more intensive protocol includes daily plasmapheresis on three successive days of 60 mL/kg, followed on days 4, 5, and 6 by three pulses of 12 mg/kg of IVC. Patients are then treated over a 6-month period with escalating doses of oral CYC and 1 mg/kg/ day of prednisone that gradually is tapered. This treatment regimen is highly toxic even when GCSF is employed to treat leukopenia, and deaths have resulted. However, in a patient population with mixed indications for therapy including pneumonitis, nephritis, retinal vasculitis, and pericarditis, there was marked overall reduction of disease activity in

12 of 14 treated patients, and at final assessment, eight patients were reported to be clinically well for a mean follow-up time of 6 years. Seven patients developed amenorrhea. This highly complex protocol appears to achieve satisfactory results in most patients when in expert hands, but it has been extremely toxic (92 ,93 ,94 ,95). It may represent an intermediate intensity of treatment between conventional and high-dose cyclophosphamide regimens.

Aggressive Cyclophosphamide-Containing Regimens

High-dose CYC regimens sufficient to arrest production of hematopoietic cells are being tried in lupus. Because stem cells are resistant to CYC, the marrow recovers after a period requiring support with cells and colony stimulating factors. Brodsky et al. (96) reported treating patients with severe SLE with 200 mg/kg CYC with complete responses in half the patients. There were no deaths. Another study by Petri et al. examined the effect of high dose CYC in a group of 14 lupus patients ages 21 to 45 who had failed prior immunosuppressive therapy (5 of 14 had prior CYC) (97). In this population, 9 of 14 had active renal disease, of whom 5 had diffuse proliferative glomerulonephritis and 4 had membranous disease. The serum creatinine levels ranged from 0.4 to 2.0, and SLEDAI scores reflected a range of lupus activity: 5 patients had scores of 0 to 4, 5 patients had scores of 5 to 8, and 4 patients had scores of 10 to 16. Treatment in this protocol was with CYC 50 mg/kg × 4 days, followed by granulocyte colony stimulating factor six days after the last dose of CYC. Results of the trial revealed that a complete renal response was achieved in 5 patients, a partial response in 7 and no response in 2, one of whom had renal failure. SLEDAI score significantly improved (pretreatment average score was 6.8, posttreatment average score 2.7), and prednisone doses were also significantly decreased in the posttreatment group (20 mg to 5 mg). This open trial suggests that this regimen is efficacious but does not allow comparison with alternate regimens such as monthly bolus CYC plus methylprednisolone or MMF.

Autologous stem cell transplantation utilizing high-dose CYC with or without additional immunosuppressives is being evaluated in a number of rheumatic diseases. One study reported enrollment of nine patients in a protocol (98), of whom one died during induction and seven ultimately underwent transplantation. Fluid overload occurred in all patients; three required dialysis or hemofiltration and two were intubated. All patients responded clinically and were able to discontinue immunosuppressive medications. The dramatic disease suppression reported appears to exceed that of high-dose cyclophosphamide regimens. However, the short-term toxicity appears to be greater. Across the world the mortality of stem cell transplantation for rheumatic diseases exceeds 10%. This figure may improve with modifications of treatment regimens and criteria for patient selection.

Summary of Cyclophosphamide Therapy for Lupus

- There is no evidence that IVC is more effective than oral CYC in patients with lupus in the long run, but it is unquestionably less toxic.
- Prednisone has been employed with oral or IVC in all studies showing efficacy. Daily oral CYC without prednisone was not effective in a single controlled study.
- Addition of bolus methylprednisolone to monthly bolus CYC for lupus nephritis has the potential to improve efficacy with only modest increased risk of toxicity.
- The cumulative dose of CYC predicts the risk of gonadal injury and risk of secondary malignancies. Meticulous surveillance for malignant and premalignant conditions (especially those resulting from human papilloma virus [HPV]) is indicated in CYC-treated patients.
- Failure of active lupus to respond to IVC does not imply that the disease manifestation is inherently untreatable. If the deterioration is clearly a result of active lupus nephritis, addition of bolus methylprednisolone, which has proven to be beneficial in a controlled trial, addition of another immunosuppressive to IVC, high-dose CYC or stem cell transplantation, may be appropriate. In the near future, use of biologic agents in this situation is likely to become the norm.
- Approximately 80% of patients with nephritis respond to IVC treatment after 6 monthly pulses, of whom at least 20% will flare if the frequency of IVC is reduced to every 3 months and continued for an additional 2 years. If no immunosuppression is given after the first 6 months, the flare rate approaches 50%. Alternatively, maintenance therapy with AZA or MMF may provide equivalent or superior protection against flares with reduced toxicity. In the event of a partial but unsatisfactory response, continued monthly treatment after initial 6 months may be successful. Sequential therapy should be considered in all patients receiving CYC as induction therapy for lupus nephritis after 6 months.
- Proteinuria and features of nephrotic syndrome usually improve substantially during the first 6 to 12 months. Patients who are not in complete remission after 6 months (e.g., proteinuria reduced but not yet <1 g) may have continued reduction of proteinuria during less intensive therapy. Patients who fail to achieve remission after 6 months have a worse prognosis and presumably require more aggressive therapy than patients who have achieved remission.
- Patients with renal insufficiency average an approximately 30% improvement in creatinine clearance during the first 6 months, but tend to backslide toward baseline values after 1 to 2 years. It is interesting to speculate that this may result from continued glomerular scarring in the apparent absence of inflammation, as described by Chaghac in total lymphoid irradiation-treated lupus nephritis patients (99). However, it is possible that inflammation recurs as immunosuppression is reduced or that other processes

such as occult hypertension and hyperfiltration may be important factors.

- The indications for IVC therapy for neurologic disease are poorly characterized. The possibility that an antiphospholipid antibody syndrome exists is not in itself a contraindication to the treatment of apparent inflammatory disease. In catastrophic neurologic disease in which the etiology is unclear, combined anticoagulation and immunosuppression should be considered.

Nitrogen Mustard

Nitrogen mustard (mechlorethamine) is a highly potent alkylating agent with prolonged immunosuppressive and immunomodulatory effects. Although it is infrequently used at the present time, a detailed review is in order because it appears to be quite efficacious in severe lupus and may have different properties than IV cyclophosphamide. For instance, studies in patients with rheumatoid arthritis show that nitrogen mustard, when administered intravenously, is effective in suppressing disease activity, whereas IV cyclophosphamide given monthly probably is not.

Nitrogen mustard is administered in its active form and has potent caustic, immunosuppressive, and carcinogenic effects on exposed tissue. These properties are responsible for the high incidence of thrombophlebitis and local irritation at IV infusion sites, as well as for the risk of severe tissue necrosis if the drug is extravasated. These effects also occur during topical application and are responsible for the reports of successful therapy for both cutaneous lupus and other cutaneous diseases, such as mycosis fungoides. Topical application of nitrogen mustard is associated with the induction of primary or secondary malignancies at the application site.

Nitrogen mustard is considered to be a more potent stimulant of nausea and vomiting than cyclophosphamide. Reviews that deal with this side effect generally were written before the introduction of highly effective antiemetic agents or antiemetic combinations, such as the combination of ondansetron, lorazepam, diphenhydramine, and dexamethasone. Judicious use of these agents when coupled with vigorous hydration, both immediately after drug administration to reduce local tissue damage effects and during the period of time that nausea and vomiting may occur, could considerably reduce the immediate toxicity of this drug.

The bone marrow effects of nitrogen mustard differ from those of cyclophosphamide. Hematologic toxicity of nitrogen mustard following the administration of a single course of this drug has been reported to be relatively modest by Dubois (100), who treated patients only after a bone marrow examination to rule out bone marrow hypocellularity. In this setting, the nadir of the leukocyte count is said rarely to fall below 2,500 cells/mL, although the period of leukopenia is far longer than that associated with cyclophosphamide, on the order of 6 weeks. This appears to result in part from the increased toxicity of nitrogen mustard to stem cells. Although reports of secondary hematologic malignancies in nitrogen mustard–treated patients with lupus are rare, patients who were treated with nitrogen mustard for hematologic diseases have an increased incidence of secondary hematologic malignancies, including myelodysplastic syndromes. The rarity of such reports in patients with connective tissue disease may relate in part to the lower cumulative doses that usually are administered in this population, which may relate in turn to the cumbersome nature of this therapy.

Nitrogen mustard is given as a course of approximately 0.3 to 0.4 mg/kg (15 to 20 mg), either as a single IV dose administered in a large amount of fluid over 1 hour and followed by vigorous hydration, or as two divided doses given 12 hours apart. In some patients, courses have been repeated as often as every 6 weeks.

Clinical responses to nitrogen mustard have been observed as rapidly as within several days, and they are more protracted than those to IV cyclophosphamide. During a period when the prognosis of lupus nephritis was poor, outcomes were in general better than those obtained with prednisone alone, although they were worse than those obtained today with improved supportive care and adjunctive agents (Table 61-2). It therefore is difficult to evaluate nitrogen mustard in the context of modern therapeutic adjuncts.

In 1954, Dubois (100) reported the treatment of 33 patients with 20 mg of IV nitrogen mustard. He initially treated most patients with steroids or antimalarials, only adding nitrogen mustard after 2 months of unsuccessful therapy with prednisone. Twenty-six of the 33 patients had renal disease. Interestingly, none of the patients who did not have nephritis benefited from treatment; 13 of 16 nephrotic and 7 of 10 nonnephrotic patients with renal disease improved. In many cases, Dubois observed diuresis within a few days in patients with nephrotic syndrome. Long-term survival was greater in those patients with nephritis who responded to the treatment. Wallace et al. (101) reviewed the therapy of nephritis in the same practice, including 44 patients receiving 74 courses of nitrogen mustard. After 40 days, improvement of multiple parameters, including urinary sediment, serum cholesterol, steroid dosage, serum creatinine, and urine protein excretion was noted (Table 61-2). The response rate again was noted to be higher in patients with nephrotic syndrome. Favorable responses to one or two courses were said to have lasted for years in some patients.

In 1973, Dillard et al. (102) described the treatment of 17 patients with diffuse proliferative glomerulonephritis using nitrogen mustard. Five patients died of renal failure, four within 6 weeks. The remaining 12 patients improved, seven of whom had no exacerbation of renal disease after a mean follow-up of 33 months. All were reported to have normal renal function and protein excretion at follow-up. Nitrogen mustard was felt to be steroid sparing and to be associated with reduction of the level of activity in serial renal biopsies, although the severity (i.e., chronicity) score was not reduced by therapy. Results were said to be unsatisfactory in patients with high levels of both disease activity and scarring.

Wallace and Metzger (103) reported that administration of one or two courses of nitrogen mustard, 0.4 mg/kg,

resulted in improvement of disease activity in two patients with severe nephritis and nephrotic syndrome who failed to respond to monthly IVC, one of whom had failed 32 prior courses of IVC. Topical application of nitrogen mustard has been advocated in severe cutaneous lupus; however, this can result in local induction of malignancy (104).

Summary of Nitrogen Mustard Therapy for Lupus

The role of nitrogen mustard in the management of severe lupus is unclear at present. In patients with nephritis, it appears to offer the potential to dramatically reduce severe disease activity for several months after a single intervention, perhaps in patients who have failed cyclophosphamide or pulse steroid therapy.

Chlorambucil

Chlorambucil is an alkylating agent that differs from nitrogen mustard because it does not produce local irritative effects, and from cyclophosphamide because it is not metabolized to acrolein and does not cause hemorrhagic cystitis. In patients with lupus, it is administered orally in daily doses of from 2 to 12 mg. It has been used as an IV bolus in patients with multiple sclerosis (105) or sarcoidosis (106). Chlorambucil is toxic to dividing cells (107), but it also may have immunomodulatory effects. In idiopathic membranous nephritis, reduction of the number of T cells and reduction of the $CD4^+/CD8^+$ ratio resulted from alternating monthly oral prednisone and oral chlorambucil (108). Monthly pulse chlorambucil in multiple sclerosis was reported to disproportionately reduce circulating B cells (105). The dose, rate of administration, and underlying disease probably alter the observed effects of chlorambucil on immune function, as is the case with cyclophosphamide.

Side effects of chlorambucil that may differ from cyclophosphamide include both marrow suppression and malignancy. Recovery of stem cells is less rapid than after cyclophosphamide administration. Published series suggest that substantial difficulty is encountered during treatment resulting from suppression, which may be irreversible, of individual circulating cell lines (red cells, leukocytes, and platelets) (109).

Chlorambucil is a potent oncogen. Its oncogenicity relative to cyclophosphamide is controversial; however, it is in our opinion more dangerous than IVC. Somatic and germ cell mutations (107 ,109), leukemias, myelodysplastic syndromes, and cutaneous malignancies are increased. Of 144 patients with polycythemia vera who were treated with chlorambucil, 11% developed leukemia after 5.4 years, representing a 13.5-fold increase over patients treated with phlebotomy alone (110). Patapanian et al. (111) identified significant excess malignancy compared with controls in 39 patients with rheumatoid arthritis after 5 years; three hematologic and eight cutaneous malignancies were identified.

Studies of this potentially useful agent are too limited to permit comparison with other immunosuppressive drugs. However, it almost certainly is effective against lupus when combined with prednisone.

In 1973, Snaith et al. (112) reported improvement of steroid-resistant nephritis in five of five patients and of active lupus in a sixth using chlorambucil, 2 to 5 mg/day. Amenorrhea developed in four patients. In 1974, Epstein and Grausz (113) reported improved survival in 16 of 31 patients with lupus and diffuse proliferative nephritis who received chlorambucil in addition to prednisone, but they also reported serious toxicity, including marrow aplasia in five patients, leading to one death. Egypt, Sabbour and Osman (114), in a retrospective study of a group of patients with very high mortality (58% over 5 years), found prednisone plus chlorambucil to be associated with better survival than prednisone alone or prednisone plus oral cyclophosphamide. Survival and reduction of proteinuria were markedly better in chlorambucil-treated patients than in other groups; however, the chlorambucil-treated patients were treated much more recently than other groups

Summary of Chlorambucil Therapy for Lupus

There has been little recent work with chlorambucil in lupus; however, it has been extensively studied in idiopathic membranous and proliferative nephritis and nephrotic syndrome. Regimens containing chlorambucil generally have been felt to be effective, and in one study they were found to be superior to monthly pulse cyclophosphamide (115 ,116).

Azathioprine

Azathioprine (AZA) has been in use for more than 50 years for organ transplantation and to treat autoimmune diseases. Although less potent and slower in onset of efficacy than CYC as treatment for acute severe autoimmune disease, AZA is being recognized both as a steroid sparing agent and as a “maintenance” drug to be used after initial control of severe systemic autoimmune diseases with CYC.

AZA is inactive as administered and is metabolized intracellularly to the purine antagonists 6-mercaptopurine (6MP) and 6-thioinosinic acid. The immunologic effects of AZA and 6MP differ despite the fact that 6MP is the major active metabolite of AZA, suggesting that additional metabolites of AZA may also be active. AZA reduces the numbers of T cells, B cells, and natural killer cells, thereby inhibiting both cellular and humoral immunity, suppressing autoantibody formation and inhibiting prostaglandin synthesis.

In contrast to cyclophosphamide, AZA has not been extensively studied in the past decade as an initial therapeutic agent in lupus nephritis. Recent studies have emphasized the potential role of AZA as a maintenance drug after induction of remission in lupus nephritis. No one has yet demonstrated that MMF, which is viewed by many rheumatologists

as the most promising conventional immunosuppressive drug for lupus nephritis, is superior to AZA for maintenance of remissions.

The caveats that apply to older studies of nephritis (i.e., potentially confounding effects on renal survival of differences in management of hypertension, use of angiotensin-converting enzyme [ACE] inhibitors or other measures to prevent progression of renal disease) apply to the evidence regarding AZA as a first-line agent (Table 61-1). The following controlled studies of azathioprine have yielded disparate results, suggesting that azathioprine is effective in some, but not all, patients.

In the large NIH trial, low-dose azathioprine added to low-dose cyclophosphamide plus prednisone was as effective as IV cyclophosphamide (administered every 3 months) plus prednisone, with comparable mortality and toxicity (49 ,50 ,117). Compared with oral cyclophosphamide, renal survival was the same, but there was a trend that failed to reach statistical significance for the combination regimen to be associated with lower mortality. Thus, azathioprine appears to have a cyclophosphamide-sparing effect when used in combination with that drug. Overall outcomes in the NIH study of azathioprine alone plus prednisone (without cyclophosphamide treatment with azathioprine) were intermediate between prednisone and cyclophosphamide-containing regimens and failed to achieve significance, although they were better than prednisone alone. In our opinion, the slow onset of action of AZA may be partially responsible for its failure as initial therapy for active nephritis, and the studies below do not necessarily suggest that it will not be effective as an agent either in early relatively mild nephritis or as a maintenance agent after initial control of severe nephritis has been achieved.

Donadio et al. (118) randomized 16 patients to azathioprine plus prednisone versus prednisone alone. After 6 months, there was improvement in histologic measures of disease activity (i.e., karyorrhexis, proliferation, fibrinoid deposition, hyaline thrombi, necrosis) in both groups, but there was no difference in outcome after 6 months or after 2 to 3 years (119). Hahn et al. (120) randomized severely ill patients with lupus to prednisone with or without azathioprine over a 2-year period and found no differences in outcome. However, patients with nephritis entered into the azathioprine group were more likely to have severe renal disease with diffuse proliferative glomerulonephritis on biopsy, possibly resulting in underestimation of the efficacy of this drug.

In a study that illustrates the difficulty of distinguishing the toxicity of one drug regimen from the efficacy of alternate therapy, Cade et al. (121) used four different regimens to treat 50 patients with lupus, including prednisone alone, prednisone plus azathioprine, azathioprine alone, and azathioprine plus heparin. Unfortunately, 13 of 15 prednisone-treated patients died, with a mean survival of 19 months, after receiving 60 to 100 mg of prednisone daily for 6 months. In the azathioprine plus prednisone group (which received lower doses of prednisone), 9 of 13 patients survived, with a mean survival of 38 months. Compared with the very high-dose prednisone group, azathioprine alone or in combination with either prednisone or heparin produced superior results. In a double-blind, crossover trial, Ginzler et al. (122) compared azathioprine plus prednisone with prednisone alone and found no benefit.

Recent studies suggest that azathioprine may be most useful either in early stages of nephritis to prevent development of more severe lesions or as maintenance therapy after IVC (as noted earlier). Esdaile (123) conducted an elegant study in the 1980s at Yale of patients who received immunosuppression for lupus nephritis. Almost all immunosuppressed patients were treated with azathioprine. When patients who received early biopsies and treatment were compared with those who had delayed biopsies and treatment, there was strikingly increased preservation of renal function and reduced mortality in the early treatment group. These patients, who were less sick than those reported in earlier trials described above, appeared to be more responsive to treatment with azathioprine, suggesting that there may be a role for immunosuppressives that are safer than IVC when new-onset, relatively mild nephritis is diagnosed. In our experience, however, some AZA-treated patients develop progressive renal disease that responds dramatically to the discontinuance of azathioprine and institution of IVC.

Azathioprine has been used for a variety of nonrenal indications in active SLE. During a controlled trial in patients with active nonrenal lupus, Sztejnbok et al. (124) added AZA 2.5 mg/kg/day to prednisone in half the patients. Azathioprine was reported to be unhelpful in controlling acute disease but to be steroid sparing and reduce mortality. A study randomizing patients with well-controlled lupus to continuation or withdrawal of azathioprine has demonstrated more exacerbations in patients who discontinued the drug (125).

Azathioprine has been reported to be effective in severe cutaneous lupus in several series (126 ,127 ,128 ,129) and to have a steroid-sparing effect. It has been reported to be useful in treating chronic active hepatitis complicating lupus, as well as nonvirally mediated chronic active hepatitis in non-lupus patients. The relatively slow onset of action as well as the lack of dramatic responses of disease activity to this drug mandate consummate clinical judgment on the part of the treating physician when decisions are made regarding whether use of this agent has been effective.

Two recent studies that have highlighted the potential role of AZA as a maintenance agent are reviewed in the section on combined therapies.

Summary of Azathioprine Therapy for Lupus

Azathioprine appears to be less effective than either cyclophosphamide or nitrogen mustard in arresting active, very rapidly progressive nephritis, but it may be effective in both early nephritis and as a maintenance drug after IVC. It appears to be both corticosteroid and cyclophosphamide

sparing. Although slow in onset of action, AZA is a very useful agent in mild to moderately severe SLE. Recent important studies utilizing AZA to maintain remission after induction of remission with IVC in patients with lupus nephritis are described in the section on “Sequential Therapy for Lupus Nephritis .”

Cyclosporine

Cyclosporine (CS) has complex immunologic effects, predominantly inhibition of T cell gene activation, transcription of cytokine genes, and lymphokine release. Additionally, it inhibits the recruitment of antigen-presenting cells and antigen presentation. It is unclear which, if any, of these effects is relevant to the pathogenesis of SLE, except perhaps high-level control of T cell activation of antibody production (130). CS is administered orally or intravenously, with marked variation in bioavailability after oral dosage. Absorption requires formation of an emulsion with bile and can be altered by a variety of gastrointestinal conditions, including diarrhea, malabsorption, and delayed gastric emptying. The drug is highly lipophilic, and levels may be increased in patients with hypocholesterolemia. It is eliminated by cytochrome P-450 with formation of multiple metabolites and excreted in the bile. CS levels are influenced by numerous medications, including rifampin, phenytoin, phenylbutazone, and phenobarbital, which decrease concentrations; and calcium channel blockers, progesterone and macrolide antibodies, and ketoconazole, which increase concentrations. As a consequence, monitoring CS levels is recommended at currently employed doses.

A major adverse effect is nephrotoxicity. Reduction of glomerular filtration may be underestimated because of compensatory hyperfiltration and the increasing contribution of tubular secretion of creatinine to the measured creatinine clearance as renal function declines (131). This side effect appears to be dose related, but it is not completely absent even in studies using doses as low as 2 mg/kg. In a population of 192 adults and children, including 152 with diabetes, who were treated with CS for a mean of 13 months before biopsy, 41 had biopsies that were consistent with CS-induced nephropathy (132). There is an association of nephropathy with maximal dose, mean dose, and cumulative dose before biopsy, but not with blood levels. Deray et al. (133) evaluated 16 patients with autoimmune uveitis who were treated with CS, 5 mg/kg/day initially. CS was adjusted according to the serum creatinine. There was a progressive decline of creatinine clearance throughout the study from the baseline of 120 mL/minute to 75 mL/minute at 24 months. The glomerular filtration rate (GFR) decreased from 116.8 to 75.3 mL per minute. There was a significant increase in total cholesterol levels. Altman et al. (134) treated patients with rheumatoid arthritis sequentially with the following regimens: first with a nonsteroidal anti-inflammatory drug (NSAID), then with CS, 5 mg/kg, then with both. At the end of the study, there was a significant increase in blood urea nitrogen and creatinine levels in 9 of 11 patients, and an additive effect of the two drugs was postulated. This side effect, in our opinion, is the major potential limiting factor in its use in SLE.

Compared with alkylating agents, bone marrow suppression is uncommon. Lymphoproliferative syndromes frequently are observed in CS-treated patients with organ transplants, but they are rare in patients with autoimmune disorders. CS appears to have little, if any, ovarian toxicity and has been employed in a limited number of pregnancies without obvious birth defects. Hypertension has been observed in 50% to 80% of transplant recipients. CS impairs the excretion of potassium, uric acid, and magnesium, and it is a notorious cause of refractory gout. It can cause hypomagnesemia and has been implicated in CNS toxicity, including headache, tremors, and occasionally, focal neurologic defect. Hirsutism, gingival hyperplasia, and gastrointestinal disturbances may occur.

Application of CS in SLE has been the subject of only a few controlled trials. Balletta et al. (135) randomized ten patients with lupus nephritis to either CS, 3 mg/kg/day, plus prednisone or to prednisone alone. After 12 months, there was no significant change in creatinine or creatinine clearance, but there was a reduction in proteinuria in the CS-treated group. In the CS-treated group, the proteinuria declined from 2.7 to 0.3 g per 24 hours, whereas in the prednisone-alone group, proteinuria increased from 2.1 to 2.6 g per 24 hours.

In an open randomized trial, Fu et al. (56) treated 40 children with World Health Organization (WHO) class III or IV lupus nephritis with either CS (2.5 to 5 mg/kg) alone (without corticosteroids) or prednisolone 2 mg/kg plus cyclophosphamide 2 mg/kg orally (P + C) for 1 year. At entry, all children had growth retardation following more than 1 year of corticosteroids. Subjects received an intense regimen of corticosteroids just prior to randomization until lupus activity diminished. There was comparable control of disease activity and resolution of proteinuria. Hemolytic complement (CH_{50}) and C3 levels were actually lower at the end of treatment, however. The authors concluded that CS controlled clinical but not serologic activity. The alternate regimen, although quite toxic, was probably effective, suggesting that CS as monotherapy also had some activity in order to achieve comparable results.

A number of open trials have suggested efficacy, using 2.5 to 10 mg/kg CS. Favre et al. (136) treated 26 lupus nephritis patients with CS 5 mg/kg/day, adjusted to achieve through levels of 250 to 350 mg/mL, plus prednisone, reporting a reduction of disease activity and proteinuria compared with baseline after 2 years. They reported an overall reduction of disease activity, stable serum creatinine levels, and slightly improved GFR, with striking reduction of proteinuria. By their estimation, activity was reduced on biopsy, and chronicity was slightly improved. In 1986, Feutren et al. (137) added CS, 5 to 10 mg/kg/day for 9 to 18 months, to prednisone in nine patients with systemic disease, and they reported apparent benefit in six patients and reduction of mean prednisone dose from 0.8 to 0.3 mg/kg/dy without changes in DNA antibody titers or complement C4 levels. Proteinuria declined

from 1.6 to 0.9 per 24 hours. In another trial, Isenberg et al. (138 ,139) treated five patients with a relatively large dose, 10 mg/kg/day, of CS, but these patients failed to respond, except for improvement of arthralgias in two. There were complications, including angioedema associated with the decline of Cl esterase inhibitor levels (139), in three patients, increased serum creatinine in three, and a sensation of being unwell in all patients. The study was terminated as a result.

Radhakrishnan et al. (140) treated ten lupus patients with membranous nephropathy, complicated in three cases by mesangial lesions, with CS, 4 to 6 mg/kg/day. Serial biopsies revealed an increase in the chronicity index and an increase in the stage of the membranous nephropathy, which is somewhat analogous to the chronicity index that is applied in proliferative disease. Several patients had reduction of proteinuria.

Tokuda et al. (141) treated 11 women with lupus with initial CS doses of 3 mg/kg/day. Hypertension occurred in 40%, and modest clinical improvement was observed.

Hussein et al. (142) treated five women with lupus nephritis, two of whom were pregnant, using CS plus low-dose prednisone. They noted stable renal function in all but one patient and hypertension in three.

In an open trial, Caccavo et al. (143) treated 27 lupus patients who had an unsatisfactory response to either corticosteroids alone or corticosteroids plus an immunosuppressive for 24 months with an initial dose of CS 2.5 to 5 mg/kg. Disease activity, minor manifestations, cytopenias, and neurologic disease improved. Proteinuria was reduced, and the mean serum creatinine increased from 0.89 to 0.96 mg/dL (p = NS).

Dostal et al. (144) treated 11 patients with nephritis with CS, 5 mg/kg/day. After 1 year there was improvement of proteinuria, slight reduction of mean serum creatinine levels, and reduction of the mean Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) from 26 to 4. Hypertension occurred in seven patients. At repeat biopsy, inflammation improved, and “chronic” changes were increased in five patients and reduced in three.

Tam et al. (145) administered CS 5 mg/kg/day to 17 patients with class IV nephritis, of whom 12 completed 24 months of treatment. (Three of the remaining patients had azathioprine added.) Reduction of proteinuria was gradual, with only partial resolution at 1 year and further improvement in the subset of patients who reached year 5. Biopsy findings were of interest: no patient had evidence by repeat biopsy of persistence of inflammation at 1 year. There was no increase in chronicity (mean chronicity index 5.1 at baseline vs. 5.7 at 1 year).

Morton and Powell (146) reviewed treatment of mostly nonrenal lupus in 43 patients with CS (mean 4.1 mg/kg/day). Hypertension occurred in 14 patients, and a rise of creatinine more than 30% in nine. The drug was stopped in 39 of 47 treatment courses because of toxicity or lack of efficacy. Hallegua et al. have suggested that CS is helpful in membranous nephritis (147).

Cyclosporine has been reported to be well tolerated in pregnancy (142), useful when added to cyclophosphamide for nephritis (148), and has been combined with immunoadsorption in pregnancy (149).

Summary of Cyclosporine Therapy for Lupus

Although the mechanism by which cyclosporine may alter the course of lupus is uncertain, it is likely that it favorably affects some manifestations of lupus, such as proteinuria, over the relatively short period of 1 to 2 years, and may reduce overall disease activity. The outcome of studies in membranous nephropathy will be of great interest. In both controlled and open trials, glomerular filtration has not improved, as would be expected in bolus cyclophosphamide-treated nephritis patients, and some patients have developed hypertension and reduced GFR. Stabilization of GFR might reflect amelioration of nephritis combined with mild toxicity from the drug. Experience with other immunosuppressives (e.g., prednisone, daily cyclophosphamide) emphasizes the importance of long-term follow-up in determining risk versus benefit. CS still does not have the track record of successful long-term use in lupus shared by cyclophosphamide and azathioprine, and is better suited to empiric use when irreversible injury is not imminent.

Methotrexate

Methotrexate (MTX) is a folate antagonist, which when administered at the doses used in rheumatic diseases has less obvious immunosuppressive effects than alkylating agents or azathioprine. In fact, it may act by other means. There is no convincing association of changes in lymphocyte subsets, surface markers, lymphocyte function, or autoantibody levels with therapeutic effects on rheumatic diseases. It appears to have multiple anti-inflammatory effects including increased adenosine levels at sites of inflammation, inhibition of leukotriene B2 formation, interleukin-1 (IL-1) effects, fibroblast proliferation, and preferential cyclooxygenase-2 inhibition (150 ,151). Other putative mechanisms of action include inhibition of neutrophil function, interference with the action of IL-1, suppression of lipoxygenase formation, and inhibition of the intracellular enzyme 5-aminoimidazole-4-carboxamide ribonucleotide transformylase (152). It is unclear which mechanisms of action are important in lupus treatment.

Side effects of MTX, particularly hepatotoxicity and cytopenias, are well known. Selected aspects will be reviewed. MTX toxicity is increased in patients with depressed renal function, and increases in patients maintained on a stable dose of MTX who sustain a decline in renal function (e.g., from active nephritis) (151 ,153). Side effects occur less frequently in rheumatoid arthritis patients given 1 mg/day of folate, and drug efficacy (against rheumatoid arthritis) appears unaltered. MTX increases homocysteine levels and cardiovascular risk in lupus patients, an effect that is also reduced by simultaneous administration of folate (142 ,143 ,144 ,145 ,146 ,148 ,149 ,150 ,151 ,153 ,154 ,155).

Pulmonary toxicity, which has been reported in 2% to 7% of patients with rheumatoid arthritis, is characterized by cough, bilateral or unilateral pulmonary infiltrates, and dyspnea (156 ,157 ,158 ,159 ,160). There is a nonspecific interstitial inflammatory cell infiltrate and an increased number of T cells on bronchoalveolar lavage (158). Reported mortality is 17%, with a 50% recurrence rate on rechallenge and up to 50% mortality associated with recurrences. Pneumocystis prophylaxis for patients receiving MTX plus moderate- to high-dose corticosteroids should reduce the number of episodes of PCP pneumonitis requiring distinction from MTX pneumonitis. MTX is teratogenic and an abortifacient (161 ,162). Rheumatologists have been shown to inadequately identify and address the need for contraception in women taking MTX (163). MTX-induced malignancies appear to be rare. A reversible lymphoproliferative disease can occur, and has been reported to evolve into Hodgkin disease (164).

Other forms of MTX toxicity, including acute hepatic injury, hepatic fibrosis, stomatitis, and, in high doses, central nervous system toxicity, have been reviewed elsewhere (152).

Methotrexate has gained considerable popularity in the treatment of systemic rheumatic diseases, including rheumatoid and psoriatic arthritis, Wegener granulomatosis (157 ,165), and Takayasu disease (166). In Wegener, the efficacy of MTX appears to be less than that of daily oral CYC (as also is the case for IVC).

In SLE, there is a paucity of data. Two early studies, the treatment of seven patients with 7.5 mg/week for 6 weeks by Dubois (167), and administration of either daily oral MTX, 2.5 mg, or 50 mg of IV MTX weekly by Miescher and Riethmuller (168), produced equivocal results. Rothenberg et al. (169) treated 10 patients with MTX, 7.5 to 10.0 mg weekly, of whom 7 patients improved. Improvement was noted in myositis, rash, pleurisy, arthritis, and proteinuria, but leukopenia was observed in three patients. Wilke et al. (170) treated 17 patients with lupus using a mean MTX dose of 1 g over 8 months. They reported benefit in 57% of patients and toxicity in 70%, with an increase of toxicity associated with use of diuretics or NSAIDs. They noted elevation of liver function tests and gastrointestinal side effects, but not cytopenia. In an open trial in pediatric patients, Abud-Mendoza et al. (171) treated 10 patients with MTX, 5 to 10 mg weekly, in addition to their previous regimens, which in four cases included both prednisone and cyclophosphamide. Of the 10 patients, 2 had a poor outcome, and 5 had excellent responses with discontinuation of cyclophosphamide and other drugs. Wilson et al. (172) treated 12 SLE patients who lacked renal or CNS disease with MTX and noted apparent clinical improvement without change in antibodies to DNA or complement. Corticosteroid dose was reduced in six patients. Hashimoto et al. (173) treated two patients with myositis, fever, and pancytopenia complicating lupus, and they noticed a fall in the number of CD20 positive cells. Walz et al. (174) found MTX to be well tolerated and effective in a review of five patients.

Rahman et al. (175), in a retrospective controlled study, concluded that there was a 60% reduction of the joint count in MTX-treated lupus patients with antimalarial-resistant synovitis versus 12% in the control group. In this study, MTX was not steroid sparing. In a retrospective study, Kipen et al. (176) also concluded that MTX had not been steroid sparing. Gansauge et al. (177), in an open trial of MTX 15 mg/week administered to 22 patients, noted overall improvement.

In a double-blind, randomized, placebo-controlled trial, Carneiro and Sato (178) treated 41 patients with MTX, 15 to 20 mg/week versus placebo. After 6 months, compared with the placebo group, MTX-treated patients had significantly more resolution of arthritis, rash, and hypocomplementemia (the most frequent clinical features at entry), and more improvement of the SLEDAI score. Mean prednisone doses at follow-up were increased in the placebo group and significantly decreased in the MTX group.

Summary of Methotrexate Therapy for Lupus

Methotrexate is relatively safe and well tolerated and helps some lupus patients, particularly those with synovitis. Monitoring of renal function is essential to ensure safety.

Mycophenolate Mofetil

MMF has established itself as a successful immunosuppressive medication in multiple applications. In recent years, MMF has been used in SLE with increasing success and has been the focus of much study. MMF has a unique mode of action that may be particularly applicable to control of SLE. MMF is the morpholinoethyl ester of mycophenolic acid (MPA). MPA was originally isolated from *Penicillium* species in 1896. In the 1960s, MPA was found to have antifungal and anticancer activities. In the 1970s, MPA was studied as a treatment for psoriasis. Although it was effective, poor and erratic absorption along with a high incidence of gastrointestinal toxicity limited further study (179).

MPA is a potent, noncompetitive, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a necessary enzyme in the de novo pathway of purine synthesis. The de novo synthesis pathway is uniquely essential (180) in activated lymphocytes, whereas most other cells use the salvage pathway of purine synthesis. Not only do lymphocytes primarily depend on the de novo synthesis pathway, but activated lymphocytes predominately use the second isoform of IMPDH against which MPA is most specific (181 ,182). Activities of MPA include inhibition of both T- and B-lymphocyte proliferation in vitro in response to mitogenic stimulation (183) and inhibition of antibody formation in humans to horse antilymphocyte globulin (180) and tetanus toxoid (184). Suppression of IgG anti–antithymocyte γ-globulin (ATGAM) antibody production by MMF is greater than that of azathioprine (AZA) (180). MPA also interferes with the production and/or function of adhesion molecules necessary for the migration of lymphocytes to areas of inflammation (185). MMF has also been shown to suppress the induction of the inducible form of nitric

oxide synthetase (iNOS) (186). Studies of various animal models of atherosclerosis and arterial injury demonstrate protective effects of MMF with decreased vessel wall thickening and cellular proliferation (187 ,188 ,189).

MMF is rapidly hydrolyzed to MPA and is 94% bioavailable by the oral route. The drug is reversibly converted in the liver to an inactive glucuronide—mycophenolic acid glucuronide (MPAG)—and excreted into the gastrointestinal tract. Much of MPAG is deglucuronidated by intestinal flora and undergoes enterohepatic recirculation. It is eventually excreted primarily through the kidney (179). Serum drug levels are increased in patients with renal insufficiency. In renal transplant studies, doses of 2.0 g/day or 3.0 g/day were studied with little gain in efficacy but increased toxicity at the 3.0 g/day dose (190 ,191 ,192). A review of patients at our institution suggests that, in practice, rheumatologists are using dosage ranges between 0.75 and 2.5 g/day for the treatment of SLE, and about 50% of patients cannot tolerate 2 g/day (193).

MMF has proven to be a potent antirejection agent in experimental animal models (183 ,194). MMF appears to be effective in experimental autoimmune disease including Heymann nephritis and experimental autoimmune uveitis (195 ,196). Studies in the Medical Research Laboratory lymphoproliferative (MRL/lpr) and New Zealand black (NZB) × New Zealand white (NZW) F1 mouse models of SLE have shown suppression of development of lupus glomerulonephritis, a decrease in glomerular immunoglobulin deposits in MMF-treated mice, and improved survival (197 ,198 ,199).

MMF currently has Food and Drug Administration (FDA) approval for prevention of acute allograft rejection in kidney transplantation and for use in cardiac transplantation. MMF has been widely accepted as a more potent agent than AZA in pediatric and adult transplantation. Three large, multicenter, randomized controlled studies of human cadaveric renal transplants have compared MMF with either AZA or placebo. Findings among the studies showed statistically significant decreases in acute rejection and steroid use, suggesting MMF is superior to AZA or placebo (190 ,191 ,192). Studies of lung, liver, and heart transplant elicited similar results (179). A successful trial of renal transplant utilizing MMF without prednisone was reported (200).

Successful treatments reported in adults with autoimmune diseases include a randomized trial suggesting superiority to or against AZA in Crohn disease (201). MMF has been explored in the treatment of uveitis, rheumatoid arthritis, Takayasu arteritis, psoriasis, pemphigoid, and pyoderma gangrenosum (202 ,203 ,204 ,205 ,206 ,207 ,208).

The role of MMF in the treatment of SLE is expanding. MMF for SLE has been investigated in both uncontrolled and controlled studies for both renal and nonrenal disease. MMF may be valuable as induction therapy of lupus nephritis (LN), potentially replacing CYC as initial treatment for severe LN and may also prove to be useful in the treatment of LN that is not CYC responsive. In addition it is likely that MMF (and/or AZA) will become much more widely used for maintenance of remission following induction therapy with CYC—“sequential therapy.” The uncontrolled studies in SLE have overall shown improvement in LN and other lupus symptoms. Several short-term controlled trials in LN have generally shown MMF to be similar to or even superior to standard treatment.

In 1999, Dooley et al. (209) evaluated 12 patients with relapsing or resistant LN, most of whom had prior IVC therapy, and followed them for a mean of 12.9 months. They found significant improvements in proteinuria and improvement or stabilization of serum creatinine in all patients. Gaubitz et al. (210) studied ten patients with SLE whose systemic and renal manifestations of disease were inadequately controlled on corticosteroids and AZA or CYC. All patients had improvement in clinical disease activity measured by the Systemic Lupus Activity Measure (SLAM) with scores decreasing from mean 15.5 ± 5.5 to 8.0 ± 3.3 after 6 months of therapy. The mean corticosteroid dose decreased significantly over 6 months. Pashinian et al. (211) reported on eight severe SLE patients who had failed one or two immunosuppressive agents. After 6 months, four of six patients had improvement in SLEDAI scores, and all patients with nephritis had decreased proteinuria (211). Petri (96) studied 22 SLE patients requiring immunosuppressive therapy who were treated with MMF. Although eight failed and required cyclophosphamide, most showed sustained improvement in overall disease activity, prednisone, C4, and anti–double-stranded DNA (dsDNA). The 10 patients with nephrotic-range proteinuria showed a mild but not significant improvement in proteinuria while on MMF.

Since these early studies, a number of investigators have published their results of uncontrolled studies using MMF in LN refractory to other treatments. Most of these studies, focused on proliferative LN, have a recurring theme of overall but not universal improvement in renal function and SLE serologic markers of disease activity.

In a retrospective review, Kingdon (212) evaluated thirteen SLE subjects who had a long history of difficult-to-treat LN of various types (two with membranous LN and 11 with proliferative LN, four of whom also had membranous nephropathy). Generally, improvements were seen in serologic disease activity markers in nine patients, corticosteroids were reduced in 8 of 10 patients, and the mean proteinuria decreased. Karim et al. (213) evaluated a very similar group of 21 subjects and found significant improvements in SLEDAI score and reduction in corticosteroid dose, but little change in serologic markers of lupus activity. These positive responses are extending to those difficult SLE patients with membranous LN. Recently, Karim et al. (214) published a retrospective study of 10 subjects with predominantly membranous LN. After a mean of 18.8 months of treatment, 24-hour urinary protein excretion decreased from a median of 2.26 g to 0.66 g. Kapitsinou (215) described treatment of 18 LN patients, including 6 patients with membranous nephritis with 2 g/day MMF. All but 5 had prior CYC treatment. Except in the membranous nephritis patients, the results were described as favorable with 10 of 18 complete and 4 of 18 partial remissions after a mean follow-up period of 15 months. The

membranous (MLN) patients had nonstatistically significant resolution of proteinuria (from a mean of 1.9 +/- 1.4 g/24 hour to 1.0 +/- 1.0 g/24 hour), and the mean creatinine clearance was not improved. This observation in a small number of patients highlights the necessity of separately analyzing the outcome of lupus patients with MLN; it is possible that the expectation of reduction of proteinuria to less than 0.5 g/24 hour may be too stringent and fail to identify patients who benefit from treatment.

The controlled trials using MMF for, primarily, LN have been well designed and have added substantially to the body of literature on this medication. In the first well known randomized trial comparing treatment of class IV nephritis with MMF plus prednisone (P) for 1 year versus daily oral CYC plus P for 6 months followed by AZA plus P for 6 months, responses were favorable and equivalent in both groups, with reduction of proteinuria and stabilization of serum creatinine levels (57). Neither regimen resulted in improvement of creatinine levels, which has been seen in some series. In a separate report, Chan (216) followed up these subjects for a median of 63 months. The subjects in the MMF group received on average 28.3 months of treatment with MMF. In this long-term follow-up, neither group showed a significant change in serum creatinine over time. In another study of induction therapy with MMF compared to IVC, Hu et al. concluded that MMF was superior to IVC after 6 months of therapy based not only on clinical response but also on serial biopsies (217). In an important multicenter controlled trial in the study of MMF for LN, Ginzler et al. randomized 71 subjects to 3.0 g/day of MMF and 69 subjects to monthly IVC by the NIH protocol for induction therapy of LN. Complete and partial remissions were more frequent and toxicity was lower in the MMF group at 24 weeks (218). Although MMF may have a promising role as induction therapy, most recent interest has been directed toward its use as sequential therapy after induction with IVC (see earlier sections on sequential therapy) (66).

Overall, MMF has had lower toxicity than alkylating agents. In controlled trials for the prevention of renal transplant rejection, diarrhea was increased in patients receiving MMF, with an incidence of up to 36%, compared to 21% for patients receiving AZA and 14% for patients receiving placebo. Few patients (up to 2%) developed severe neutropenia (absolute neutrophil count <0.5 × 103/L). The incidence of malignancies among the patients enrolled who were followed for 1 year was similar to the incidence reported in the literature for renal allograft recipients. There was a slight increase in the incidence of lymphoproliferative disease in the MMF treatment groups compared to the placebo and AZA groups. In three controlled studies for prevention of rejection, similar rates of fatal infections or sepsis (<2%) occurred in patients while receiving MMF or control therapy in combination with other immunosuppressive agents (43 ,219).

The following data are from a compilation of six studies involving MMF and SLE in which adverse events were reported. Out of 81 patients, 21 (26%) had gastrointestinal symptoms—nausea, vomiting, and/or diarrhea. Four patients (5%) had recurrence of herpes stomatitis. Two patients each had vertigo and prurigo. One patient had severe leukopenia, severe anemia, pancreatitis, and a flare of existing vaginal candidosis. Pneumonia and asymptomatic leukopenia each occurred in two patients (209 ,210 ,211 ,220 ,221). In the study published from our group (193) to evaluate the overall tolerability of MMF in patients with SLE, we identified 54 SLE patients followed for a mean of 12.4 person-months on MMF. Twenty-one of 54 patients (38.9%) had a total of 28 gastrointestinal adverse events. Twenty-four of 54 (44.4%) patients had a total of 37 infections, only one of which required hospitalization. Leukopenia occurred 3 times but never required dose adjustment. Adverse events occurred at a similar rate at all MMF doses. Pisoni et al. (222) published a similar report in which they evaluated 93 SLE patients retrospectively. Forty-three percent of patients (37 subjects) developed an adverse event, of which gastrointestinal intolerance was found in 25 subjects and infections in 20. Only 14 patients (16%) discontinued the drug, though, because of adverse events. Ginzler et al. reviewed the tolerability and toxicity in their randomized trial of MMF versus IVC (218). Most adverse events were GI or infection related. Seventeen patients in the IVC group had upper GI distress, 6 of which required hospitalization. Nineteen MMF patients had mild or moderate GI symptoms. Hematologic toxicity was unusual and seemed to be similar in both groups with the exception of lymphopenia, which developed in 28 patients on IVC versus 18 on MMF. The authors reported a trend toward decreased serious infections in patients on MMF.

Summary of Mycophenolate Mofetil Therapy for Lupus

- Several relatively short term studies suggest that MMF is less toxic and may be at least as effective for LN as IVC used as initial therapy for severe nephritis. MMF has not been compared to the combination of monthly IVC + monthly bolus methylprednisolone, which is probably our most effective “conventional” therapy.
- Sequential therapy utilizing IVC followed by MMF is, in our opinion, highly likely to be as effective as monthly IVC followed by q 3-month IVC and is less toxic.
- Although MMF has a better track record than AZA as an initial agent to treat active lupus nephritis and is in our opinion likely to be shown to be faster acting, its superiority and/or better safety profile compared with AZA as a maintenance agent has not been established. It is possible that AZA will work equally well in this setting.
- There are no guidelines for using MMF when there is severe renal insufficiency. In this setting, IVC may be more easily and effectively administered.
- Overcoming the problems of high variability of MMF levels in individual patients and difficulty measuring levels in individual patients may improve results in future trials.

- There is emerging evidence that MMF may prevent atherosclerosis, at least in transplant patients. An “antiatherosclerotic” effect would make MMF highly desirable as a treatment for lupus.

Leflunomide

Leflunomide is a relatively new inhibitor of de novo pyrimidine synthesis. It has been used extensively for the treatment of rheumatoid arthritis (RA), and has been shown to be as effective as methotrexate and sulfasalazine (223 ,224). Leflunomide is a cytotoxotic isoxazole derivative and is structurally unrelated to other immunomodulatory drugs (225). Leflunomide is rapidly converted to its active metabolite, A77–1726, a malononitrilamide, which is a known inhibitor of the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), a key enzyme in the de novo synthesis pathway of the pyrimidine ribonucleotide uridine monophosphate (rUMP) synthesis which prevents activated lymphocytes from moving from the G1 to the S phase (226 ,227). A77–1726 has other known anti-inflammatory roles as an inhibitor of cyclo-oxygenase (COX)-2 activity and an inhibitor of leukocyte adhesion. Furthermore, leflunomide may also have antiviral activity and has been proposed as therapy for CMV in renal transplant patients (228).

Studies with leflunomide for treatment of lupus and other systemic autoimmune diseases are underway. One study of leflunomide in 11 patients with mild to moderate SLE revealed improvements in ECLAM score in 8 patients and reduction in corticosteroid dosing in 4, but did not reach statistical significance (229). In a retrospective study of eighteen patients, 15 of whom met ACR criteria for SLE and had moderate disease activity, leflunomide was administered for 2 to 3 months (230). There was a significant decrease in SLEDAI score and ESR from baseline among the 14 patients finishing the study, with no serious adverse events reported. However, four patients stopped leflunomide because of GI complications, a rash, and an unrelated hospitalization. Another study of SLE patients with refractory arthritic symptoms revealed that 6 of 20 patients achieved a complete response to leflunomide at a dose of 40 mg/day within 1 month, suggesting that leflunomide may have a role particularly in joint-based symptoms of lupus not responsive to treatment with methotrexate or hydroxychloroquine (231).

In addition to observational studies, there are several controlled trials of leflunomide in SLE. One double-blinded placebo controlled study randomized twelve lupus patients with mild to moderate disease activity, who were taking less than 0.5 mg/kg/day of prednisolone, to receive either leflunomide or placebo for 24 weeks (232). The primary outcome was a change in the SLEDAI, and secondary outcomes included changes in proteinuria, complement levels, anti-dsDNA and prednisolone dosage. The results of the study reveal that there was significant reduction in the SLEDAI in both the leflunomide and placebo groups, but the reduction in the leflunomide group was significantly greater compared with the placebo group (11.0 +/- 6.0 in the leflunomide group and 4.5 +/- 2.4 in the placebo group, $P = 0.026$). The secondary endpoints were similar in both groups.

A second controlled trial was a prospective multicenter study evaluating safety and efficacy of leflunomide in the treatment of 51 patients with proliferative lupus nephritis (233). Patients enrolled in this study had biopsy-confirmed proliferative lupus nephritis and were divided into three treatment groups. Those patients with recent onset nephritis who had never received treatment with immunosuppressive drugs received either leflunomide or IV cyclophosphamide. A third group consisted of patients with recurrent nephritis who had received immunosuppressive therapy within 3 months; they received leflunomide. As reported in the English language abstract, the results of the study after 6 months revealed no difference in the response or remission rates of patients initially treated with either leflunomide or cyclophosphamide. Furthermore, renal parameters such as proteinuria, serum albumin and creatinine, as well as SLEDAI improved similarly in both groups. Among the 14 patients enrolled with relapsed nephritis, the total response rate was 60% and complete remission rate was 6.7% following treatment with leflunomide. Four patients withdrew from the study due to adverse events, including herpes zoster and severe lung infection.

Leflunomide is orally administered at a dose of 10 to 20 mg/day. Although early studies with leflunomide employed a loading dose of 100 mg daily for three days, the practice has been largely abandoned to reduce toxicity. The drug has a relatively long half-life (15 days) and is well absorbed, undergoing extensive enterohepatic recirculation. Prior to treatment, complete blood counts and liver function tests should be obtained and monitored at monthly intervals for the first 6 months and at least every 2 months thereafter.

Several potential side effects of treatment associated with leflunomide, including diarrhea, nausea, and alopecia, have been noted to decrease in frequency with continued treatment (234) and if a loading dose is not used (235). Severe hepatotoxicity has also been reported, although its actual incidence remains controversial. Unacceptably high rates of transaminase elevation, cirrhosis and liver failure were reported in initial studies and postmarketing data regarding leflunomide (236). However, a subsequent FDA review of this data found that that most patients with hepatic involvement were concomitantly taking other potentially hepatotoxic drugs such as methotrexate, or had confounding comorbidities such as viral hepatitis or alcohol abuse. A review of 3,325 leflunomide-treated patients found that abnormalities in liver function testing were cited as reasons for drug discontinuation in 5% of patients (237). Furthermore, the review identified no deaths attributable to leflunomide.

An important aspect to consider when using leflunomide in lupus patients, many of whom are young women, is that it is a potent teratogen rated category X for pregnancy by the FDA, and therefore absolutely contraindicated in women who are at risk for becoming pregnant. Leflunomide can persist after administration for up to 2 years (161), and should therefore be used with reluctance in any woman of

childbearing age. To assure safety following discontinuation of leflunomide, patients must be instructed to avoid pregnancy until undetectable plasma levels (<0.02 µg/mL) are demonstrated. If need be, the drug can be removed from the body by administration of cholestyramine (161).

Conclusion

Refinement of the use of immunosuppressive agents and the introduction of both sequential therapies and ovarian protection regimens to reduce the toxicity of CYC therapy is taking place in the context of the introduction of new and potentially highly potent biologic agents, such as rituximab, anti-BLyS antibodies, and CTLA4-Ig. These biologic agents may prove to be effective either as monotherapy or in combination with traditional immunosuppressive agents (or each other). Use of these drug combinations has the potential to markedly reduce reliance on alkylating agents and/or corticosteroids, presumably dramatically decreasing the toxicities associated with conventional agents.

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Chapter 62

Nonpharmacologic and Complementary Therapeutic Modalities

Daniel J Wallace

In addition to medication, physical measures, and psychologic support, the treatment of systemic lupus erythematosus may necessitate the use of other nonpharmacologic modalities. These include dialysis and transplantation for end-stage renal disease, lasers for cutaneous lesions, apheresis for life-threatening complications of the disease, and lymphocyte depletion for selected patients with refractory disease. Because most lupus patients use some form of alternative or complementary remedies, this topic is also reviewed herein.

Dialysis and End Stage Renal Disease

Patients with end-stage renal disease (ESRD) from chronic SLE represent 1.5% to 2.0% of all dialysis patients in the United States (1 ,2). Three thousand to 4,000 patients with lupus are dialyzed annually; 10% succumb each year (2). As patients with SLE are surviving longer, the incidence of ESRD is increasing. Between 1982 and 1995, it increased from 1.16 to 3.08 cases per million person years (3).

Patients with SLE who develop renal failure have improved mental well being but worse physical functioning and general health compared to lupus patients not in renal failure (4).

Uremia

Uremia was the major cause of death in patients with SLE until the 1960s, when dialysis became available. Only a small percentage of dialysis patients have SLE. Between 1977 and 1985, 5,726 persons in Australia and New Zealand were placed on dialysis; only 63 (1.1%) had SLE (5). Despite therapeutic advances, 26 of 128 patients with nephritis who were followed by Wallace between 1980 and 1989 evolved ESRD that required dialysis (6 ,7); all but three of the 26 had nephrotic syndrome. Between 1972 and 1993, 104 of 566 patients diagnosed with SLE in Okinawa, Japan, evolved ESRD (8). The reader is referred to Cheigh and Stenzel's excellent literature review (9).

Uremia and dialysis both are associated with a decrease in the systemic activity of SLE in many, but not all, patients (10 ,11). Mojcik and Klippel summarized the results in 179 patients in seven studies, and concluded that clinical, serologic, and steroid requirements decreased (12). Szeto noted that most disease flares occur during the first year of dialysis (13), and a high level of disease activity during this time also was found by Bruce et al. (14). Ziff and Helderman (15) have speculated that the toxic effects of uremia on the immune system are responsible for its ameliorative effects on extrarenal disease. It also is possible that the disease has run its course in some individuals and subsided but, by that time, the chronic renal damage is irreversible.

Reversibility

Some patients who develop renal failure from lupus nephritis can discontinue dialysis. This was true in 41% of the 41 patients of Kimberly et al.; 11 were dialyzed for less than 2 months (16). On the other hand, 37% died. Five were transplanted successfully. Coplon et al. (17) reported on their experience with 28 dialysis patients followed between 1969 and 1980. Of these, 8 were dialyzed for a mean of 4.3 months before discontinuation, 6 deaths occurred in the patients with the highest steroid requirement, 7 were transplanted, and only 3 had extrarenal disease. The first few months on dialysis appear to be critical. A high mortality rate is observed, but many of those who survive can either discontinue dialysis or become candidates for transplantation (18). Acute tubular necrosis superimposed on lupus nephritis can induce transient renal failure (19).

Prognosis

Survival on dialysis is good. Ziff and Helderman's 30 patients had a 67% 5-year survival rate (15), Jarrett et al.'s had 59% (20), and Cheigh et al.'s had 65% in the late 1970s (21). These figures compare favorably with those of non-SLE dialysis patients. Some reports have documented a better survival rate: 71% in Australia and New Zealand at 5 years (5), and 89% in 55% Dutch patients at 5 years (22). Males had a poorer renal survival than females (23), and African American women fare poorly with 58% of 19 dead at 5 years (24). In these studies, nonrenal SLE activity was minimal. Most deaths were related to infection or vascular-access problems.

At the University of California, only three of 12 patients on dialysis for lupus nephritis needed corticosteroids after

31 months' mean observation (25). In 1990, Cheigh et al. (26) presented a follow-up of 59 patients with ESRD who were seen at New York Hospital between 1970 and 1987. Of these, 86% were female, and mean age was 27.4 years at ESRD onset. They were followed for a mean of 6.5 years. SLE disease activity at years 1, 5, and 10 was 55%, 6.5%, and 0%, respectively. The 5- and 10-year survival rates were 81.1% and 74.6%, respectively. Erythropoietin decreases the morbidity of patients with SLE on dialysis (27). In Taiwan, a 2003 report demonstrated 73% and 38% 10-year survival rates among 26 patients with ESRD (28). Hospitalizations for cardiovascular and cerebrovascular morbidity as well as leading to mortality does not differ between lupus and nonlupus patients with ESRD (29).

Ginzler et al.'s multicenter trial of 1,103 patients with SLE (30) showed that dialysis has little impact in evaluating causes of death in SLE, although dialysis patients have a much higher rate of infection (10 ,31). Socioeconomic considerations also are important. In the largely indigent group at Los Angeles Harbor-University of California at Los Angeles (UCLA) Medical Center, six of nine dialysis patients died at 1 to 28 months, and five of these six had disease flares (32). The quality of life of patients on dialysis has been reviewed. They tended to have good mental well being but reduced physical function and general health (33).

Hemodialysis versus Peritoneal Dialysis

The success of hemodialysis in ameliorating disease activity may result from its ability to remove circulating pathogenic immune complexes, complement, and other factors (34). Hemodialysis also has anti-inflammatory effects, decreases T-helper lymphocyte levels, and diminishes mitogenic responsiveness (35 ,36). Three cases of a patient developing SLE while on hemodialysis have appeared (37 ,38 ,39), and although rare, successful pregnancies while on hemodialysis are noted occasionally (40). Nephrogenic fibrosing dermopathy is a rare complication of hemodialysis in SLE patients (41).

In contrast, peritoneal dialysis does not cause these changes. Several studies have documented more reactivation of SLE, higher anti–double-stranded DNA (anti-dsDNA) levels, more thrombocytopenia, and higher steroid requirements with peritoneal dialysis (13 ,19 ,35 ,36). In one center, four of six patients who were started on peritoneal dialysis had to be switched to hemodialysis (42), although this has not been found by others (43). Nossent et al. (22 ,44) noted among 55 patients that peritoneal dialysis was associated with poorer survival, more serositis, cytopenias, and serologic activity. Switching to it from hemodialysis could reactivate lupus. In a gender matched, controlled study comparing nondiabetic lupus patients on peritoneal dialysis with those who did not have SLE, the lupus patients had a higher infection rate (45). SLEDAI scores are higher in peritoneal dialysis than hemodialysis lupus patients (46).

It is my experience that barring extenuating or unusual circumstances, hemodialysis is preferable to peritoneal dialysis.

Transplantation

Patients with lupus account for 3% of all renal transplantations in the United States (47). Perhaps a result of medical comorbidities, lupus patients are less likely than others to be transplanted with ESRD (48). Nevertheless, 772 of 32,644 patients who received a kidney transplant in the United States between 1987 and 1994 had lupus nephritis (49). By 1996, the overall graft survival for all diseases at one year had improved to 93.9% from living donors and 87.7% from cadaveric grafts, compared to 88.8% and 75.7%, respectively, in 1988 (50).

Graft and Patient Survival in Adults

By 1975, patients with SLE in the United States were being transplanted, and 150 were transplanted between 1975 and 1980 (51 ,52). These studies concluded that allografts from a living, related donor have a much better survival rate. Two-year graft survivals averaged 50%. In Australia and New Zealand, 19 transplants performed between 1977 and 1985 were associated with 95% and 83% survival rates at 1 and 5 years, respectively, and with 75% and 70% 1- and 5-year graft survival, respectively (5). In 1987, Roth et al. (53) reported a 93% patient survival and an 84% graft survival at 6 years among 15 patients who were transplanted at their institution. Of 2,510 renal transplants performed at the University of Minnesota between 1969 and 1987, 33 (1%) were for SLE (54), as were 20 of 616 (3%) at Albert Einstein College of Medicine (55). Of Cheigh et al.'s 59 patients with lupus and ESRD (discussed earlier), 18 were transplanted over a 17-year period (26). Massry's group at Los Angeles County/University of Southern California Medical Center transplanted 64 indigent (60% Hispanic or black) patients with SLE between 1979 and 1992 (56); 80 received cadaveric transplants. Five-year graft and patient survivals were 61 and 86, respectively. Currently, 5-year graft survivals average 70% to 80% (22 ,26 ,54 ,55 ,57 ,58 ,59 ,60 ,61 ,62 ,63). Criswell's group has undertaken three large-scale reviews of transplantation in the cyclosporin era and compared their results with 97 patients to others in a literature review (64 ,65 ,66). Graft survival compared to nonlupus controls was 82 (vs. 88%) at 1 year but only 19 (vs. 35%) at 10 years. Allograft failure risk was doubled and associated with HLA mismatches, smoking, and delayed allograft functioning (67). An excellent, current review has appeared (68).

Outcomes in Children

254 children between the ages of 1 and 21 with lupus nephritis were transplanted in the United States between 1987 and 1997 (69). Cadaveric allografts did not do as well, and these children had an increased mortality rate. Bartosh et al. retrospectively analyzed 100 renal transplants among 94 lupus patients enrolled in the North American Pediatric Renal Transplant Cooperative Study. The 3-year

graft and patient survival was 69 and 89%, respectively (70). Indigent pediatric populations do not do as well. Among 17 children with SLE who were transplanted in Brooklyn (71), 80% and 45% 1- and 4-year graft survivals, respectively, were reported. The availability of cyclosporine has resulted in a decreased use of azathioprine to prevent rejection. One study comparing these drugs in SLE transplants (19 received azathioprine and 17 cyclosporine) documented the statistically significant superiority of cyclosporine in graft survival (72), whereas another came to opposite conclusions (73).

Antiphospholipid Antibodies

Patients with antiphospholipid antibodies are clearly at an increased risk for thromboembolic events, which can have an adverse effect on graft survival (74 ,75 ,76 ,77), the course of dialysis (78) and vascular access viability (79). Stone et al. identified 25 patients with antiphospholipid antibodies who were transplanted at University of California, San Francisco. Fifteen had thromboembolic events, and ten died (80). Antiplatelet drugs should be employed, and the addition of warfarin may be necessary.

Serologic Features and Disease Recurrence

Patients undergoing transplantation may have persistent elevations of antinuclear antibody and anti-DNA antibody titers as well as reduced complement levels. These serologic abnormalities are of little importance and do not affect the outcome of the graft (10 ,51 ,53). These abnormalities were present in four of seven patients who were transplanted by our group between 1980 and 1989. Despite this, disease recurrence in the transplanted kidney is rare. Various centers have noted nephritis in 0 of 12 patients (81), 0 of seven (10), 1 of 17 (71), 2 of 15 (53), 1 of 28 (22), 1 of 14 (62), and 1 of 18 patients (51), for a total of 6 in 111 patients (8). However, Goral et al. have suggested that up to half of transplanted lupus nephritis patients biopsied have some evidence for recurrent disease activity, even though it is usually mild (mesangial or membranous) and rarely threatens the graft. Isolated case reports of disease recurrence suggest that a disproportionate number of these patients had undergone peritoneal dialysis or had active disease at the time of transplantation (82 ,83 ,84 ,85 ,86). Criswell's group noted 9 of 97 cyclosporin treated patients had recurrent lupus nephritis by biopsy (64). Only three had abnormal serologies and one was symptomatic. Their literature review suggested that using serologic parameters or serum-complement levels to evaluate for recurrence was inaccurate (66). Little specific information of the impact of tacrolimus and sirolimus on transplant outcomes have been published.

Despite this, most of the allografts were still functioning well (87). Extrarenal lupus activity usually is quiescent after renal transplantation (88). One case of de novo SLE in a renal transplant patient has appeared (89).

Pregnancy

According to the National Transplantation Pregnancy Registry, 60 pregnancies were reported among 38 lupus patients (90). 77% were successful, though many were complicated by pre-eclampsia and hypertension.

In conclusion, to achieve the optimal transplant environment, patients should be in remission, be on hemodialysis or no dialysis, and receive an allograft from a living, related donor. Cyclosporine, tacrolimus, or sirolimus with mycophenolate mofetil (MMF) and low-dose prednisone now constitutes the immunosuppressive regimen of choice, but this regimen has not been specifically studied in lupus.

Dialysis and Transplantation in SLE: Summary

- Up to 10% of SLE patients evolve ESRD. Their 5-year survival with optimal care is 80% to 90%.
- Hemodialysis has theoretical advantages over peritoneal dialysis, is associated with fewer infections, and perhaps less lupus activity.
- The majority of lupus patients have their disease activity improve when uremic.
- Graft survival for SLE patients in the United States at 1 year is less than the 93.9% national average, and usually is in the 80% to 90% range.
- Transplantation is most successful if lupus is not active at the time of surgery.
- Patients with a history of antiphospholipid antibody-related events have a poor outcome.

Laser Therapy

Carbon-dioxide lasers have been used to treat discoid lupus lesions and telangiectasias. These lesions can be vaporized, but cellular alterations in nonvaporized cells that are several hundred microns away may be responsible for decreased disease activity (91 ,92). Argon lasers also have been used (93), for atrophic facial scars, and telangiectasias although flares have been reported with its use (94 ,95 ,96).

Lymphocyte Depletion

Basic Principles

Evidence that the lympholytic actions of alkylating agents, corticosteroids, and radiation were responsible for ameliorating certain disease states has led to investigations of the role of thoracic-duct drainage, total-lymphoid irradiation, and lymphapheresis in rheumatic diseases. Lymphoid tissue occupies up to 3% of the total body weight; this includes 1% lymphocytes, or 10^{12} lymphocytes per 70 kg. Lymphocytes are widely distributed and consist of both long- and short-lived populations. T cells compose roughly 90% of the lymphocytes in the thoracic duct lymph, 65% in peripheral blood, 75% in the mesentery, and 25% in the spleen; most of

these are long-lived. Therefore, thoracic duct drainage and localized radiation remove lymphocyte populations in a different manner than those removed by lymphapheresis (97).

Thoracic Duct Drainage

Pioneered by researchers at UCLA in the early 1970s, cannulation of the thoracic duct followed by removal of billions of lymphocytes clearly improved disease activity in patients with SLE (98). The procedure is not practical for clinical use, however, because it is technically difficult, expensive, frequently complicated by infection, and only can be done once.

Lymphapheresis

Online lymphocyte depletion has not been studied adequately as a treatment modality for SLE. No commercially available membranes selectively remove lymphocytes without other leukocytes, so only cell separation by centrifugation methods have been used. Because most patients with SLE are lymphopenic, and this is aggravated by the concomitant use of corticosteroids or cytotoxic drugs, it often is difficult to remove the lymphocyte fraction on cell separators. Our studies in patients with rheumatoid arthritis have shown that a 5×10^9 lymphapheresis performed three times a week for 6 weeks can induce a significant lymphopenia that persists for 4 to 6 months (99). Unlike plasma removal, 15% of the body's total blood volume must be extracorporeal to perform a lymphocyte cut on centrifugation devices, and this requires a near-normal cardiovascular and pulmonary status. Additionally, blood transfusions may be required. Despite these drawbacks, however, Spiva and Cecere (100) reported that a combination of lymphapheresis and plasmapheresis was clinically beneficial in 16 of 19 patients with SLE on concurrent immunosuppression, and that helper-to-suppressor T-cell ratios were decreased.

Total Lymphoid Irradiation

Between 1980 and 1997, a total of 17 patients with lupus nephritis and nephrotic syndrome refractory to conventional drug therapy received 2,000 rad of total lymphoid irradiation over a 4- to 6-week period at Stanford University (101 ,102 ,103 ,104 ,105 ,106). Clinical responses were seen within 3 months and sometimes persisted for years. At follow-up ranging from 12 to 79 months, seven patients were off corticosteroids and without nephrosis; however, one patient died, one ultimately required chronic dialysis, and four developed neutropenia, one developed thrombocytopenia, three developed bacterial sepsis, and four developed herpes zoster. T-helper populations (i.e., $CD4^+$ cells) decreased, and selective B-cell deficits documented by diminished pokeweed mitogen-induced immunoglobulin secretion were observed. Both total and serum immunoglobulin-specific IgE levels were not altered. The survival rate at 7.5 years was identical to that of a historical control group treated with steroids and immunosuppressives, with an equal prevalence of serious complications. Genovese et al. published a long-term follow up on these patients in 2002 (107). Six of 21 had died, and four developed cancer. 57% were dialyzed, and 33% had developed opportunistic infections.

Trentham et al. (108) at Harvard also used total lymphoid irradiation (for rheumatoid arthritis), but they no longer advocate its use. These authors agree that although short-term benefits are apparent, the high probability of disease recurrence after several months to years limits the physician's options in giving alkylating agents to patients who have already been irradiated (109). Further, a high infection rate is observed, and newer therapeutic strategies (e.g., parenteral cyclophosphamide) probably are superior to total lymphoid irradiation. An Israeli group noted unsatisfactory results in two patients who underwent total lymphoid irradiation (110).

Total lymphoid irradiation has no place in the management of SLE.

Photopheresis

In extracorporeal photochemotherapy, commonly known as photopheresis, leukocytes obtained at apheresis are treated with ultraviolet A (UVA) irradiation after the patient has received a photoactivatable drug, p-methoxypsoralen (111). Leukocytes reinfused into the patient can function but have diminished responses. Although only 5% of one's total circulating lymphocytes are treated, photopheresis clearly is beneficial for cutaneous T-cell lymphomas. Knobler et al. (112 ,113) observed modest improvements in eight patients with SLE in an uncontrolled study and Richard et al. found little benefit (114).

Summary

No online lymphocyte depletion method has been shown to be safe and effective in managing lupus.

Plasmapheresis

Basic Principles

Apheresis refers to the removal of a blood component (e.g., red-blood cells, lymphocytes, leukocytes, platelets, plasma) by centrifugation or a membrane cell separator, with return of the other components to the patient. Removing 1 L of plasma decreases plasma proteins by 1 g/dL, but because of compartmental equilibration and protein synthesis, 2.5 L of plasma must be exchanged weekly to decrease protein levels. In the intravascular space, 50% of the total IgG and 67% of the total IgM are found. Nine exchanges of 40 mL/kg over 3 weeks leave only 5% native plasma. The removal rate of plasma proteins and components depends on charge, solubility, avidity to other plasma proteins, configuration, synthesis, and uptake rates. In immunologic disorders, the recovery of immunoglobulin levels can be slowed by the concurrent use of immunosuppressive agents. If none are used, then antibody rebound, or the tendency of certain antibody levels to rise rapidly above their prepheresis baseline after initially decreasing, is observed; this often correlates with a disease flare (91). Plasma usually is replaced with a combination of albumin, salt, and water. Certain complications of lupus (e.g.

thrombotic thrombocytopenic purpura) necessitate the use of fresh-frozen plasma replacement, because a plasma factor is deficient. When performed by personnel at experienced blood banks or dialysis facilities, plasmapheresis usually is safe; serious complications (e.g., hypotension, arrhythmia, infection) occur less than 3% of the time in this group of sick patients (115). The reader is referred to my detailed reviews of the subject (116 ,117 ,118).

Rationale In Systemic Lupus Erythematosus

Plasmapheresis can remove circulating immune complexes and immune reactants (e.g., free antibody, complement components), alter the equilibrium between free and bound complexes, and restore reticuloendothelial phagocytic function (119). Three different centers have documented reversal of the reticuloendothelial system blockade by plasmapheresis (120 ,121 ,122). Plasmapheresis may improve suppressor-cell functioning (123), promote a Th1 shift (124), reduce IL-10 levels (125), reduce GM-CSF secreting peripheral blood mononuclear cells (126) and selectively remove IgG anti-DNA (127). Steven et al. (128) demonstrated improved bacterial killing by monocytes after plasmapheresis, and in patients with mild disease, Tsokos et al. (129) found no change in proliferative responses to mitogens or lymphocyte subpopulation percentages. In 17 steroid- and immunosuppressive-resistant patients with lupus nephritis, however, Wallace et al. (130) reported normal B- and T-cell counts but diminished mitogenic responsiveness and CD4 levels (compared with preplasmapheresis values) after 15 exchanges.

Clinical Studies in Systemic Lupus Erythematosus

The use of plasmapheresis was reported first by Jones et al. in 1976 (131). Follow-up observations brought the conclusion that patients who are the most seriously ill and have the highest levels of circulating immune complexes respond best (132 ,133 ,134 ,135). Patients who are treated concomitantly with plasmapheresis, prednisone, and cyclophosphamide do better than those who are treated with prednisone and azathioprine (136), and those who are on prednisone alone may become worse (137). The procedure is well tolerated in children and pregnant women with SLE (138 ,139). Over 1,000 case reports of apheresis improving specific aspects of SLE and antiphospholipid syndrome have been published. Only controlled studies or large series are cited in this section.

Lupus Nephritis

The most impressive results were reported in patients with lupus nephritis who had active disease and minimal scarring. Of 31 Finnish patients in this subset, 24 responded to treatment (140). Kincaid-Smith's group (141) rebiopsied eight patients with acute crescentic proliferative lupus nephritis several weeks after they received prednisone and cyclophosphamide and underwent plasmapheresis, and they found dramatic improvements in seven. A literature review in 1986 noted that 69% of 42 cases of diffuse proliferative nephritis improved after plasma exchange (142). These and other promising reports (143 ,144 ,145) led to two controlled trials. Lewis' group randomized 86 patients with new-onset proliferative nephritis to oral cyclophosphamide and prednisone, with or without plasmapheresis (146 ,147 ,148 ,149). Both groups improved, and no differences in the outcome were noted. Numerous methodologic flaws minimize the value of this study, however (150). Wallace et al. (130 ,150) restricted their study to 27 patients with nephrotic syndrome who were resistant to a minimum 3-month trial of steroids and cytotoxic drugs. Of these, ten were randomized to continue their therapy, and plasmapheresis was added to 17. After 2 years, seven had a good outcome (i.e., normal serum-creatinine level and resolution of nephrotic syndrome), and seven had a poor outcome (i.e., dialysis or death). All seven who had a good outcome underwent plasmapheresis (p = 0.026). Of the seven responders, five had undergone apheresis. The poor responders could not be predicted in advance by any of 30 variables used (130 ,150).

Antiphospholipid Syndrome and Congenital Heart Block

Interest has focused on the removal of anticardiolipin antibody and the lupus anticoagulant by plasmapheresis during pregnancy or in patients who have experienced recurrent thromboembolic episodes (151). Results have been mixed (152 ,153 ,154 ,155 ,156 ,157 ,158 ,159). Plasmapheresis is safe during pregnancy (160) and can be used weekly for the temporary removal of anticardiolipin (161 ,162 ,163 ,164). It is especially helpful if large amounts of the IgM isotype are present (165). The apheretic removal of anti-Ro/SSA in mothers whose fetuses show signs of congenital heart block has been reported but no conclusions can be made from published studies (166 ,167 ,168 ,169).

CNS, TTP, Cryoglobulinemia, Hyperviscosity, and Pulmonary Hemorrhage

Six French patients with central nervous system lupus had favorable responses (170), and this was confirmed by Neuwelt et al. in a larger study (171). The usefulness of plasmapheresis in cryoglobulinemia, thrombotic thrombocytopenic purpura, and hyperviscosity syndrome is well established (117 ,172 ,173), and these complications occasionally occur in patients with SLE. Several reports have suggested that plasmapheresis is useful for pulmonary hemorrhage (174 ,175 ,176).

Pulse Synchronization Therapy

Euler's group in Germany has devised an innovative approach for the treatment of seriously ill patients with SLE (177). It involves deliberately inducing antibody rebound with plasmapheresis, followed by high-dose intravenous cyclophosphamide to eliminate the increased numbers of malignant clones. Their pulse synchronization

technique has resulted in some spectacular successes with long-term, treatment-free remissions (146 ,177 ,178 ,179 ,180 ,181 ,182 ,183 ,184). The net result was that pulse/synchronization does not work using conventional cyclophosphamide doses for lupus nephritis (185) or for the disease in general (186 ,187). Using higher-dose cyclophosphamide can be more effective, though much riskier.

Membrane Technologies

New membrane technologies have enabled selective plasmapheresis to be performed. Membranes that remove cryoproteins (188), anti–single-stranded DNA (189 ,190), IgG containing circulating immune complexes (191), and anti-dsDNA by immune adsorption (192 ,193 ,194 ,195 ,196 ,197 ,198 ,199 ,200) have been developed. Unfortunately, membranes activate complement and may present additional risks of hemolysis. In my experience, any theoretic cost saving obtained by avoiding albumin replacement is countered by the frequent clogging of expensive membranes, which necessitates termination of the procedure or use of a second membrane. Also, many patients with SLE do not have anti-DNA, and it is only one of many putative autoantibodies that may accelerate the disease process. Flares with treatment also have been reported (201). Further, selective membranes remove fewer plasma proteins than conventional plasmapheresis; this usually is not the practitioner's intent. Some promising approaches, such as a C1q column immunoadsorption have definite clinical effects (202). Adacolumn adsorbs granulocytes and monocytes and in pilot study of 15 patients appears to be well tolerated and not associated with an increased infection rate (203 ,204).

Summary

At this time, plasmapheresis should be used only for patients with renal disease who are resistant to corticosteroid and cytotoxic drug therapy, specific disease subsets in which its efficacy is established (e.g., those with hyperviscosity syndrome, cryoglobulinemia, or thrombotic thrombocytopenic purpura), and in those with acute, life-threatening complications of SLE, in each instance in combination with corticosteroids and cytotoxic therapy (Table 62-1).

Table 62-1: Indications for Apheresis in Systemic Lupus Erythematosus

I. Absolute—benefits are clear-cut and often life-saving
   1. Thrombotic thrombocytopenic purpura
   2. Cryoglobulinemia
   3. Hyperviscosity syndrome

II. Relative—acceptable to use when clinically indicated
   1. Severe organ-threatening disease that is underresponsive to steroid and cytotoxic drug therapy.

III. Investigational
   1. Antiphospholipid antibody removal in pregnancy
   2. Anti-Ro/SSA antibody removal in pregnancy

IV. Contraindicated
   1. Mild lupus or nonorgan-threatening lupus

Ultraviolet UVA-1 Radiation

McGrath et al. (205 ,206 ,207 ,208 ,209) have reported modestly beneficial effects of the longer wavelengths of UVA-1 radiation (340 to 400 nm) in open-label, double-blind, placebo-controlled, and long-term follow-up studies. Disease activity indices, cutaneous lesions, and anti-dsDNA levels improved. No side effects were reported. In a small double-blind placebo controlled study of 12 patients, Polderman et al. showed modest improvements in SLAM and SLEDAI scores with minimal toxicity (210 ,211). UVA1 photons may promote DNA repair, cell mediated immunity, apoptosis, and reduce B-cell function leading to anti-inflammatory effects (212 ,213).

In summary, cold UVA-1 light may be marginally beneficial in selected cases of SLE.

Complementary and Alternative Medicine

Up to 40% of Americans use CAM to treat chronic conditions, spending $27 billion a year (214). Moore et al. followed 707 lupus patients from Canada, the United States, and the United Kingdom. In 2000, they reported that one half used CAM (215). The users were younger and better educated, exhibited poorer levels of self-related health status and satisfaction with medical care, but did not have worse disease than nonusers. CAM was defined as including relaxation techniques, massage, herbal medicine, lifestyle diets, self-help groups, imagery, folk remedies, spiritual healing, chiropractic, megavitamin therapy, homeopathy, energy healing, commercial weight loss, biofeedback, acupuncture, and hypnosis. Ernst's review also included autogenic training, Alexander technique, aromatherapy, colonic irrigation, Feldenkrais, magnet therapy, meditation, osteopathy, reflexology, Tai Chi, and vitamins (216). Leong et al. interviewed 192 Chinese lupus patients in Singapore and found that 38% used CAM to manage the disease, whereas 28.6% employed it for cultural or other health reasons (217). In Mexico, patients who used alternative therapies had more disease severity and/or complications (218). Physicians managing lupus should ask their patients whether they are using CAM approaches, since certain may have a negative impact on the disease or interfere with other medications. For example, certain Chinese and Peruvian herbs and echinacea also contain chemicals that are harmful in SLE, and many herbal preparations contain sulfa (219 ,220 ,221).

The use of CAM approaches is discussed in other sections of this monograph. This includes diet and vitamins (Chapter 57), biofeedback or cognitive behavioral therapy for Raynaud and cognitive dysfunction (Chapters 38 and 57),

physical measures (Chapter 57). In this writer's opinion, any approach that promotes spiritual healing, relaxation, or improves blood flow (e.g., biofeedback) or uses the mind-body connection (e.g., CAM approaches that influence the sympathetic nervous system) are usually beneficial in SLE. Unfortunately, there is almost no evidence-based literature on the subject. Areas not covered elsewhere are summarized below (222). Other drugs and modalities that have been tried include tuberculin (223); Chinese herbs, especially T Wilfordii Hook F (224 ,225 ,226 ,227 ,228 ,229 ,230 ,231 ,232); arsenic (233); heliotherapy (234); hemotherapy (235); auricula acupuncture (236); hyperbaric oxygen (237), sarei-to (238), acupuncture (239), pulsed magnetic fields (240), and Pycnogenol phytotherapy (241). Witchcraft has even been used successfully (242). Tables 62-2 and 62-3 summarize CAM approaches that have been utilized in autoimmune disease patients.

Table 62-2: Complementary and Alternative Treatments Used by Lupus Patients

1. Manipulative, physical, and manual therapies

a. Acupuncture
b. Alexandra technique
c. Chiropractic
d. Osteopathy
e. Pilates
f. Reflexology
g. Tai chi
h. Yoga

Comment: Any technique that strengthens muscles and promotes aerobic conditioning is acceptable. Some also diminish pain. Fibromyalgia can be aggravated by some of these techniques, and caution and modifications are advised if synovitis is present (see Chapter 57).

2. Methods that promote relaxation, cognitive improvement, and greater disease understanding

a. Aromatherapy
b. Autogenic training
c. Biofeedback
d. Cognitive behavioral therapy
e. Guided imagery
f. Feldenkrais therapy
g. Hypnotherapy
h. Meditation
i. Self help groups
j. Spiritual healing

Comment: Any activity that works with the mind-body connection, decreases anxiety, or promotes restful sleep is usually acceptable (see Chapters 38 and 57).

3. Detoxification regimens

a. Colonic irrigation
b. Magnet therapy
c. Chelation therapies

Comment: Unproven for any aspect of lupus. Colonic irrigation is dangerous in patients who had thin bowel lining from corticosteroids; chelation therapies can be dangerous.

4. Herbal, dietary, and nutraceutical approaches

a. Homeopathy
b. Herbal anti-inflammatory regimens
c. Lifestyle diets
d. Elemental or hypoallergenic diets
e. Elimination or fasting diets
f. Folk remedies
g. Megavitamin and or vitamin regimens
h. Commercial weight loss programs
i. Dietary fatty acid regimens

Comment: Diets and vitamins are discussed in Chapter 57; specific supplements are listed in Table 62-3. Supplements are not FDA-regulated and their bioavailability varies as well as packaging and preservatives. A well-balanced diet is important.

5. Ayurvedic system (Indian healing system that combines aspects of the above approaches)

Table 62-3: Herb Chart (for Antiarthritics, Skin Treatments, and Gastrointestinal Treatments)

Herb	Claimed Uses	Active Ingredients	Potential Side Effects
Alfalfa	Antiarthritic	Nonprotein amino acid (L canavanine) and some saponins	In large quantities, could produce pancytopenia (decreased white blood cell count, anemia); could reactivate systematic lupus erythematosus
Arnica	Analgesic, anti-inflammatory (external application)	Sesquiterpenoid lactones (helenalin, dihydrohelenalin)	May cause contact dermatitis; cannot be taken internally; causes toxic effects on the heart and increases blood pressure
Black cohosh	Antirheumatic, sore throat, uterine difficulties	Substances that bind to estrogen receptors of rat uteri; also acetin, which causes some peripheral vasodilation	Information on toxicity is lacking; could cause uterine bleeding
Burdock	Treatment of skin conditions	Polyacetaline compounds that have bacteriostatic and fungicidal properties	Side effects may result from addition with belladonna
Calamus	Digestive aid, antispasmotic for dyspepsia	Unknown	Use only Type 1(North American) calamus, which is free of carcinogenic iso a sarone (may promote cancers)
Calendula (marigold)	Facilitate healing of wounds lacerations)	Unknown	Unknown
Capsicum	Counterirritant used to treat chronic pain (herpes zoster, facial neuralgia, or surgical trauma)	Capsaicin (proven analgesic in osteoarthritis, used externally)	Use caution in application; avoid getting into eyes or other mucous membranes; remove from hands with vinegar
Catnip	Digestive, sleep aid	*Cis-trans*-nepetalactone (attractive only to cats)	Unknown; does not mimic marijuana when smoked
Chamomilles, yarrow	Aids digestion, anti-inflammatory, antispasmotic, anti-infective	Complex mixture of flavonoids, coumarins, d-bisabolol motricin and bisabololoxides A + B	Infrequent contact dermatitis and hypersensitivity reactions in susceptible people
Chickweed	Treatment of skin disorders, stomach and bowel problems	Vitamin C, various plant esters, acids, and alcohols	Unknown
Comfrey	General healing agent, stomach ulcer treatment	Atlantoin, tannin, and mucilage, some vitamin B_{12}.	Hepatotoxicity (liver); can lead to liver failure, especially when the root is eaten; also causes atropine poisoning due to mislabeling
Cranberry	Treatment of bladder infections	Antiadhesion factors (fructose and unknown polymeric compounds) prevent adhesion of bacteria to lining of bladder	Increased calories if used in large doses (12–32 ounces per day) as a treatment rather than as a preventative (3 ounces per day)
Dandelion	Digestive, laxative, diuretic	Taraxacin (digestive), vitamin A	Free of toxicity except for contact dermatitis in people allergic to it
Devil's claw	Antirheumatic	Har pagoside	None
DongQuai	Antispasmotic	Coumarin derivatives	Large amounts may cause photosensitivity and lead to dermatitis, possible bleeding
Echinacea	Wound healing (external), immune stimulant (internal)	Polysaccharides, cichoric acid, and components of the alkamide fraction	None reported, but allergies are possible; be sure product is pure and not adulterated with prairie dock (can cause nausea, vomiting); may flare lupus

Evening primrose	Treatment of atopic eczema, breast tenderness, arthritis	*Cis*-gamma-linoleic acid (GLA) (some suggestive data)	No data; borage seed oil (20% as GLA) may be a substitute and does have toxic side effects (liver toxicity, carcinogen)
Fennel	Calms stomach, promotes burping	*Trans*-anethole, fenchone, estragole, camphene, L-pinene	Do not use the volatile oil—causes skin reactions, vomiting, seizures, and respiratory problems, no side effects with use of seeds
Fenugreek	Calms stomach, demulcent	Unknown	None
Garlic	GI ailments, reduces blood pressure, prevents clots	Allin (sulphur-containing amino acid derivative), ajoene	Large doses are needed (uncooked, up to 4 grams of fresh garlic a day), which may result in GI upsets; can "thin" the blood (anticoagulant)
Gentian	Appetite stimulant	Glycosides and alkalids; increases bile secretion	May not be well tolerated by expectant mothers or people with high blood pressure (possibly increasing pressure)
Gingko biloba	Helps dementia	Antioxidant	Well tolerated
Ginseng	Adaptogen, cure-all, anti-stress agent	Triterpenoid saponins	Be sure the product is pure; some insomnia, diarrhea, and skin eruptions have been reported; possible immune stimulant (antagonizes other medications)
Goldenseal	Digestive aid, treatment of genitourinary disorders	Alkaloids (hydrastine and berberine)	In *huge* doses, may cause uterine cramps
Honey	Sore throat, antiseptic, anti-infective, antiarthritic, sedative	Fructose, glucose, sucrose, tannin	Do not give to children under 1 year of age; may cause botulism in infants
Lovage	Diurectic, promotes burping	Lactone derivatives (ph thalides)	Some photosensitivity with volatile oil of lovage
L-Tryptophan	Sleep aid, antidepressant	Essential amino acid that increases chemical serotonin, leading to some sleepiness	Be sure product is pure; contaminants may cause a serious blood disorder and a scleroderma-like illness
Mistletoe	Stimulates smooth muscle (American), antispasmotic and calmative (European)	Phoratoxia and viscotoxin (depending on the plant species)	Berries are highly toxic, and the leaves may also cause cell death; in animals lowers blood pressure, weakens, constricts blood vessels
Nettle	Antirheumatic, antiasthmatic, diuretic, against BPH	Histamine, acetylcholine, 5-hydroxytryptamine	Skin irritation from the active ingredients
New Zealand green-lipped mussel	Antiarthritic	Amino acids, mucopolysaccharides	No toxicity or side effects except in those allergic to seafood
Passion flowers	Calmative, sedative	Unknown or disputed	None
Peppermint	Calms stomach, promotes burping, antispasmotic	Free menthol and esters of menthol	Do not give to infants and young children, who may choke from the menthol
Pokeroot	Rheumatism, cure-all	Saponin mixture (phytolaccatoxin), mitogen, pokeweed mitogen (PWM)	Vomiting, blood cell abnormalities, hypotension, decreased respiration, gastritis
Rosemary	Antirheumatic, digestive, stimulant	Camphor, borneol, cineole, diosmin (a flavonoid pigment)	Large quantities of the volatile oil taken internally cause stomach, intestinal, and kidney irritation

Rue	Antispasmotic, calmative	Quinoline alkaloids, coumarin derivatives	Skin blisters and photosensitivity following contact; gastric upsets when taken internally; may be an effective antispasmotic but is too toxic to be used
St. John's wort (Hypericum)	Antidepressant, anti-inflammatory, wound healing	10% tanin, xanthones, and flavonoids that act as monoamine oxidase inhibitors (antidepressants)	Photosensitivity dematitis in those who take the herb for extended periods; Prozac-like; increases serotonin
Sairei-to	Antiarthritic	12 herbs in combination	Diarrhea, abdominal pain, rash
Sassafras	Antispasmotic, antirheumatic	Safrole	Active ingredient is carcinogenic in rats and mice
Senna	Cathartic	Dianthrone glycosides (sennosides A + B)	Diarrhea, gastric, and intestinal irritation with large and/or habitual doses
Tea tree oil	Antiseptic (external application only)	Terpene hydrocarbons, oxygenated terpenes-4-01)	No side effects except skin irritation in sensitive individuals
Valerian (garden heliotrope)	Tranquilizer, calmative	Unknown	None noted
Yucca	Antiarthritic	Saporins	None noted

Source: Compiled by Elaine E. Furst, R.N., and Daniel E. Furst, M.D. Modified from V. E. Taylor: *The Honest Herbalist, 3rd ed*, Binghamton, NY: Haworth Press, 1993, pp. 336–351.

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Chapter 63

Fibromyalgia in SLE and the Use of Complementary and Alternative Medicine

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Introduction

Fibromyalgia (FM) is a common painful musculoskeletal condition associated with widespread tenderness or other somatic conditions, which has been described for centuries in the literature. Between 10% and 12% of the general population has chronic widespread pain. Women are affected more than men, and the prevalence of widespread pain increases with age (1). The mandatory symptom of this syndrome is widespread pain not explained by an inflammatory or degenerative musculoskeletal disorder. There are no “objective markers” of disease. The presence of many tender points in soft tissue locations validates the diagnosis. To this day, there remains significant controversy within the field of rheumatology about the existence of this syndrome and its true definition (2). A small but distinguished minority of academicians including rheumatologists have expressed the view that FM is a nondisease or a “medically unexplained syndrome.” This controversy highlights the concept that FM is not a single disease, but a group of disorders that is classified under the functional somatic syndromes. The disease fibromyalgia represents a group of heterogeneous individuals suffering from chronic pain (3 ,4).

Given the heterogeneity of patients with this condition, it has been difficult to study fibromyalgia. Various diagnostic criteria for FM were proposed and field tested. The 1990 American College of Rheumatology classification criteria (5) for FM have been adopted by most investigators in the past 10 years. In that study, 293 consecutive patients with FM were compared with 265 control patients who had regional chronic musculoskeletal pain or a systemic rheumatic disease. Training sessions were used to increase interrater reliability, and independent blinded assessors recorded the history and performed the physical examination. The symptom of widespread pain and the finding of mild or greater tenderness in at least 11 of 18 specified tender points on digital palpation provided a sensitivity of 88% and specificity of 81% in distinguishing FM from other causes of chronic musculoskeletal pain. Thus, the definition of fibromyalgia for classification purposes requires the presence of widespread chronic pain along with 11 of 18 tender points on digital palpation. However, the American College of Rheumatology (ACR) study committee found no difference in patients with FM who had a concurrent medical condition. Therefore, in regard to classification criteria, no distinction is made as to comorbid illness or possible associated trauma (5).

Distinguishing Fibromyalgia from SLE:

Fibromyalgia is much more common than SLE. Using current classification criteria, the estimated prevalence of FM in the general community is 2% for both sexes, 3.4% for women, and 0.5% for men. The prevalence increases with age, reaching greater than 7% in women aged 60 to 79 years. Fibromyalgia is the second most common diagnosis in rheumatology clinics (2). The prevalence of SLE in white women in the United States is approximately 10 to 50 per 100,000. It is 4 to 5 times more prevalent in African American women. Thus, fibromyalgia is 20 times more common than SLE, though a ratio of 65:1 or 75:1 is plausible (6).

Secondly, the prevalence of a positive ANA is much higher than the prevalence of connective tissue diseases. Hence, there is a high prevalence of positive ANA's in FM. In a study of 485 healthy volunteers, 20% of women and 7% of men and 31% of women over 40 were ANA positive (7). Slater et al. studied 1010 consecutive patients in a tertiary care center who underwent ANA testing by performing chart reviews. They found that 15% of all patients and 40% of patients over 65 years had a positive ANA. Overall, the sensitivity of the ANA test for SLE was high, but the positive predictive value was low for SLE or other rheumatic diseases (8). Given that the specificity of the ANA is low, it is not a reliable tool to differentiate inflammatory from noninflammatory conditions.

In light of the unreliability of ANA testing, the history and physical examination are extremely important in differentiating between inflammatory conditions and other conditions that mimic symptoms of autoimmune disease. Differentiating lupus from fibromyalgia can pose some problems to clinicians because patients often complain of the

same symptoms such as fatigue, arthralgias, sun sensitivity, neuropathies, memory deficits, morning stiffness, and chest wall pain. As an example, through a careful history, it is important to rule out seborrhea and rosacea when investigating a malar rash. Similarly, clinicians should inquire about the duration of sun exposure and factor in the altitude, latitude, and the skin pigmentation of an individual when inquiring about photosensitivity (6). When performing a physical examination, the clinician should look for evidence that supports the diagnosis of lupus or for evidence that the symptoms have another explanation. For example, the malar rash typically spares nasolabial folds, which are involved in rosacea and seborrhea, oral ulcers in lupus are more common on the hard palate, and acrocyanosis is commonly seen in lupus, particularly when the examination room is cool.

Lastly, clinicians should have a high index of suspicion for fibromyalgia, and feel comfortable making the diagnosis of fibromyalgia based on history and physical examination alone. Completely normal blood tests are consistent with a diagnosis of FM. Clinicians should also be attuned to the fact that fibromyalgia symptoms are reported in patients with increased stress, depression, anxiety, lack of sleep, lack of exercise and traumatic life experiences.

Prevalence of Fibromyalgia in SLE

Different studies have estimated that the prevalence of fibromyalgia in SLE is between 5% and 60%. Pistiner et al., reporting on 570 patients with SLE in the 1980s, reported the incidence of fibrositis to be 22% (9). Middleton et al. reported that 22% of patients in their cohort met ACR criteria for fibromyalgia and another 23% had clinical FMS without meeting the ACR criteria (10). Similarly, Moreland et al. (11) reported that 25% of SLE patients in their cohort had fibromyalgia using the Yunus criteria (12).

Other groups have reported a lower incidence of fibromyalgia in SLE; notably, a study by Friedman et al. (13) studied the prevalence of fibromyalgia prospectively and longitudinally in a group of 266 SLE patients. The prevalence of FM was then calculated, as was the prevalence of FM-like manifestations (widespread pain with at least 6, but fewer than 11 of 18 tender points). Fibromyalgia was seen only in 5% of patients, and fibromyalgia like illness was noted in another 13% of patients. If the patients with fibromyalgia like illness were included in the analysis, the prevalence of FM is approximately the same as prior studies.

Internationally, there is a variable prevalence of fibromyalgia. A Spanish study (14) reported that only 10% of Spanish patients with SLE had FM. It was suggested that the low prevalence of could be the result of geographical, psychological, sociocultural, and therapeutic factors. Handa et al. (15) reported that only 8.2% of patients in their cohort of Indian SLE patients were found to have fibromyalgia. Age, sex, marital status, educational level, disease duration, disease activity, and the organ involvement in patients with SLE and FM were comparable to those in patients not having fibromyalgia. They hypothesized that fibromyalgia appears to be distinctly uncommon in Indian patients with lupus and posited that a strong social support network, racial variation, and the lack of disability benefits in India could explain the differences between their results and other reports. However, even though family support is strong is Turkey, the prevalence of FM in Turkish patients with SLE is about 25% and is comparable with North American populations (16).

Only one study has shown a 61% prevalence of FM in SLE (17). The author hypothesized that other studies may have underestimated the prevalence of FM because the patients may have been treated with pain medications and nonsteroidal anti-inflammatory drugs (NSAIDs), which in turn would have increased their pain threshold and altered the tender point examination.

Overall, these studies point that the prevalence of FM is around 20% in patients with SLE, unless the prevalence of FM in the general population being studied is different from the prevalence of FM in the United States.

Etiology of Fibromyalgia in SLE

The etiology of fibromyalgia is not completely understood. Current thinking suggests that fibromyalgia is caused by a combination of factors such as central sensitization, dysfunction of the autonomous nervous system, and inappropriate secretion of neurotransmitters, cytokines, and hormones. The symptoms are exacerbated by stress, lack of sleep, and psychological dysfunction. A brief summary of some of the current theories on the etiopathogenesis of fibromyalgia is presented below.

Central Nervous System Disturbances

Patients with fibromyalgia have a generalized hypervigilance to both pain and auditory stimuli, with alteration in nociception. The heightened pain response at tender points is now considered to be a manifestation of altered central nervous system (CNS) processing of nociceptive stimuli (2). In one study, FM patients who were given electrocutaneous stimuli had a secondary hyperalgesia in the upper extremities (18). In other studies, elevated levels of substance P and abnormal antinociceptive peptides were demonstrated in the CSF of patients with FM (19 ,20). Further evidence for a central role can be inferred from brain imaging studies in women with FM, which demonstrate low regional blood flow to the caudate nucleus and the thalamus; areas previously determined to be involved in pain perception (21).

Sleep and Endocrine Abnormalities

Slow-wave–sleep abnormalities are fairly prominent in fibromyalgia. The most frequent finding has been alpha intrusion in non–rapid-eye-movement (NREM) sleep. Research has also found alterations of various neurohormones in FM. There is also an exaggerated corticotropin response to corticotropin-releasing hormone and variable disturbances of

sympathetic nervous system activity in FM. Low levels of somatomedin C, which reflects growth-hormone release, also have been noted in patients with FM. Evidence of sympathetic-parasympathetic imbalance, specifically related to neurally mediated hypotension, has been found in FM and CFS. Cervical spine pain, benign joint hypermobility, and steroid withdrawal also have been identified by some authors as contributing to the pathogenesis of FM (2).

The exact etiology of the development of fibromyalgia in SLE is not understood. In patients with SLE, there is hyperactivity of the immune system, which leads to immune complex formation and deposition in tissues. These immune changes can theoretically lead to pain either directly, because of inflammation, or indirectly, by causing tissue damage. However, several studies have shown that pain and fatigue, which are features of fibromyalgia in SLE patients do not correlate with disease activity measures (22 ,23). Thus, severity of SLE tissue damage is not linked to the development of fibromyalgia and indeed the presence of fibromyalgia in SLE patients does not correlate with SLE disease activity measures (24).

One possible theory for the etiology of fibromyalgia is the presence of severe fatigue in lupus patients, which in turn may lead to inactivity, which is a predisposing factor for the development of fibromyalgia. In one study, although the presence of fatigue did not affect disease activity of SLE, fatigue correlated strongly with depression, FM, and lower health status in SLE patients. This may represent poor coping abilities in these patients (22).

Various authors have hypothesized that steroid therapy is an import trigger for the development of diffuse pain (25). Handa et al. reported that corticosteroid withdrawal or dose reduction was the probable precipitating factor in nearly one third of their patients, who had concomitant FM and SLE (15). Smythe et al. reported that in a group of 54 patients, 26 of whom had received corticosteroid therapy for at least 3 years, there was a predisposition to develop tenderness of the shins in the patients who received steroids (26). This finding was recently duplicated by Buskilla et al. in patients with inflammatory bowel disease who were receiving corticosteroids (27).

Clinical Implications of FM in SLE Patients

The recognition of FM as a major contributor to the symptoms of concurrent rheumatic conditions, such as rheumatoid arthritis and SLE, has changed the approach to the treatment of these conditions and provided renewed interest in the psychosocial aspects of the common rheumatic disorders (10 ,24). The prevalence of fibromyalgia in SLE has several important clinical consequences.

Though the presence of FM in SLE patients does not seem to directly influence disease activity scores, it affects the ability of a clinician to correctly determine disease activity. Moreland et al. reported that there were no significant differences in disease expression between SLE patients with or without concomitant FM. However, they reported that patient and physician disease activity ratings did not correlate with SLAM scores in SLE patients with FM. This implies that concomitant FM affects the clinical ability to predict activity of disease (11). In another study, it was found that SLE patients with FM, suffered significantly more headaches, morning stiffness, diffuse alopecia, muscle pain, arthralgias, and renal involvement but the presence of FM did not correlate with lupus activity. Although the authors of this small, uncontrolled study noted that FM can interfere with assessment of lupus activity, they maintained that FM itself did not affect SLE activity (28). These findings were recently confirmed in a prospective study of FM in SLE, where clinical measures of disease activity, disease damage, specific organ dysfunction, sociodemographic factors and serologic features were not correlated with FM in their early SLE cohort (13).

There is good evidence that the presence of FM appears to affect the quality of life in a negative manner for lupus patients. Health-related quality of life is impaired in patients who have SLE and those that have FM. Patients with FM in fact have higher levels of impairment compared with SLE patients in several domains (29). In a cross-sectional study of 119 patients with SLE, SLE outcome measures such as disease activity or damage did not correlate with quality of life, but quality of life measured with the SF-36 reflected the presence of FM (24). Another study reported that SLE patients were dissatisfied with their quality of life, and this dissatisfaction was more prominent in those SLE patients who had FM (30).

Moreover, the presence of FM can decrease the productivity of patients who have SLE. Middleton et al. reported that SLE patients with FMS were more likely to be unemployed and receiving disability benefits but did not differ in terms of disease activity measures (10). Friedman et al. reported that SLE patients with FM, had poorer self-reported physical functioning (13).

Conclusions

Fibromyalgia is highly prevalent in patients with SLE. The exact etiology of fibromyalgia in SLE is not known. The clinical examination is the most important way to distinguish fibromyalgia from SLE. The presence of fibromyalgia in SLE patients does not adversely affect their outcomes, but seems to portend a worse quality of life for these patients. Thus, the recognition and treatment of fibromyalgia in SLE patients can have a high impact on their functional status and sense of well being and should be part of the assessment of lupus patients.

The Use of Alternative Therapies in Lupus with Fibromyalgia

Introduction

Complementary and alternative medicine, as defined by the National Center for Complementary and Alternative Medicine (NCCAM), is a group of diverse medical and health care systems, practices, and products that are not

presently considered to be part of conventional medicine. While some scientific evidence exists regarding some CAM therapies, for most there are key questions that are yet to be answered through well-designed scientific studies—whether these therapies are safe and whether they work for the diseases or medical conditions for which they are used (31). Table 63-1 gives a classification of CAM therapies.

Table 63-1: Classification of CAM Therapies

Type	Description	Examples	Comments
Alternative Medical Systems	Alternative medical systems are built upon complete systems of theory and practice.	Homeopathy, ayurvedic medicine and traditional Chinese medicine.	No human clinical trials of these medical systems as a whole exist.
Mind-Body Interventions	Mind-body medicine uses a variety of techniques designed to enhance the mind's capacity to affect bodily function and symptoms	Meditation, prayer, mental healing, and therapies that use creative outlets such as art, music, or dance	No human clinical trials
Biologically Based Therapies	Biologically based therapies in CAM use substances found in nature, such as herbs, foods, and vitamins	Dietary supplements, herbal products and nutraceuticals	Some human trails, summarized in the text
Manipulative and Body-Based Methods	CAM are based on manipulation and/or movement of one or moreparts of the body	Chiropractic, massage therapy or osteopathic manipulations	No human clinical trials
Energy Therapies	Energy therapies involve the use of energy fields. They are of two types: biofield therapies, and biomagnetic based therapies	Qi gong, Reiki, and therapeutic touch	No human clinical trials

Prevalence of CAM Usage in SLE

Twenty-five percent of the general population of the UK, and 15% of Canadians use the services of a complementary medicine provider and about 35% of Americans use some form of complementary therapy (32 ,33 ,34). In rheumatology practices, the use may be even higher, with about two thirds of rheumatology clinic patients reporting CAM use (35), especially for certain conditions such as fibromyalgia and osteoarthritis. A follow-up study of the same patients did not show any difference in outcomes between patients who used CAM and those who did not (36).

CAM usage is very prevalent in SLE patients. Seventy of 107 consecutive SLE patients (65%) attending one of two rheumatology clinics in a Mexican city used alternative medical therapies (37). A large study of 707 patients with SLE from Canada, UK, and the United States, showed that 49.8% of SLE patients used alternative therapies with similar usage rates in all three countries. This study may have underestimated the actual use of CAM therapies, because they assessed CAM use over the last 6 months, rather than a “year prior use” definition used by other studies and it has previously been reported that patients who use CAM may not want to disclose their use of CAM because of fear that their physicians may not approve its use (38).

Generally, users of CAM therapies are younger and better educated than nonusers (38 ,39). SLE patients who use CAM exhibit poorer levels of self-rated health status and satisfaction with health care, though objectively they do not have worse disease. SLE patients who use CAM do not have higher objective disease measurement scores nor do they incur higher indirect costs (such as loss of productivity), thus suggesting that they are not sicker. However, interestingly, they incur larger costs for conventional care, thus suggesting that the use of CAM in lupus patients may be a marker for care-seeking behavior associated with higher consumption of conventional medical resources (38).

Utility of an Evidence-Based Approach to CAM

There is considerable public demand that alternative medical therapies be covered by existing public and private health insurance plans. However, critics of this movement suggest that the costs of nonconventional therapy will substantially increase the costs of caring for lupus patients, and the

integration of unproven therapies will not make the delivery of care more cost-effective. The lay public believes that herbal medicines are safe because they are natural as compared to allopathic medicines, which are manufactured. Although most physicians remain skeptical of nontraditional therapies, patients appear to be satisfied with the complementary approach to their own care (36). Because CAM therapies can have potential side effects and may interact with conventional medications, it is important to obtain a history of use of CAM from patients. In order to engage their patients about CAM usage in a constructive manner, it is imperative that physicians educate themselves about those therapies, for which sound evidence exists and those therapies that may be harmful. Thus, nonconventional therapies should be subjected to rigorous scientific study and the outcomes of diseases treated with complementary therapies measured, so that those therapies with evidence of effectiveness may be integrated with conventional therapies for SLE.

In the next section, the available scientific evidence for the use of nonconventional therapies for SLE is discussed. English language literature from 1966 to 2005 was searched using the PubMed database. Key words included SLE, complementary and alternative therapies, all the common forms of CAM used by lupus patients (see Table 63-2), and the common and scientific names of herbs used for the treatment of SLE. Only those therapies for which human clinical trials have been published have been reviewed below.

Table 63-2: Complementary and Alternative Therapies Used by SLE Patients.

Therapy	Comments
Relaxation techniques	No evidence for efficacy
Massage therapy	No evidence for efficacy
Herbal medicine	Some evidence for efficacy (see text)
Lifestyle diets	No evidence for efficacy
Self-help groups	No evidence for efficacy
Imagery	No evidence for efficacy
Folk remedies	No evidence for efficacy
Spiritual healing	No evidence for efficacy
Chiropractic	No evidence for efficacy
Megavitamin therapy	Some evidence for efficacy (see text)
Homeopathy	No evidence for efficacy
Energy healing	No evidence for efficacy
Acupuncture	Weak evidence for efficacy
Hypnosis	No evidence for efficacy
Copper bracelets/magnets	Weak evidence for pulsed magnetic fields

Adapted from Moore AD, Petri MA, Manzi S, et al. The use of alternative medical therapies in patients with systemic lupus erythematosus. Trination Study Group. *Arthritis Rheum* 2000;43(6):1410–1418.

Dietary Factors in SLE

Although dietary factors are not strictly under the purview of CAM and have been discussed in another chapter (Chapter 57 , Principles of Therapy and Local Measures), alternative practitioners recommend specific diets to patients and hence a brief summary of the main dietary studies in lupus is listed below.

Caloric and protein restriction may have a beneficial role in lupus. Severe caloric restriction in lupus rats is associated with delayed onset of glomerulonephritis (40). The exact etiology of how this works is not known, but animal studies show that caloric restriction is associated with lower levels of proinflammatory cytokines, decreased circulating immune complexes and decreased production of prostaglandin E2 (41). Restricted protein intake is also associated with higher survival in lupus rats. Similarly, diets low in the amino acids phenylalanine (found in beef and dairy products), casein, and tryptophan have been shown to be beneficial in lupus mice. Other animal studies indicate harmful effects of diets rich in saturated fats and omega-6 fatty acids, and L-canaverine (found in alfalfa) in lupus mice. Zinc deficient diets given to MRL/1 mice resulted in improved survival (41).

Nutritional Supplements

Several nutritional factors have been studied in lupus patients including omega 3 fatty acids, vitamin E, vitamin A and selenium. The rationale for their use and the clinical data is presented below.

Rationale for Use

Omega-3 fatty acids inhibit the production of eicosanoids (proinflammatory compounds), whereas omega-6 fatty acids such as arachidonic acid are metabolized into proinflammatory eicosanoids. Omega-3 fatty acids can displace arachidonic acid from cell membranes and compete with arachidonic acid for cyclooxygenase and lipoxygenase enzymes, themselves being poor substrates for cyclooxygenase, the rate limiting step in the synthesis of production of PGE2, a proinflammatory eicosanoids. Omega-3 fatty acids also decrease T-cell activity and cytokine concentration, which has the effect of decreasing the process of peroxidation (the final common pathway in inflammatory tissue damage) and thus free radical damage to tissues. Vitamin E, selenium, and other antioxidants may work further downstream in this process to prevent oxidative tissue damage (41).

Clinical Studies of Supplements

Fish oil supplements retard the development of lupus in mice, and prolong their survival. Fish oil may work through several mechanisms, including its high content of omega-3

fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Although the majority of animal studies show that omega-3 fatty acids ameliorate the severity of autoimmune disease, only modest anti-inflammatory effects have been reported in humans with lupus. In one study, 8 out of 17 SLE patients given 3.7 g of EPA/DHA and 2 of 17 controls improved at 3 months (42). In an uncontrolled study of 12 patients given 6 to 18 g of fish oil, (1.8 to 5.4 g of EPA/DHA), no significant improvement in immune complexes, anti-DNA titer, or prostacyclin was noted (43). In a double-blind cross-over study of fish oil therapy, 26 patients with lupus nephritis were followed for 2 years. The fish oil dietary supplementation had no significant effect on proteinuria, isotope glomerular filtration rate, disease activity index, or steroid consumption. However, it did have a significant effect on lipid levels (44). Pending further studies, fish oil has no proven benefit in human lupus.

Vitamin E use for lupus has been reported since the 1940s, and a review of the literature shows that large doses of vitamin E may be beneficial in some cases. Several studies reporting positive effects of Vitamin E in SLE and other reporting negative results have been published (41). Thus, it is unclear whether the use of vitamin E is effective for SLE. A recent meta-analysis of trials of high dose vitamin E for cardiovascular protection reported higher rates of death in patients taking high dose vitamin E, suggesting that prescribing high dose vitamin E (greater than 400 IU/day) may be unwise at this time (45).

Vitamin A has been reported to be beneficial in SLE. Three patients with skin flares were given 50 mg of beta-carotene three times daily, and experienced clearing of all lesions within 1 week of treatment (46). Other researchers reported benefits of high-dose vitamin A therapy in lupus patients, in a number of immune function parameters (47). However, patients should be advised to avoid ingesting high doses of vitamin A from animal sources, which are fat-soluble and can cause significant toxicity.

Selenium supplementation, when administered along with tocopherol succinate, to lupus mice increases their survival, probably through its effect on increasing glutathione peroxidase activity. However, excess selenium ingestion can lead to toxicity with symptoms of diarrhea, vomiting, hair loss, skin lesions and nervous system dysfunction (41).

Herbal Medications

Flaxseed oil, composed of 70% omega-3 fatty acids, has been shown to have a direct effect on the antibody profile of SLE patients. In a small uncontrolled study, nine patients were enrolled, eight of whom completed the study. After the baseline studies, patients were given 15, 30, and 45 g of flaxseed/day sequentially at 4-week intervals, followed by a 5-week washout period. The 30 g flaxseed/day dosage was well tolerated and conferred the most benefit without side effects in terms of renal function as well as inflammatory and atherogenic mechanisms important in the pathogenesis of lupus nephritis. There were no outcome data reported in this study (48). Patients should be cautioned about the possibility of allergic reactions with flaxseed.

Evening primrose oil, which has a high concentration of linolenic acid (GLA), has been reported to increase survival time in autoimmune mice. No human studies of evening primrose oil in lupus patients have been reported.

Tripterygium wilfordii hook F (TwHF) is a plant that has been used in China for over 2,000 years. TwHF has been found to inhibit IL-2, interferon-1, and prostaglandin E2 (49). During the past three decades, thousands of patients with a variety of autoimmune diseases have been treated successfully with TwHF. However, it should be emphasized that although efficacy has been claimed, few of these claims have been substantiated in randomized controlled trials. TwHF preparations have been used in the treatment of SLE, in 5 open trials in a total of 249 patients. In these trials, treatment with TwHF improved clinical manifestations of SLE, including fatigue, arthralgia, fever, skin rash, lymphadenopathy, hepatomegaly, and laboratory abnormalities, such as proteinuria, thrombocytopenia, antinuclear antibodies, and renal function. In two studies, TwHF treatment reduced prednisone requirements in SLE patients. The results suggest that TwHF might be an alternative or additional drug for those SLE patients in whom steroid therapy is insufficiently effective or contraindicated (50).

The most common side effects of TwHF are diarrhea, nausea, vomiting, hair loss, dryness of mouth, headaches, leukopenia, thrombocytopenia, rash, skin pigmentation, angular stomatitis, oral ulcers, gastritis, abdominal pain, weight gain, weight loss, diastolic hypertension, and vaginal spotting (51). Serious side effects have been reported with the use of TwHF, including infertility, and suppression of lymphocyte proliferation. A young man reportedly died from cardiac toxicity, and teratogenicity has also been reported (50). The possibility that serious side effects may be underreported in clinical trials coupled with the lack of any large randomized controlled trials, places a large question mark on the utility of this medication in management of lupus.

Other Preparations

Other drugs and modalities that have been tried but are only of historical interest include tuberculin, (52) arsenic (53), heliotherapy (54), and hemotherapy (55). Auricula acupuncture (56), hyperbaric oxygen (57), and sarei-to (58) have been reported in case series. Acupuncture is used widely by SLE patients, but a review of published studies showed that the studies lacked sufficient quality (59). Pulsed magnetic fields (60), and pycnogenol phytotherapy (61) have been reported to be useful in lupus as has witchcraft (62).

Conclusions

CAM usage is very prevalent in SLE. There are very few human clinical trials for the commonly used CAM methods in SLE. There is no strong evidence that any of the CAM

therapies have any disease modifying effects. However, there is some suggestion that a few of these therapies may be helpful as adjunct treatments. Generally, any technique that strengthens muscles and promotes aerobic conditioning is acceptable. Some of these also diminish pain, whereas fibromyalgia can be aggravated by some of these techniques, and caution and modifications are advised if synovitis is present. Any activity that works with the mind-body connection, decreases anxiety or promotes restful sleep is usually acceptable. Some therapies such as colonic irrigation can be dangerous in patients who have a thin bowel lining from corticosteroids, and others such as chelation therapies which use intravenous infusions of chemicals can be dangerous.

Given that patients are attracted to CAM, it is important for physicians to maintain an open mind, and have an honest discussion with their patients about the evidence while directing them toward more healthy lifestyles in general, and away from potentially harmful or expensive therapies for which no evidence exists.

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Chapter 64
Hormones and Gender-Related Issues

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Jill P. Buyon

Summary and Precis

The underlying causes of SLE are without a doubt multifactorial, evolving from several alternative triggering events that impair the orderly balance of immune responses in susceptible hosts (1). Hormones are critical to the homeostasis equilibrium of immunity, and hormonal factors might play a significant role in the development of lupus-like disease. During young adulthood (childbearing years), the ratio of women with lupus to men may be as high as 9:1, but this female preponderance is not so striking in children and the elderly (2). Some interesting reports have suggested that a variant of SLE can be seen in men with Klinefelter syndrome (3 ,4) and that men with SLE have unique androgenic profiles (5). Other studies have suggested that estrone metabolites in both men and women seem to favor 16-hydroxylated metabolites (6 ,7), that women with SLE oxidize testosterone to androstenedione at a greater rate than males, and that lupus, like many other chronic diseases, can result in low levels of the weak androgen, dehydroepiandrosterone (DHEA) and its inactive sulfated form, DHEA-S, which is thought to provide a reservoir for sex steroids in healthy individuals (8 ,9 ,10 ,11 ,12). However, despite characteristic hormonal abnormalities, it should be stressed that SLE is not strongly associated with infertility or any other identifiable gender-linked disparities in either men or women.

Until recently, the use of hormonal therapies for lupus patients was in broad disfavor, which seemed logical based on the observations cited above, and a significant experimental literature in murine models (13). Additionally, there were published reports of patients suffering disease flares while receiving exogenous hormones, one of which was a retrospective study in patients with pre-existing renal disease (15 ,16 ,17 ,18 ,19 ,20 ,21 ,22 ,23 ,24).

However, hormonal imbalance need not dictate that all exogenous hormone combinations would be necessarily toxic. The perceived advantages to hormonal use in some clinical situations (effective birth control, osteoporosis prevention, ovulation induction, and preservation of fertility in patients receiving cyclophosphamide), suggested a need to determine if such therapies could be used safely or at least within a rational therapeutic window. The potential impact of this possibility should not be underestimated. The rate of elective abortion in SLE patients approaches 23% in some reports (25 ,26 ,27). Although the cardiac and neuropsychologic effects of estrogen replacement have become controversial, osteoporosis is a major cause of damage in SLE patients (Fig. 64-1) and up to 20% of patients may have vertebral fractures when carefully studied (28). Premenopausal patients are not optimal candidates for bisphosphonate therapy, particular in the setting of inadequate birth control. Finally, there may be subsets of SLE patients who could derive a therapeutic benefit from hormone supplementation. This hypothesis arises from the intriguing clinical observation is that SLE patients have a tendency to experience disease exacerbations around the time of menses, when estrogen levels drop, as opposed to being elevated. Two reports have even suggested that, despite the experience that hormones may trigger flares in some individuals, oral contraceptive pills (OCPs) can in fact control cyclical or other disease activity in certain SLE patients (29 ,30).

Finally, some theoretical benefits may derive from supplementation with DHEA, which exerts androgenic effects on its own, but is predominantly metabolized to female hormones in women. To date, pivotal trials of this agent have failed to meet their primary endpoints, despite data suggestive of potential therapeutic responses in some patients. The current review will summarize clinical experience with female hormone supplementation and weak androgen therapy in SLE, and discuss the immunologic basis for their use. Other endocrine disorders associated with SLE will be briefly reviewed, although the literature is less extensive in these areas.

Female Hormone Supplementation for Birth Control or Postmenopausal Replacement

Hormonal contraception is the most effective form of birth control and is the preferred method of the majority of Caucasian and African-American women between 15 to 44 years (31). In 1988, approximately 10.7 million women in the United States were taking oral contraceptives (32). This form of contraception has the added advantage that it may protect against ovarian and endometrial cancers (33).

Combination OCP contain both synthetic estrogen and progesterone preventing ovulation for the most part by inhibiting gonadotropin secretion, progesterone-suppressing luteinizing hormone, and estrogen suppressing follicle-stimulating hormone. Additionally, estrogen stabilizes

endometrium to prevent bleeding, and progesterone increases cervical mucus viscosity, decreases tubal peristalsis, and inhibits the endometrium from supporting a pregnancy. Each of the progestational effects is enhanced by the estrogen supplement contained in the combined pill (33).

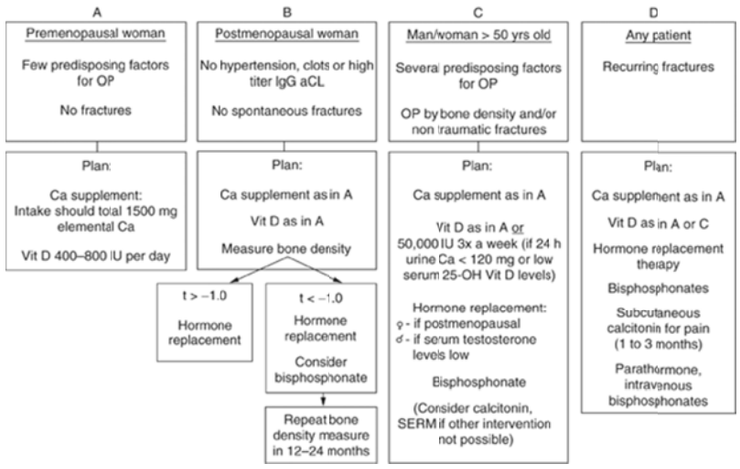

Figure 64-1. Algorithm for prevention and treatment of osteoporosis in patients with systemic lupus erythematosus.

Progesterone-only pills are potential alternatives to classic combination oral contraceptives in patients with SLE, and two studies found no instigation of flares with these treatments (14 ,34). The arguments for progesterone-only in SLE are clear. In the NZB/NZW experimental murine lupus model, progesterone prolonged survival in castrated males, was less deleterious in castrated females than estrogen, but was associated with high levels of autoantibodies in both genders (13).

Additionally, progesterone may be less thrombogenic than estrogen (35), a significant consideration for lupus patients who are already at increased risk for thrombosis and accelerated atherosclerosis. Unfortunately progesterone-only pills may be less effective as contraceptives than the combination pill (31) and are associated with more ectopic pregnancies than combination contraceptives. Thirdly, progestins may inhibit beneficial effects of estrogen on adverse lipid profiles (36 ,37), which are often seen in lupus patients. Unpredictable breakthrough bleeding is also seen with progesterone treatment, and this has been reported to occur in lupus patients (Table 64-1) (34 ,38 ,39).

Potential Benefits and Risks of Hormone Therapy for Women with SLE

Estrogen elicits both direct (40) and indirect effects on bone metabolism. Positive changes in calcium homeostasis are achieved by enhancing synthesis of 1,25 (OH)2D and absorption of calcium in the intestines (41). Additionally, the bone-resorbing cytokines interleukin-1 and interleukin-6 are inhibited by estrogens (42 ,43). The net effect is not only

to prevent bone loss but also to increase bone mass in individuals with osteopenia (44 ,45 ,46 ,47 ,48). Most of the increase in bone mass occurs during the first 12 months of therapy (47 ,48) consistent with a model that the primary mechanism is inhibition of bone resorption, allowing the ongoing complementary process of bone formation to reinstitute the bone mass over a prolonged period (49).

Table 64-1: Comparative Plasma Levels of 17 ß-Estradiol (pg/mL)

Menstrual cycle	
Peak (day 12–14, late follicular)	200–500
Nadir (day 1, early follicular)	40–100
Pregnancy	
Peak (38–40 wk)	16,000–30,000
Ovulation induction	1,000
Menopause	5–20
Estrogen replacement	
Premarin 0.625 mg	40–100
Transdermal (after 6 months of use)	40–100

Many endogenous and exogenous factors contribute to the development of osteoporosis, including genetics, caffeine, alcohol, smoking, body stature, physical activity levels, renal disorder, thyroid disease, and treatment with glucocorticoids (50). Glucocorticoid use contributes significantly to risk of osteoporosis in women with SLE. Ramsey-Goldman et al. surveyed the frequency of fractures and associated risk factors in 702 women with lupus who had been followed for 5,951 person-years (51) and found that fractures occurred in 12.3% of the patients, an almost fivefold increase compared with a background population. Older age at diagnosis and longer duration of steroid use were important variables. Two recent studies addressed bone mineral density (BMD) in premenopausal women with SLE. Sinigaglia et al. reported osteoporosis in 22.6% of 84 premenopausal patients, and both disease duration and glucocorticoids were associated risks (52). Gilboe et al. observed similar results in a study of 75 SLE (combined pre- and postmenopausal) patients and concluded that premenopausal patients taking glucocorticoids were at particularly high risk (53). Not surprisingly, observational studies suggest that women who receive OCP may have higher adjusted BMD than women who do not (54). The American College of Rheumatology (ACR) Task Force on Osteoporosis Guidelines has in fact recommended OCP to prevent glucocorticoid-induced osteoporosis in premeno-pausal women with oligo- or amenorrhea (55).

Despite recent controversies, to be discussed below, a significant literature supports the hypothesis that estrogen supplementation, in as yet to be determined formulations, given to appropriate patients, may provide significant protection against the development of atherosclerosis. Prior to menopause, the incidence of atherosclerosis-related events is eight times less common for women than for men (56). Following menopause and its associated decrease in ovarian hormone production, the rate of cardiovascular disease accelerates rapidly, so that by age 65 the risk in women is equal to that of men.

Estrogens exert several biological properties that should be cardio-protective, including favorable effects on the lipid profile, stabilizing effects on vascular endothelium, and improved fibrinolysis (57 ,58 ,59). Estrogens increase HDL-cholesterol and decrease LDL and lipoprotein (a) (60). Estradiol decreases circulating P-selectin, which connects leukocytes to endothelial cells and activated platelets (61) and reduces the vascular response in an in vitro model of immune injury, the rabbit cardiac allograft, which may mimic the pathogenesis of SLE accelerated atherosclerosis (62). Levels of plasminogen-activator inhibitor type 1 (PAI-1) (fibrinolysis inhibitor) has been linked to risk of cardiovascular disease (63 ,64 ,65 ,66). Exogenous estrogen can significantly reduce PAI-1 levels in postmenopausal women (67 ,68 ,69 ,70). Observational studies additionally suggest that estrogen levels correlate with decreased homocysteine, a risk factor for vascular occlusion (71). Finally, estrogens have an antioxidant effects, inhibiting important steps in atherosclerotic plaque development (60 ,72).

Most clinical data on the benefits and risks of hormone replacement is retrospective and uncontrolled. The only published controlled, prospective trial of HRT effects on cardiovascular outcomes, the Heart Estrogen-Progestin Replacement Study (HERS) had a negative result, showing no net benefit of estrogen plus progestin treatment over a 4-year period in women with previously established coronary artery disease (73). Additionally, hormone replacement was associated with an increased risk for coronary thrombotic events, limited to the first year of treatment, in this higher risk group of women (73). This does not prove, with finality, an absence of cardioprotective benefits for estrogen in general, but rather illustrates the complexities in the acute and chronic effects of estrogen and the contextual caution with which any further exploration of its use in lupus (or in any group of women who may have pre-existing heart disease) needs to be considered. The increased acute estrogen-induced thrombotic risk may outweigh its protective effects on atherosclerosis in women with established plaque, at least for the first years before longer term plaque-preventing effects can begin to have a major impact. It should be stressed that several clinical and animal studies in lower risk populations, have weighed in with positive cardioprotective outcomes, including the large Nurses Health Study (74 ,75 ,76 ,77 ,78 ,79). These considerations are of critical importance to patients with SLE given mounting evidence that accelerated development of atherosclerosis is a significant risk for morbidity and mortality in these patients (80 ,81 ,82 ,83 ,84 ,85 ,86 ,87). However, the subset of patients with antiphospholipid antibodies, with a substantial literature supporting a measurable risk of thrombosis, may be a group for whom hormonal supplementation is currently out of the question, supported by the observation of Asherson et al. describing 10 patients with aPL, all of whom developed vascular complications while taking oral contraception (88).

Several biologic properties of estrogens and aPL may contribute to similar pathways leading to thrombogenesis potential. It is generally accepted that platelets play a critical role in the dynamic regulation of arterial forms of thrombosis, whereas disturbances of the clotting cascade most frequently result in venous thrombosis. Patients taking estrogens or combined OCP may have increased platelet aggregation (89), but these effects are not clearly distinguishable from the associated factor of concomitant cigarette smoking (90). Estrogens can increase the concentration of coagulation factors VII, IX, X, XII, and prothrombin (reviewed in (91)). Most patients taking OCP have decreased partial thromboplastin times and prothrombin times, primarily as a result of increased levels of fibrinogen (35). Both estrogens and aPL can inhibit prostacyclin production by endothelial cells (92 ,93). Synthetic estrogens are more “procoagulant” than natural preparations, and oral

estrogen formulations influence coagulation to a greater degree than transdermal preparations, since the latter avoid the first-passage effect of oral estrogens (91). Importantly, the impact of estrogen on various clotting factors is dose dependent, with little if any effect at doses less than 50 µg of ethinyl estradiol (94). The U.S. Food and Drug Administration (FDA) issued a bulletin stating that the rate of DVT in women taking low-dose OCP containing 20 to 40 µg ethinyl estradiol was 4 per 10,000, which approximates that of women not taking OCP, 3 per 10,000 (95).

Experimental data suggest that “antiphospholipid” antibodies include specificities that recognize a number of coagulation-regulating proteins, the best characterized of which is $ß_2$GPI (96 ,97 ,98). which inhibits contact activation of the intrinsic coagulation pathway (99), platelet prothrombinase activity (100), and ADP-induced platelet aggregation (101). Since $ß_2$GPI has been shown to possess multiple inhibitory functions in coagulation pathways, its interaction with aPL may eventually lead to an explanation for the thrombotic diathesis observed in patients with these antibodies. Preliminary data support a decrease in the circulating levels of $ß_2$GPI in the second trimester of pregnancy (102), but the effect of exogenous hormones on these levels is unknown.

A known prothrombotic effect of OCPs is a decrease in the anticoagulant, protein S (103). In addition, inflammatory diseases such as SLE, may also contribute to an acquired protein S deficiency, whereby protein S may be excessively bound by a complement protein, C4BP, which is its major plasma inhibitor. In most acute phase responses, the relationship between free (active) and bound (inhibited) protein S is preserved (104), but this breaks down in dysregulated inflammatory states such as sepsis, disseminated intravascular coagulation (DIC), and flares of lupus (105 ,106 ,107 ,108). Autoantibodies to protein S are common in patients with lupus and the antiphospholipid syndrome, further suggesting relationships between hormonal regulatory pathways and effects of antiphospholipid-related autoantibodies on coagulation. It could be hoped that in the future, rational pharmacology might develop from the application of better knowledge about the pros and cons of hormonal agents on pathways destabilized by antiphospholipid-related antibodies.

The Use of Gonadotropin Agonists to Protect from Cyclophosphamide-Induced Infertility

Empiric use of leuprolide acetate, a gonadotropin-releasing hormone agonist, to protect against ovarian failure in lupus patients receiving cyclophosphamide had evolved through preliminary reports and anecdotal exchanges in some centers over a number of years. Therefore a recent published series involving 40 women with severe SLE receiving cyclophosphamide (half of whom were treated with a depot leuprolide acetate) was of interest (109). One out of 20 patients who received leuprolide developed premature ovarian failure versus 6 of 20 controls (30%), who had been matched by age and cumulative dose of cyclophosphamide. This was a statistically and clinically significant finding. Despite the theoretical issue of whether gonadotropin agonists could provoke SLE flare (110) and one case report of lupus nephritis after leuprolide treatment, (111) it is usually considered reasonable to consider this kind of therapy while patients are receiving aggressive immune suppression. On the other hand, the use of leuprolide is not without other risks. The association of this treatment with changes in coagulation parameters and/or thrombotic manifestations (112 ,113 ,114 ,115 ,116) should be carefully factored in to decision making for this high risk population. Extended use of leuprolide is also associated with bone mineral loss (117 ,118), although the latter is likely to be of minor concern in the context of the disease and treatments for which it would be considered here.

Do Hormones Exacerbate SLE Disease Activity?

What is the evidence that oral contraceptives might exacerbate lupus disease activity? The largest database evaluating this question examined oral contraceptive history and onset of SLE in 121,645 women (119). In this robustly powered study, the use of OCP was associated with a small risk (1.4) for the development of SLE, however the clinical significance of this in the context of a relatively rare disease remains uncertain. Some case reports have described lupus onset in the context of OCP use as well (15 ,16 ,17). However, in at least one retrospective series, lupus patients taking OCP did not experience increased flares as compared to a control group (38). A survey study on the use of OCP in patients with an established diagnosis of lupus found that there were no differences in the percentages of lupus vs. healthy control participants who had ever used oral contraceptives, but far less use after the diagnosis of lupus, despite the fact that overall these pills were reported to be well tolerated with a calculated flare rate of 0.45 per 100 patient months (23). Importantly, oral contraceptives have evolved over the years, so that previous doses of up to 50 µg ethinyl estradiol have been largely replaced with preparations containing 30 to 35 µg.

The SELENA study was a two-armed prospective study to evaluate the safety of oral contraceptives and estrogen replacement therapy in lupus patients. In the oral contraceptive arm (120), 183 premenopausal patients were randomized to placebo or triphasic 35 mg ethinylestradiol/0.5 to 1 mg norethindrone for twelve 28-day cycles. Severe flares were rare occurring in 7/91 (7.7%) of patients taking oral contraceptive versus 7/92 (7.6%) on placebo. Mild/moderate flares were also equivalent: 1.41 versus 1.40 flares/person-years (oral contraceptive vs placebo) and there was no significant difference in the 12-month combined flare rates (70% vs. 60%; $P = 0.42$). There was 1 DVT in the OC arm, and 1 ocular thrombosis and 1 superficial thrombophlebitis in the placebo arm. One death (placebo) occurred after trial cessation.

It was concluded that OC do not increase the rate of severe or mild/moderate flares in SLE, although patients with unstable SLE activity or at increased risk for thrombosis were excluded from this study.

Table 64-2: Guidelines for Use of Oral Contraceptives in Women with Systemic Lupus Erythematosus

1. Inactive or stable/moderate disease
2. No history of venous or arterial thrombosis
3. IgGaPL <40, IgMaPL <40, IgAaPL <50, no circulating lupus anticoagulant (unknown if presence of low-to-moderate titer of aPL in the absence of a previous thrombosis is contraindication)
4. Nonsmoker
5. Normotensive
6. For combined pill, use lowest dose of ethinyl estradiol (30–35 μg)
7. Patient without migraine headaches
8. Can add low-dose aspirin therapy to hormone regimen if there is concern about risk factors

In the hormone replacement arm of the SELENA study, 351 menopausal lupus patients were randomly assigned to either placebo or Premarin plus discontinuous progesterone for 12 months (121). Severe flares were infrequent in both groups and were not significantly increased in women taking hormone therapy. Women taking hormone replacement had more mild to moderate flares than those taking placebo (1.14 flares vs. 0.86 flares/person/year) although the clinical significance of this was not concerning. Four women taking the hormones and one woman taking placebo had thromboembolic (blood clotting) events. Women with high levels of anticardiolipin antibodies, lupus anticoagulant, or previous incidences of thrombosis were excluded from the study at the onset. On the basis of these data it was concluded that adding a short course of hormone therapy might be associated with a small risk for increasing the flare rate of lupus, but most of the flares recorded were mild to moderate, and in many or even most patients the contained risks observed in this study might be favorably weighed against the potential amelioration of peri-menopausal symptoms (Table 64-2).

Could Androgens Benefit Women with SLE?

Deficiency of the weak androgen dehydroepiandrosterone (DHEA), and its primary metabolite DHEA-sulfate have been reported in chronic medical conditions, including SLE (8 ,9 ,10 ,11 ,12), and DHEA might have impact on a number of immunologic functions relevant to SLE. Endogenous circulating DHEA levels vary widely by gender, age and ethnicity, and can be affected by changes in corticosteroid levels, alcohol intake, smoking, body mass index, medications, and thyroid function (122 ,123). With this level of complexity in DHEA metabolism it is not surprising that clinical confirmation of efficacy in SLE has been inconsistent and controversial, hampering drug development for this theoretically promising treatment.

The pharmacokinetics and metabolism of DHEA depends on the target organ. Aromatase and 17-β-hydroxylase activity are increased in breast and other adipose tissue, suggesting that obesity might increase the conversion of DHEA to estrogens (124). Selective expression of 17-β-hydroxylase genes in various organs suggests that the liver, ovary, endometrium, and testis are prominent sites for estrogen synthesis and that placenta, liver, testis, endometrium, prostate, adrenal and skin are regions where androgen synthesis takes place (125). The skin has a high expression of enzymes required to transform DHEA into dihydrotestosterone, whereas conversion of DHEA in the vagina would be mainly to estrogens (126 ,127). The net effects of DHEA supplementation could be estrogenic or androgenic, then, and either conducive or inhibitory to health or disease depending on the hormonal and metabolic background and the responsiveness of the target organ(s) to hormonal effects. This concept is critical to understand, since low levels of DHEA are associated with breast cancer risk in premenopausal patients, whereas high levels have been associated with breast cancer risk in postmenopausal patients. It stands to reason, then, that when considering DHEA for SLE, it might require individualized approach to optimize dosing and administration of this agent in specific patients.

Theoretical Background for DHEA Supplementation in Lupus

Estrogens have been observed to increase autoantibody production whereas androgens can have the opposite effect (128). In mouse models of lupus, low DHEA is associated with the Th2 dominant cytokine profiles characteristic of B cell autoimmunity (129). Exogenously administered DHEA induced a change in the cytokines to a Th1-characteristic profile (130 ,131). DHEA and corticosteroids sometimes have opposite effects on gene expression critical to immunity (132), and in the case of the immune system, a finely tuned system of checks and balances, these kinds of opposing actions might be therapeutic or harmful, depending on the degree of change, and the immunologic background.

Low DHEA levels in lupus patients have been reported in a number of studies (8 ,9 ,10 ,11 ,12), but it remains unclear to what degree this is pathogenic or simply reflective of chronic disease, given the many disorders, including normal aging in which DHEA levels are seen to drop. Low DHEA levels have been found at the onset of lupus, prior to corticosteroid treatment, although an inverse relationship to corticosteroid levels has also been described (9 ,10 ,11 ,12). This suggests that adrenal androgen deficiency might be intrinsic to SLE and might be worsened by corticosteroid treatment, despite the therapeutic

benefits that also occur with corticosteroids. In pregnant SLE patients, estradiol, progesterone, and DHEAS concentrations seem to be decreased as compared to healthy controls (133).

DHEAS delays the onset of lupus-like disease, prevents formation of antibodies to dsDNA, and prolongs survival in the NZB/W F1 female murine lupus model (134 ,135 ,136). It increases levels of the cytokine IL-2 in both murine models and human studies (10 ,11 ,137). An association between interferon gamma production and DHEAS levels has been reported in healthy individuals, but was found to be lacking in lupus patients (9), suggesting a peculiar defect in lupus T cells. Additionally, DHEA has been found to inhibit apoptosis of blood cells isolated from SLE patients, which, in general might be guessed to increase, not decrease risk for lupus-like disease (138).

Clinical Trials of DHEA in Lupus Patients

van Vollenhoven reported the treatment of ten female patients with mild to moderate SLE who were given 200 mg/day of DHEA orally (139). After 3 to 6 months of treatment, global disease activity (SLEDAI), physician's global assessment were improved, and corticosteroid requirements were lessened. In a subsequent double-blind, placebo-controlled, randomized trial in 28 female patients with mild to moderate SLE, 200 mg/day of DHEA was associated with improvement in the SLEDAI score, and improved patient's and physician's global assessments (140). Prednisone dose was also decreased in the DHEA-treated group. In another report, 50 female patients with mild to moderate SLE were treated with oral DHEA 50 to 200 mg/day. DHEA therapy increased serum levels of DHEA, DHEA sulfate, and testosterone and was associated with. decreased disease activity and lowered prednisone doses (141). In a multicenter randomized, double-blind, placebo-controlled trial conducted in Asia, 120 adult women with active SLE were treated with 200 mg/day DHEA or placebo for 24 weeks leading to significantly decreased flares in the DHEA-treated group, and improved patient's global assessment suggesting the possibility that this treatment could have an impact on the quality of life in these patients (142). However, global disease activity using the SLAM was not affected by treatment in this study.

In a double-blind, randomized trial 191 female SLE patients receiving moderate doses of prednisone (10–30 mg/day) were given either placebo, 100 mg or 200 mg of oral DHEA and subjected to a protocol steroid taper. Patients with sustained reduction of prednisone (< or = 7.5 mg/day) were considered responders, and this definition was met by 41% of the placebo-treated group, 44% of the 100-mg prasterone group, and 55% of the 200-mg group (*p* = ns). However, when only those patients who had active disease at baseline were evaluated, 29%, 38%, and 51%, respectively, were responders and there was a statistically significant difference between the 200 mg treatment group and the placebo group (143).

Only one trial has evaluated DHEA for severe lupus. This was a double-blind, placebo-controlled, randomized clinical study in 21 patients with nephritis, serositis or hematological abnormalities (144). Patients were treated with DHEA 200 mg/day versus placebo for 6 months, followed by a 6-month open label period, given in addition to corticosteroids and immune suppressive therapies as warranted. Improvement in the principal baseline manifestation was seen in 7 of 9 evaluable patients on DHEA versus 4 of 10 of the evaluable patients on placebo and the mean SLEDAI improvement was also greater in the DHEA-treated group. These differences were not statistically significant, although the DHEA group was found to have greater disease activity at baseline. Furthermore, this study was underpowered for the detection of what could potentially be clinically significant differences.

A significant positive relationship between DHEAS and bone mineral density has been reported in premenopausal women with SLE, as well as a negative relationship between DHEAS and glucocorticoid dose and iPTH (145). Significant differences in bone mineral density changes were found in treatment versus placebo groups for lupus patients treated with DHEA (144), and thus DHEA seemed to have a potential bone-protecting effect in lupus patients. This was not borne out in a recent study in which osteoporosis protection would have been the primary outcome.

The most common side effects of DHEA are linked to its androgenic effects and include acne, hirsutism, and the potential for unfavorable effects on lipid metabolism. As mentioned previously some studies have confirmed androgenic effects on lipids, in particular that DHEA lowers high-density lipoprotein (HDL) (146). Most of the expected side effects from DHEA were borne out and no new side effects of concern were reported in the lupus studies (140 ,141 ,142 ,143 ,144 ,145 ,146), suggesting an overall good safety profile. In considering the long-term use of this agent however (which might come under serious consideration for patients with lupus), the possible growth-stimulating effects on hormone-dependent malignancies particularly of the prostate or breast should be kept in mind as well as the theoretical potential for DHEA to exacerbate lupus in some clinical situations via its immunostimulating and antiglucocorticoid effects (147). This might be a relevant issue in patients with more severe lupus which may be more likely to involve a complex, mixed immune disorder or in patients who rely on glucocorticoids for clinical stability. Finally, it is possible that DHEA or its metabolites may have effects on intracellular signaling pathways that are as yet largely unexplored (10 ,11). For this reason side effects might still occur in susceptible patients that are currently unforeseen.

Inflammatory Mediators and Female Hormones

The regulation of the menstrual cycle and/or invasion and implantation of the uterine wall by an embryo, requires an ebb and flow of inflammatory mediators, which is unique

to the adult female environment. The need to protect a fetus from immune attack without initiating graft-versus-host disease also sets a high bar for balanced immune regulation and counter-regulation. This explains the fundamental requirement, in women, for vascular-based inflammatory responses by hormones. For example, progesterone is important in suppressing the inflammatory reaction that would be expected in response to the presence of a foreign body, such as an embryo. Populations of macrophages and neutrophils in the uterus are under the control of estrogen and progesterone, and progesterone can antagonize the ability of estrogen to recruit macrophages and neutrophils into the mouse uterus (148). Progesterone may also suppress interleukin-8 and cyclooxygenase-2 expression (149), suggesting that progesterone withdrawal at the time of menstruation might promote these inflammatory mediators in preparation for the increased tissue inflammation that accompanies the extrusion process.

Complex Effects of Sex Hormones on Inflammation

Estrogen replacement in postmenopausal women may increase C-reactive protein (150 ,151) while decreasing a number of other inflammatory mediators (152 ,153 ,154 ,155). In the context of a rapidly advancing literature, it may be that a complex network of endometrial cytokines are normally regulated by hormones produced during the ovulatory cycle (156). However, signal cascades are two-way streets, so, inflammatory mediators will also have impact on hormones. For example, lipopolysaccharide, a potent stimulator of monocytes, induces a lengthening of the follicular phase in monkeys associated with decreased estradiol concentrations and increased pituitary release of LH and FSH (157). Complex relationships between hormones and vasculature, inflammatory mediators and vasculature, and hormones and inflammatory mediators are intrinsic to reproduction and the preservation of the species, which sets the stage for the female preponderance of autoimmune diseases.

Sex Hormones, Immune Activation, and the Vascular System

Estrogen receptors are found on human monocytes, B cells, and T cells, indicating a direct role for estrogens in the regulation of immune cell activation (158). General B cell activities seem to be enhanced by estrogen whereas T cell reactivity may be suppressed (159). In rats, endotoxin-induced expression of adhesion molecules, associated with an influx of inflammatory cells, increases in pregnant rats as compared to hormonally cyclic animals (160). Estrogen and/or progesterone are now known to enhance numerous inflammatory events, but, importantly, the differences in their effects against different hormonal background is simply not known (161 ,162 ,163 ,164). On the other hand, estrogen has been found to decrease class II expression in vascular allografts, accompanied by decreased inflammatory cell infiltration (165). Low doses of 17-ß-estradiol have been found to inhibit interleukin-6 secretion by human endothelial cells (166).

Inflammation and inflammation-induced coagulation mechanisms are sometimes predictors of cardiovascular events (167 ,168 ,169 ,170 ,171 ,172 ,173). Because of this there may be indirect effects of sex steroids on the risk of vasculature thrombosis through immune-modulating effects. For example, estrogen can improve markers of fibrinolysis and vascular inflammation in arteries of postmenopausal women (152) as well as having other anti-inflammatory properties which may have beneficial impact on cardiovascular risk (174). Atherosclerotic plaque demonstrates features similar to inflammation. Inflammatory cytokines induce adhesion molecules in blood vessel walls, augmenting inflammatory cell adhesion and subsequent development of atherosclerosis. One study has observed a statistically significant increase in several of these adhesion molecules when men and untreated postmenopausal women with coronary artery disease were compared with postmenopausal women with coronary disease who were receiving estrogen replacement (175). This contradicts other findings, mentioned above, which suggest that estrogen can augment adhesion molecule activity (160 ,176). These issues may resolve around the multiple signaling pathways and dose-responsiveness of estrogen therapies. Estrogen, then, may either augment or help to limit the inflammatory response to injury by modulating the expression of endothelial adhesion molecules. And, as discussed in the first part of this chapter, it may take a sophisticated set of biomarkers in order to utilize estrogen for the benefit and minimize risks in postmenopausal women at risk for atherosclerosis.

Menstrual Irregularities, Sexual Dysfunction, and Pregnancy Complications

Although reproductive abnormalities may be a frequent feature of SLE, much of the available literature is in the form of abstracts, editorial letters, and/or case reviews. Some reports suggest the possibility that SLE patients can develop significant menstrual irregularities and/or endometriosis (177 ,178 ,179 ,180 ,181 ,182); however, this has not been systematically examined. Several interesting papers have correlated flares to menstrual cycles, and observed that patients seem to flare more often in the second half of the cycle or just before menses (183 ,184 ,185). Sexual dysfunction may occur in SLE secondary to direct or indirect effects of disease activity, disturbances of the hypothalamic-pituitary axis or toxic effects of immune suppressants (186). Antisperm antibodies have been recorded in both male and female patients (187 ,188), correlating to anti-DNA antibodies and disease activity. Autoantibodies are known to have the potential to interfere with fertilization, implantation, embryonic development and placental function (186). Antiphospholipid and other related autoantibodies are directly associated with embryonic or fetal losses and other pregnancy complications such as preeclampsia/

eclampsia and the HELLP syndrome (189). However, there is no evidence that fertility is impaired overall in either male or female lupus patients.

Table 64-3: Thyroid Function and Antibody Studies in Systemic Lupus Erythematosus

Parameter	Gordon and Isenberg (237)	Byron and Mowat (200)	Goh and Wang (236)	Miller et al. (239)	Ropes (238)	Boey et al. (240)
Cases (*n*)	41	64	319	332	142	129
Hypothyroid (%)	9.8	4.7	0.9	6.6	0.7	5.0
Hyperthyroid (%)	2.4	10.9	2.8	5.0	—	8.9
Hashimoto disease (%)	—	—	0.6	—	2.1	3.9
Thyroid antibodies (%)	—	—	—	20	—	32.2

Thyroid Disorders and Their Relationship to SLE

Overlap between thyroiditis and other autoimmune or rheumatic disorders is described in some studies (190 ,191 ,192 ,193 ,194); however, like other endocrine disorders in lupus, some of the most intriguing reports are in the form of abstracts or letters. Although there are few formal incidence studies, data from the British National Health Service suggests that thyroid disease may be more frequent in SLE than in the general population (195). In one autopsy study 2 of 74 patients with thyroiditis had SLE opposed to none in a matched control group (196). Symptoms and signs of thyroid disorders may overlap with those of SLE and Petri et al. have reported that 46% of patients with autoimmune thyroid disease have a positive ANA. Similarly, testing for TSH levels may uncover subclinical thyroid disease in patients with SLE (197 ,198 ,199). Studies of SLE patients suggest that hyperthyroidism can precede the diagnosis of SLE, which may indeed arise in the context of treatments for the former disorder (197 ,198 ,199 ,200). Associations between hypothyroidism, SLE, and red cell aplasia have been reported as well as combined overlap of SLE, thyroiditis and Sjogren syndrome (201 ,202 ,203). However, no common risk factors or integrated pathophysiologic insights have as yet emerged from these reports. An antimicrosomal antibody (anti-Mic-1) has been observed in patients with SLE and hyperthyroidism but not in patients with Hashimoto thyroiditis or rheumatoid arthritis (204 ,205), as well as some evidence that thyroid peroxidase antibodies in SLE may be different from those in primary Hashimoto disease (206). Anti-TSH antibodies have also been reported in association with SLE (207 ,208) supporting a model of autoimmunity as an overlapping spectrum of disorders (Table 64-3).

Other Endocrine Disorders

As above, some of the most pertinent reports on other endocrine disorders in lupus are preliminary or only available in abstract form. Positive ANA has been described in up to 41% of patients with type I diabetes (209) and mild features of SLE have been reported in up to 30% of patients with insulin receptor antibodies (210 ,211 ,212 ,213 ,214). Seven percent of patients in the Johns Hopkins SLE cohort have been reported as diabetic (215). Steroid-induced hyperglycemia is common in the SLE population and some evidence suggests an association of this complication with anti-insulin antibodies (216).

Cushing syndrome and adrenal insufficiency are of significant concern in the lupus population, given the frequent induction and/or tapering of steroids in these patients. Adrenal insufficiency has also been described in the context of cortical infarction (217 ,218 ,219 ,220 ,221 ,222), amyloid or adrenal hemorrhage (210 ,223 ,224 ,225 ,226). Not surprisingly, in two case reports, SLE disease activity improved with the onset of Cushing disease (227) and was observed to flare after a pituitary adenectomy in a patient with Cushing syndrome (228).

Hyperprolactinemia has been described in up to 20% of patients with SLE (229 ,230 ,231), associated with increased prolactin/cortisol rations, corticosteroid use, antiprolactin antibodies and/or CNS lupus manifestations. Some of those reports suggest that there may be some improvement with bromocriptine therapy. Correlations between prolactin and SLE disease activity have been found in some but not all studies (232 ,233 ,234 ,235). Interpretation of this literature is hampered by differences in assays and in the assessment of disease activity.

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Chapter 65

Clinical and Management Aspects of the Antiphospholipid Antibody Syndrome

Michelle Petri

Antiphospholipid (aPL) antibody syndrome now is recognized to be a misnomer. The field is more accurately described as antibody-mediated thrombosis, because multiple antibodies directed against plasma proteins, with or without attached phospholipid, can lead to hypercoagulability. These antibodies likely act by way of multiple mechanisms. This chapter will review: (a) the aPL and anti-plasma protein antibodies of clinical importance; (b) laboratory assays that the clinician must interpret; (c) epidemiology; (d) clinical presentations; and (e) treatment.

Characteristics of Antiphospholipid Antibodies

The original triumvirate of aPL antibodies included the false-positive test for syphilis, the lupus anticoagulant (LA), and anticardiolipin (aCL) antibody. The false-positive test for syphilis has not been associated with thrombosis in all studies, but will be described here, for completeness. The list of "new" aPL and anti-plasma-protein antibodies continues to lengthen. Several of these, including anti-$ß_2$-glycoprotein I ($ß_2$GPI), and antiprothrombin, may have clinical utility, and will be discussed.

False-Positive Test for Syphilis

The biologic false-positive test for syphilis was the first aPL to be recognized (1). Patients with a false-positive test were found to be at risk (5% to 19%) for the development of lupus or another connective tissue disease (1 ,2), but they did not seem to be at increased risk for thrombosis or pregnancy loss. The Venereal Disease Research Laboratory (VDRL) titer correlates with aCL levels only in sera from syphilis, not in sera from autoimmune patients (3). Mixing cardiolipin with phosphatidylcholine and cholesterol, as is done in the VDRL antigen, improves the binding of syphilitic aPL antibodies, but it decreases the binding by autoimmune aPL antibodies (3 ,4). It now is understood that aCL antibodies in patients with syphilis are not dependent on $ß_2$GPI (5). Patients may have the LA or aCL antibody and not have the false-positive test for syphilis, and vice versa. However, we have found a correlation of the false-positive test for syphilis with multiple other aPL antibodies, including anti-$ß_2$GPI. Additionally, in the Hopkins Lupus Cohort, a false-positive test for syphilis is predictive of later thrombosis.

Lupus Anticoagulant

A circulating anticoagulant in three patients (two of whom had probable autoimmune disease) was reported in 1948 by Conley et al. (6), who recognized that it blocked the conversion of prothrombin to thrombin. Although patients with LA who had a hemorrhagic diathesis tended to have a second coagulation defect (7), the term "lupus anticoagulant" became accepted. The second irony of this term is that as many as one half of patients with LA do not have lupus (8 ,9).

LAs, which are IgG or IgM immunoglobulins, potentially could inhibit any of four procoagulant-phospholipid complexes or two anticoagulant phospholipid-dependent reactions; however, the in vitro effect appears to be inhibition of the prothrombinase reaction (10). LA assays do not actually measure a titer of antibody, but are functional tests. However, it is likely that a large amount of antiphospholipid antibody is necessary to measure as a LA. It has been recognized for some time that LAs were heterogeneous; no one assay is able to detect 100% of LAs. Two groups, one in which LA activity could be separated from aCL activity and one in which the LA and aCL activities were inseparable, have been found (11). In the first group, LA activity was dependent on human prothrombin (12). In the second group, LA was dependent on the plasma protein $ß_2$GPI (as is aCL antibody) (11).

The in vitro detection methods for LA may not be relevant to its in vivo action. LAs prolong clotting times in vitro, because they agglutinate phospholipids in the plasma, thereby preventing their participation as cofactors in coagulation steps. If a more physiologic surface, such as

endothelial cells, is used for assembly of the prothrombinase complex, agglutination of phospholipids does not occur. For example, Oosting et al. (13) found that only 4 of 22 IgG fractions (18%) with LA activity were able to inhibit prothrombinase activity on endothelial cells.

In addition to the identification of coagulation proteins that combine with negatively charged phospholipids to form the epitopes that are targets for aPL antibodies, the nature of the phospholipid that is involved in LA activity also has been studied. Both the physical nature and the phase behavior of the phospholipid are important. LA antibodies have a greater affinity for hexagonal phase than bilayer phospholipid (14 ,15).

If LAs can be subgrouped by the plasma-protein–phospholipid targets, it follows that thrombotic mechanisms in patients with different subgroups of LA might be different. Among the potential mechanisms that have been suggested are procoagulant activity of endothelial cells (mediated by thrombin generation, fibrinopeptide A generation, and/or platelet aggregation) (16 ,17), perturbation of the thromboxane-prostacyclin balance (18), and perturbation of the protein C, protein S, and thrombomodulin pathways (13 ,19). Most recently, complement activation has been found as a cause of pregnancy lose (20) and thrombosis (21) in APS murine models.

Anticardiolipin Antibody

Realizing that cardiolipin was the major antigenic component of the false-positive test for syphilis, Harris et al. (22), in the Graham Hughes laboratory, developed a radioimmunoassay for aCL antibody. Over time, an enzyme-linked immunosorbent assay (ELISA) replaced the radioimmunoassay and underwent several revisions to optimize diluents and buffers, create multiple standards for calibration curves, and shorten incubation times (4 ,23 ,24).

Cardiolipin, which is found in mitochondria, originally was thought unlikely to be the antigen against which the antibodies detected in solid-phase assays are directed in vivo. However, cardiolipin recently has been found to be a normal plasma component, with more than 94% found as part of VLDL, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) fractions (25). Because aPL antibodies cross-react with other negatively charged phospholipids (e.g., phosphatidylserine), cardiolipin can serve as a representative antigen in the solid-phase system (26 ,27). Phosphatidylserine also may serve as the antigen (28).

The rationale for the measurement of aCL isotypes (i.e., IgG, IgM, and IgA) was the finding in several studies that IgG aCL was the major predictor of thrombosis and pregnancy loss (29 ,30 ,31), although other groups have reported similar clinical associations with IgM aCL as well (32 ,33). However, polyclonal assays for aCL also have shown predictive value for thrombosis, both in cross-sectional studies (34) and in prospective ones (35). Recent work has demonstrated that IgM aCL is associated with not only hemolytic anemia (36 ,37 ,38) but also with thrombosis (33). Although the classification criteria for APS do not include IgA aCL, recent work suggests that some patients with APS manifestations may have IgA as their only isotype (39). Additionally, in one series, IgA aCL was associated with vasculitis (40). The highest aPL titers were of the IgA isotype in one study (41). Anti-β_2GPI of IgA isotype has been associated with venous thrombosis, thrombocytopenia, valvular disease, livedo reticularis, and seizures (42). In contrast, one study found true IgA aPL antibody in only two systemic lupus erythematosus (SLE) patients, both of whom also were positive for the IgG isotype (43). Some groups have reported the highest titer, regardless of isotype, as being the most predictive of APS complications (44 ,45).

Higher-titer IgG aCL antibody has shown a stronger association with thrombosis and pregnancy loss (29 ,30 ,46). Lower titers of aCL (often with concurrent LA, but not necessarily) also occur in patients with classic manifestations of aPL antibody syndrome (34 ,45 ,47). Thus, although aCL antibody assays are able to quantify the antibody titer (as opposed to LA assays), it is not clear that the titer of aCL antibody is the only predictor of pathogenicity.

Anti-β_2GPI

In 1990, three groups simultaneously reported that a plasma protein, β_2GPI (also called apolipoprotein H), acted as a cofactor in the aCL antibody assays, improving the binding of aCL (47 ,48 ,49). This finding explained why investigators had previously noted that use of adult bovine serum (50) and fetal calf serum (51), both of which contain β_2GPI, improved the performance of the assay. β_2GPI has multiple roles in vivo, including the inhibition of ADP-induced platelet aggregation, activation of the intrinsic coagulation pathway, and activation of platelet prothrombinase activity (52 ,53 ,54). Although there was much initial discussion (55), it now is accepted that the true antigen against which most aCL antibodies are directed is a complex of negatively charged phospholipids with β_2GPI (56). Recently, some (57) but not all (58 ,59) groups have identified antibodies that they believe are directed against β_2GPI alone (60 ,61 ,62). An epitope in the fifth domain of β_2GPI is exposed when assays use polystyrene plates that previously were oxygenated by γ irradiation (63 ,64 ,65). Although levels of β_2GPI are determined by genetic factors, ethnicity, gender, and age (66 ,67), levels do not seem to influence the antibody response or risk of thrombosis (68).

The binding of autoimmune aCL antibodies is enhanced by the presence of β_2GPI, as opposed to aCL antibodies that are made in response to infections (49). Thus, it now is possible to differentiate aCL caused by infections (with little association with thrombosis (69)) from those caused by autoimmunity, which are associated with the aPL antibody syndrome (APS).

Even though β_2GPI is the target of aCL antibodies, aCL and anti-β_2GPI antibody results may be discrepant in

individual patients (70). Patients can be positive for anti-ß$_2$GPI, but negative for aCL, if they have antibodies that recognize human epitopes, or ß$_2$GPI epitopes that are hidden when cardiolipin is bound. Conversely, patients can be positive for aCL, but negative for anti-ß$_2$GPI, if their antibodies recognize cardiolipin alone, epitopes in cardiolipin bound to ß$_2$GPI, or bovine epitopes (71). In one study, 11% of SLE patients made antibodies to ß$_2$GPI alone (72).

Multiple studies have suggested that anti-ß$_2$GPI may be a more specific marker than aCL for thrombotic events (57 ,60 ,73 ,74 ,75 ,76 ,77). Because it is unusual for a patient with APS to be negative for both aCL and LA, anti-ß$_2$GPI is not yet part of the routine work-up for patients with hypercoagulability.

It is not as clear if anti-ß$_2$GPI is a more specific marker than aCL for pregnancy morbidity. An association with pregnancy-induced hypertension, preeclampsia (78 ,79), and pregnancy loss (80) has been reported. However, several studies have found no association with pregnancy loss (81 ,82 ,83 ,84).

Lupus Anticoagulant and Anticardiolipin Antibodies: Separate, but Related

With the development of assays for aCL antibodies, investigators initially wondered if these solid-phase immunoassays would detect the same antibodies as LA assays (85). Most studies found aCL antibody more frequently than LA in patients with lupus (34 ,86), even when sensitive assays for LA were used. The two antibodies are discordant in as many as 35% of patients (69 ,87). In our study, SLE patients followed prospectively over 5 years continue to show discordance for the two antibodies (Fig. 65-1). Proof that the two antibodies can be different ensued from separate isolation from patient plasmas using multiple techniques (12 ,88 ,89 ,90).

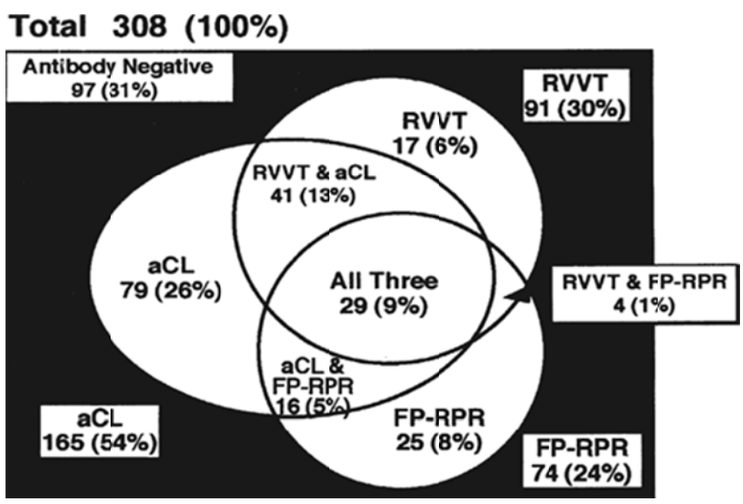

Figure 65-1. Venn diagram showing the percentage of patients in the Johns Hopkins Lupus Cohort who have had a positive acle antibody, LA (as determined by the modified RVVT assay, or a false-positive test for syphilis during proscpective followup). FP-RPR-false-positive rapid regain test.

Studies of monoclonal antibodies suggested that LAs preferentially bound hexagonal-phase phospholipids (15). The chain length and degree of saturation of the fatty-acid chains also are critical determinants. Levy et al. (91) found that aPL antibodies bound better to C18:1 phosphatidylglycerol than to C18:0 or C18:2, and that binding was greater to C18 than to C14:0 or C16:0 phosphatidylglycerol.

The demonstration that ß$_2$GPI (apolipoprotein H) is a requirement for autoimmune aCL antibody-binding in solid-phase assays (47 ,48) was the next step in understanding the different specificities of aCL and LA antibodies. Although many LA antibodies also were dependent on ß$_2$GPI (17 ,92), some were directed against other plasma cofactors, including prothrombin (12).

Antiprothrombin

Antiprothrombin antibodies represent a subset of LAs. They have been found in 33% of SLE patients in one study (93). Several retrospective studies have shown an association with thrombosis (94 ,95 ,96). The prospective ATBC study of deep-venous thrombosis (97) and myocardial infarction (98) has shown an association, as well. However, multiple other studies, including our own, have failed to find an association with thrombotic events (99). Antiprothrombin antibodies most closely associate with the profile of LA detected by the kaolin clotting time (KCT), which is less associated with thrombosis than LA detected by the dilute Russell viper venom time (RVVT) (100). In fact, antiprothrombin antibodies may cause hypoprothrominaemia and, therefore, bleeding (101).

Antiannexin

Annexin V is an anticoagulant in placental villi. Antiannexin V antibodies, by reducing annexin V at the maternal-fetal interface, may contribute to pregnancy losses in APS (102). Antiannexin V can have LA properties (103). The prevalence of antiannexin V in SLE patients ranges from 3.8% (104) to 19% (105). It has been found in association with both thrombosis and pregnancy loss (106). However, one study found no association with clinical manifestations of APS (107).

Antiphosphatidyl Serine

Antiphosphatidyl serine has been found in 32% of SLE patients in one series (108).

Antithromboplastin

Antithromboplastin has been found in 35% of SLE patients. It is associated with thrombosis, pregnancy loss, and thrombocytopenia (109).

Antioxidized Low-Density Lipoprotein

Oxidized LDL is one of the pivotal steps in the pathogenesis of atherosclerosis. Multiple studies have found an association of anti-oxidized LDL with atherosclerosis (110 ,111 ,112 ,113 ,114 ,115 ,116 ,117). In contrast, multiple groups have failed to find an association with atherosclerosis (118 ,119 ,120). In SLE, antioxidized LDL is associated with arterial, but not venous thrombosis (121 ,122). In prospective studies, antioxidized LDL has predicted myocardial infarction over 5 years in middle-aged men (112), myocardial infarction in men aged 50 years or older (123) and in middle-aged dyslipidemic men (110).

Laboratory Detection of Lupus Anticoagulant

Laboratory Definition

Laboratory criteria for LA almost certainly will continue to evolve as more knowledge about negatively charged phospholipid and plasma-protein neoantigens is obtained. The Scientific and Standardization Committee Subcommittee for the Standardization of LAs published criteria for the laboratory detection of LAs in 1995 (124). These criteria included the following:

- Prolongation of phospholipid-dependent clotting tests (e.g., KCT, dilute RVVT, tissue-thromboplastin inhibition test, plasma-recalcification clotting time, or sensitive partial thromboplastin time [PTT]).
- The clotting time of a mixture of test and normal plasma should be significantly longer than that of the normal mixed with various plasmas from patients without LA.
- There should be a relative correction of the defect by the addition of lysed, washed platelets or, preferably, phospholipid liposomes containing phosphatidylserine or hexagonal-phase phospholipids.
- It should be nonspecific for any individual clotting factor, rapidly lose apparent activity on dilution of test plasmas with saline (i.e., nonparallel lines in factor assays), usually fast acting, associated with positive aPL antibody ELISAs, and identified as an immunoglobulin whenever possible.

Some very practical summary recommendations also were made. The first was to use high-speed centrifugation (10 minutes at 5,000 g) and/or filtration to remove platelets. Platelet-poor plasma is essential to maximize the sensitivity of most assays, especially if frozen plasma is used (77). The second recommendation was to use a sensitive test to screen for LAs. The activated PTT (aPTT), for example, which is the most common screening test, often uses a thromboplastin reagent that is insensitive to LA. The third recommendation was to select an LA test that struck the right balance between sensitivity and specificity (125 ,126).

Triplett (127 ,128) has extracted what he considers to be minimal criteria for the laboratory diagnosis of LA: (a) an abnormality of an in vitro phospholipid-dependent coagulation test(s), (b) demonstration of an inhibitor (i.e., anticoagulant) as the cause of the abnormal screening test (i.e., mixing step), and (c) proof that the inhibitor is directed at phospholipid coagulation factors and not at specific coagulation factors. The use of two sensitive screening tests, such as a sensitive PTT and RVVT, may be preferable, because no one test is capable of detecting all LAs (129). The importance of using a battery of screening tests was demonstrated by McHugh et al. (130). Of 13 patients with LA, only four were abnormal on all three of the clotting assays that were employed (i.e., KCT, RVVT, and tissue-thromboplastin inhibition test). In addition, if a 1:1 patient to normal control plasma mixing study is not diagnostic, an additional mixing study with 4:1 patient to normal control plasma should be performed (128). Confirmatory tests that prove the inhibitor is directed at phospholipid include those that decrease the amount of phospholipid in the test system to accentuate the inhibitor effect (i.e., the RVVT), increase the amount of phospholipid to neutralize the LA (i.e., the platelet neutralization procedure [PNP]), or use specific hexagonal-phase phospholipids to neutralize LA (131).

Individual Assays

aPTT

The sensitivity of detection of LA using the aPTT is highly dependent on the choice of reagents (126 ,132 ,133), activator, and phospholipid to be used as a platelet substitute (134). Both the phosphatidylserine concentration (135) and the physical state of the phospholipid (i.e., hexagonal or not) (131) can affect the sensitivity of the test. Most hospital laboratories have used aPTT reagents that were insensitive to LA, but sensitive aPTT reagents now are available commercially (136). Comparative studies have demonstrated that Actin FSL (American Dade), Automated APTT (Organon Teknika Corp), and Thrombosil (Ortho Diagnostics) all are sensitive to LAs (129 ,134 ,137 ,138 ,139). A sensitive aPTT using a sensitive reagent is an excellent screening test (127). However, a sensitive aPTT may not be an appropriate screening test during pregnancy, because it is affected by rising factor VIII.

Modified (Dilute) Russell Viper Venom Time

The modified RVVT can be used as an initial, sensitive screening test for LA or as a confirmatory test (140). Its sensitivity has equaled or surpassed those of the KCT and tissue-thromboplastin inhibition tests in two studies (140 ,141), but it was not as sensitive as a sensitive aPTT in another (127). In comparison to other tests for LA, the RVVT is relatively resistant to deficiencies of clotting factors and to clotting factor antibodies. It is unaffected by antibodies to factors VIII, IX, and XI, and it remains normal in plasma that is deficient in factors VII, IX, and XI. However, factor V or X levels below 0.4 U/mL do prolong the RVVT (140). The source of phospholipid can affect the RVVT (142), as can the source of venom. These factors can lead to variability in the method of

performance and, therefore, the results of the RVVT (143). The RVVT method can be automated (144).

Kaolin Clotting Time

The KCT is an aPTT without added platelet substitute, with the kaolin acting as an activator and phospholipid surface (145). The KCT is affected by residual platelets and therefore requires a filtration step to remove platelets from the patient plasma (146). Originally, Exner et al. (147) performed multiple determinations of the KCT using different ratios of patient to normal plasma. A simplified KCT using just patient plasma has been developed, without important reduction in sensitivity or specificity (148). In some comparative studies, the KCT has not been as sensitive as a sensitive aPTT (127).

Platelet Neutralization Procedure

One approach to proving that an inhibitor is phospholipid dependent is to neutralize the inhibitor by increasing the amount of phospholipid in the assay (as in the platelet neutralization procedure [PNP]) or to accentuate the prolonged coagulation time by reducing the phospholipid (as in the tissue-thromboplastin inhibition time). The tissue-thromboplastin inhibition time is not widely used, however, because in most studies, it is both less sensitive and less specific than the PNP (133 ,137 ,141 ,149). The PNP can be combined with screening tests for LA by using freeze-thawed platelets with the sensitive aPTT or RVVT test to correct or shorten the abnormal clotting time (150).

The Anticoagulated Patient

No LA screening test is valid in the presence of heparin. In a patient who presents with a thrombotic event, plasma should be sent for LA testing before administration of heparin is begun. A more difficult problem is evaluation of the patient on warfarin. If the patient has moderate or high-titer aCL antibody, diagnosis of the APS is secure, and the LA test is not necessary for diagnosis. In some patients, however, solid-phase assays for aPL antibodies may be negative, and it may not be possible to stop anticoagulation to allow the LA testing to proceed. In this setting, mixing studies with normal plasma before LA testing (to correct deficiencies in vitamin K-dependent factors) or an LA test that already includes a mix with normal plasma (e.g., KCT or dilute aPTT) can be considered, although no consensus or criteria exist for the diagnosis of LA in this setting.

Standardization of Lupus Anticoagulant

Because of the heterogeneity of LA, it is unlikely that any one assay will be accepted as the preferred standard. Instead, a battery of the most sensitive tests (e.g., a sensitive aPTT and the RVVT) is the best approach to screening. The best assays will be those with predictive value (i.e., high relative risks) for future thrombosis or pregnancy loss.

Laboratory Detection of Anticardiolipin Antibody

The aCL antibody assay, which is the most widely used solid-phase aPL test, is a multistep procedure. In the first step, ELISA plates are coated with the negatively charged phospholipid, which usually is cardiolipin, although phosphatidylserine (28) and mixtures of phospholipid also are used (151). To prevent nonspecific antibody from binding to the plate, adult bovine serum, bovine serum albumin, or fetal calf serum are added and then washed from the plate. Diluted patient sera (the diluent should contain $ß_2$GPI, which is the cofactor for binding) then are incubated in the wells. After the incubation step, the wells are washed, and an enzyme-labeled antihuman antibody (e.g., anti-IgG, anti-IgM, anti-IgA, or polyclonal) is added. After washing, the enzyme substrate is added to develop the color reaction; the absorbance is then read.

The aCL antibody distribution is not Gaussian in most studies (34 ,152). Major differences have existed in the cutoff of negative from positive values in different laboratories. To facilitate calibration and comparative studies between laboratories, two international workshops have established and tested positive standards for the IgG and IgM isotypes, leading to the report of results in IgG isotype phospholipid (GPL) or IgM isotype phospholipid (MPL) units (23 ,24 ,153). However, use of calibrated standards may not solve the problem of standardization, because the slopes of each standard change with the buffer that is used and are different for each standard (154).

Epidemiology of Antiphospholipid Antibodies

Prevalence and Incidence of Antiphospholipid Antibodies in Normal Individuals

Published study designs primarily have been cross-sectional, with one ascertainment of aCL and/or LA status. Thus, the results represent the frequency or point prevalence of aPL, not a true prevalence (i.e., prevalence equals the incidence multiplied by the duration) or incidence. Despite the methodologic issues discussed earlier, published studies are, in large part, quite consistent regarding the frequency of aPL in normal individuals. Table 65-1 shows representative studies. The great majority of studies consisted of young women, whether pregnant or not. Most studies included aCL assays, but fewer included LA or anti-$ß_2$GPI.

Studies of prevalence (i.e., repeated measures of aCL or LA in normal individuals) are important. aPL antibody titers fluctuate in patients with lupus over time because of disease activity and treatment, meaning that an assay performed at

one point in time has less predictive value. If fluctuations occur within the normal population, this will influence the design of prospective studies on aPL antibodies in normal individuals.

Table 65-1: Frequency of Antiphospholipid Antibodies in Normal Controls

Source of Control Population	LA (%)	Anticardiolipin (%)	Anti-β_2 GPI (%)	References
Blood donors	0.0–3.6	0.0–9.4		(155,665,666,667,668)
Pregnant women	0.0–13.7	0.0–9.9	1.0–3.0	(32,35,78,148,156,157,158,159,160,161,162,163,164,165)
Healthy women		7.5		(45)
Osteoarthritis		14.0		(178)
Neurologic disorders		1.2		(296)

Frequency of Antiphospholipid Antibodies in the Elderly

Because both the frequency of antinuclear antibodies and antithyroid antibodies increases with age, it is not surprising that several studies have addressed the frequency of aPL antibodies in the elderly. As these studies employed different definitions of a positive aCL (i.e., any positive versus highly positive [>5 SD from the mean]), they are not directly comparable. However, it does appear that a positive aCL is more common in the elderly (Table 65-2). A recent study found aCL in the elderly with chronic diseases but not in the healthy elderly (155).

Frequency of Antiphospholipid Antibodies in Systemic Lupus Erythematosus

The frequency of LA (Table 65-3), aCL antibody (Table 65-4), and anti-β_2GPI (Table 65-5) in SLE has varied widely among studies. Some of this variation results from differing sensitivities of the assays, selection of patients, and bias introduced by retrospective study designs. Detection of aPL antibodies in any cross-sectional study also may be influenced by transient production of the antibody. For example, Hedfors et al. (156) reported a woman who only made aCL antibodies transiently during early pregnancy. My own group (34) and others (157 ,171) have reported patients whose aPL antibody titers or assays drop, or become negative, at the time of a thrombotic event.

Table 65-2: Anticardiolipin Frequency in the Elderly

Study	Patient (*n*)	Mean Age (y)	aPL Positive (%)
Chakravarty et al. 1990 (297)	100	75.6	0 (aCL >5 SD)
Fields et al. 1989 (669)	300	70.0	12 (aCL IgG and IgM)
Manoussakis et al. 1987 (665)	64	80.0	50 (aCL)
Juby et al. 1998 (155)			
Healthy	63		0
Chronic disease	301		13.3

Generally, the LA assays appear to be more easily suppressed by treatment for active lupus (158), but treatment also may suppress aCL levels (44 ,159). Active lupus may increase the titer of either aCL antibody or LA (159 ,160 ,161 ,162 ,163) Thus, it is not surprising that studies with multiple determinations of aPL antibodies will find a higher prevalence of antibody positivity (44 ,164) or that many patients fluctuate from their entry category of negative, low positive, or high positive (163).

Occurrence of Antiphospholipid Antibodies in Diseases Other Than Systemic Lupus

Erythematosus

APL antibodies commonly occur in infections, especially human immunodeficiency virus (HIV) (165 ,166 ,167), but in many other bacterial, protozoan, and viral illnesses as well. Most microbial aPL antibodies are IgM isotype and nonpathogenic. Most of the infection-related aPL antibodies are not dependent on β_2GPI (168) or associated with APS. In one study, however, aCL was associated with cerebral perfusion defects in HIV-positive patients (166). One patient with AIDS and aCL developed a splenic infarction (169). Another patient with AIDS and a stroke had aCL (167). In a large study of 74 HIV-infected men, however, the presence

of aCL (in 86%) or protein S deficiency (in 33%) was not associated with the development of thrombosis (165).

Table 65-3: Frequency of the Lupus Anticoagulant in Patients with Lupus

Series	Patients (*n*)	Assay Used	Frequency (%)
Clotting			
Lee & Sanders (670)	43	Whole blood or plasma clotting time	16
Meacham & Weisberger (671)	25	Clotting time	8
Zetterstrom & Berglund (672)	11	Clotting time	18
Margolius et al. (145)	23	Recalcified clotting time	13
Johansson & Lassus (673)	44	Racalcified and quick times	31
Pauzner et al. (674)	66	Recalcified clotting time	49
KCT			6
Exner et al. (147)	17	KCT, kaolin partial thromboplastin time	65
Rosner et al. (675)	66	KCT	49
Padmakumar et al. (676)	55	KCT	13
PTT			59
Regan et al. (677)	50	Partial thromboplastin time	6
Mintz et al. (678)	43	aPTT	35
Averbuch et al. (679)	36	aPTT	19
Meyer et al. (680)	91	aPTT	49
Mayumi et al. (348)	106	aPTT	16
KPTT			
Boey et al. (681)	49	Kaolin partial thromboplastin time	51
Harris et al. (22)	59	Kaolin partial thromboplastin time	49
Bennett et al. (682)	67	Kaolin partial thromboplastin time	21
Colaco & Elkon. (683)	52	Kaolin partial thromboplastin time	37
RVVT			26
Petri et al. (34)	60	RVVT	7
Multiple Assays			
Hasselaar et al. (16)	74	Partial thromboplastin time, phospholipid dilution test, KCT	49
Cervera et al. (667)	100	Prothrombin time, aPTT, KCT, RVVT, tissue thromboplastin inhibitor	30
McHugh et al. (130)	58	Kaolin-cephalin clotting time, RVVT, time thromboplastin inhibitor	22
Wong et al. (684)	91	aPTT, RVVT, platelet neutralization procedure, tissue thromboplastin inhibition	11
Cervera et al. (33)	1000	Multiple	9
Intragumtornchai et al. (160)	91	PTT, KCT, TTI, PNP	18
Sohngen et al. (685)	80	aPTT, KCT	19
Golstein et al. (686)	92	aPTT, dilute thromboplastin	22

APL antibodies also occur in other autoimmune diseases, including connective tissue diseases such as rheumatoid arthritis (170 ,171), Sjögren syndrome (172), eosinophilic fasciitis (173), scleroderma (174 ,175 ,176), and related conditions such as hemolytic anemia (177) and idiopathic thrombocytopenic purpura (178 ,179 ,180 ,181). They also have been reported in small-vessel (182), medium-vessel (i.e., microscopic polyarteritis (183 ,184 ,185)), and large-vessel (i.e., giant-cell arteritis (186 ,187 ,188) and Takayasu arteritis (189 ,190 ,191)) vasculitides. APL antibodies have been found in 38% of patients with sarcoidosis (192). Several reports of aPL antibodies in Behcet disease also exist (193 ,194 ,195), but there has been no consistent association with thrombotic disorders.

APL antibodies can occur with multiple malignant conditions (196 ,197). Whether they might be responsible for Trousseau syndrome in these patients has not been adequately studied.

APL antibodies result from many of the drugs that are known to cause drug-induced lupus, including the major tranquilizers (198), procainamide, thiazides (177), and anti-TNF biologics. They have not been associated with thromboses in chlorpromazine-treated patients (199). However, aCL (both IgG and IgM) also can be found in drug-free, multiply affected families with schizophrenia (200).

Table 65-4: Frequency of Anticardiolipin Antibody in Lupus Patients*

Series	Patients (*n*	Frequency (%)
Jones et al. (687)	200	17
Cervera et al. (33)	1000	20
Golstein et al. (686)	92	20
Hazeltine et al. (570)	65	22
Danao-Camara & Clough (688)	47	23
McHugh et al. (353)	98	24
Axtens et al. (689)	127	24
Tubach et al (690)	102	24
Petri et al. (34)	60	25
Kutteh et al. (691)	125	25
Wilson et al. (227)	44	27
McHugh et al. (130)	58	29
Buchanan et al. (692)	117	30
Guerin et al. (693)	20	30
Shimada et al. (694)	31	31
Cervera et al. (667)	100	36
Fanopoulos et al. (41)	48	37
Lopex-Soto et al. (108)	92	37
Toschi et al. (695)		37
Worrall et al. (696)	100	38
Alarc-n-Segovia et al. (205)	500	39
Ishikawa et al. (697)	31	39
Meyer et al. (157)	108	40
Fort et al. (178)	30	40
Koike et al. (698)	24	42
Kalunian et al. (45)	85	42
Wilson et al. (699)	48	42
Kaburaki et al. (105)	140	44
Sebastiani et al. (700)	64	44
Wong et al. (701)	91	44
Savi et al. (222)	80	45
Faux et al. (702)	77	45
Knight & Peter (703)	100	47
Hasselaar et al. (16)	74	47
Norberg et al. (704)	59	48
Tincani et al. (51)	51	49
Shergy et al. (161)	32	50
Sturfelt et al. (666)	59	54
Loizou et al. (30)	84	55
Harris et al. (22)	59	61
Picillo et al. (164)	102	86
Ravelli et al. (705)	30b	87

*Studies are ordered by frequency of anticardiolipin positivity.

APL antibodies have been found in 68% of a series of 25 patients with sickle cell anemia, suggesting that structural alterations in the red-cell membrane may be associated with autoantibody production (201).

Table 65-5: Frequency of Anti-β_2-Glycoprotein I in Lupus Patients

Series	Patients (*n*)	Frequency (%)
Viard, 1992 (57)	47	36
Kaburati, 1995 (706)	140	15
Romero, 1998 (707)	118	17
Fanopoulos, 1998 (41)	48	58
Tubach, 2000 (690)	102	19

Children who are on hemodialysis had a high frequency of aCL in one study. Those children with aCL had more fistula thromboses (202). In a study of 39 adult hemodialysis patients, however, the LA and aCL were β_2GPI independent, suggesting that they would not be procoagulant (203). A large study of 84 patients with end-stage renal disease found no association between thrombosis and aCL or LA (204). No thromboses occurred in the nine patients with IgG aCL or IgM aCL in a study of 42 patients who were dialyzed using cuprophane membranes (205).

Effects of Gender and Ethnicity

APS has been reported in several patients with Klinefelter syndrome (206 ,207 ,208 ,209). The effect of ethnicity has only been adequately studied in African-American and Caucasian populations; the frequency of LA and high-titer aCL is significantly less common in African Americans than in Caucasians.

Genetic Factors

Familial

In descriptions of Sneddon syndrome, which is a syndrome that overlaps significantly with APS, studies have remarked on the frequent occurrence of more than one affected family member (210 ,211 ,212). Familial cases of LA and/or aCL antibody also have been reported (210 ,213 ,214 ,215 ,216). The increased frequency of lupus or other autoimmune disease (including idiopathic thrombocytopenic purpura and autoimmune thyroid disease) in the relatives of patients with SLE is well recognized. In one study of 37 pediatric patients with SLE and 107 first-degree relatives, the occurrence of aPL in relatives was not always related to aPL positivity in the probands, and none of the aPL-positive relatives had thrombosis (217). Whether family members of patients with APS are more likely to have APS and/or autoimmune disease, and whether this results from an autosomal-dominant autoimmune gene that is incompletely penetrant, or a second APS gene, currently is under study in multiple centers. Murine models suggest that APS is multigenic (218). The valine/leucine 247 polymorphism of β_2GPI is a genetic risk factor for anti-β_2GPI (219).

HLA

Multiple HLA-DR or DQ associations with aPL antibodies have been described. In a study of 13 patients with the primary form of APS, HLA-DR4 (p <0.01) and Drw53 (p <0.05) were increased, but no correction was made for multiple comparisons (220). In a study of 20 patients with SLE and LA, an association with HLA-DQw7 (DQB1*0301), linked to HLA-DR5 and DR4 haplotypes, was found (and was increased versus that in normal controls; p = 0.002) (221). In previous studies of HLA and aCL, Savi et al. (222) found an increase of HLA-DR7 in Italian patients, and McHugh and Maddison (223) found an increase in HLA-DR4 in British patients with SLE (223). Our group's results failed to find any association of DQB1*0301 with either aCL or LA in SLE (224), and no HLA association with aCL positivity was found in a study of 139 patients with SLE (225). In a recent study, the association of aCL with DRB4 and DRB1 was investigated, with the conclusion that at the DRB1*04 locus, the *0402 allele is most common (226).

Complement Genes

One group has suggested that patients with either partial C4A or C4B null allotypes are more likely to have aCL antibody (227 ,228 ,229). In contrast, patients in the Hopkins Lupus Cohort study who were homozygous for C4A deficiency had a lower frequency of aCL and LA than patients with SLE who did not have this deficiency (230). Additionally, the subgroup of patients with C4A deficiency who had a C4A deletion were less likely to have a positive polyclonal aCL antibody (p = 0.02) than those without the deletion.

Prospective Studies

Most studies of aPL antibodies have been cross-sectional, with retrospective review of patients' case histories. One requirement to determine a pathogenic role for aPL antibodies is to document their presence before the clinical event, whether it is fetal loss (231) or thrombosis (35). In the case of fetal loss, there is further documentation of pathogenic significance, in that multiple murine models of fetal loss associated with aPL antibodies exist (232 ,233 ,234 ,235).

Multiple prospective studies in the general population have shown the predictive value of aPL antibodies for a first episode of deep-venous thrombosis (236), stroke (237 ,238), or myocardial infarction (112 ,123 ,238) as well as recurrent venous thrombosis (239) or stroke (240). Finazzi et al. found that 34 of 360 patients with aPL had a thromboembolic event over 4 years (241).

APL are present for a mean of 3 years before SLE onset and 3.1 years before a thrombotic event (242). Only 8% of primary APS patients later develop SLE, with an additional 5% developing lupus-like disease (243).

Our prospective study, the Hopkins Lupus Cohort, has shown that both aCL and LA are predictive of later thrombosis (244). The risk of venous thrombosis is 50% after 20 years (245). Most thrombotic events after cohort entry were arterial, with stroke leading the list. An occasional SLE patient makes aPL antibodies for the first time after a thrombotic event. A second large study in SLE has shown that both LA and IgG aCL are associated with thrombosis (246). Several prospective studies in cancer suggest the importance of aPL antibodies in later thrombosis. In children with acute lymphoblastic leukemia treated with L-asparaginase, four of eight with aPL antibodies had thrombosis (247). In a series of lymphoma patients, the risk of thrombosis was 5.1%/year with aPL antibodies versus 0.75%/year without aPL antibodies (248).

Prognosis and Natural History

Several studies have suggested that recurrent thrombotic events will be in the same distribution as the initial event (249). In our experience, however, the distribution may cross over, from venous to arterial and arterial to venous. Many patients seem to peacefully coexist with their aPL antibodies until a “second hit” occurs. However, several studies have failed to show that genetic hypercoagulability—specifically Factor V Leiden—is an additional risk factor in patients with aPL antibodies who thrombosed (250 ,251).

Risk factors for thrombosis include the LA, high-titer aCL, and IgG isotype of aCL. However, there are no firm rules. IgM aCL, for example, also has been associated with venous thrombosis (252). Primary APS patients, on average, have higher avidity anti-$ß_2$GPI antibodies than SLE patients (253). A risk factor for recurrence of thrombosis includes persistence of aPL antibodies for 6 months after the thrombotic event.

Seven studies, four of APS with both arterial and venous events (254 ,255 ,256 ,257) and three of APS with venous events (239 ,258 ,259), have addressed the natural history of recurrent thrombotic events in patients who already are diagnosed with APS. In the four studies that included patients with both arterial and venous thrombotic events, the risk of recurrence was high if the patient did not remain on adequate anticoagulation (254 ,255 ,256 ,257). There was a 50% risk of recurrent deep-venous thrombosis within 2 years in one study (258).

The natural history and prognosis of patients with APS who present with stroke have been studied extensively. In a retrospective review, Levine et al. (260 ,261 ,262) found a high frequency of recurrent events. In the Antiphospholipid Antibody and Stroke Study, the odds ratios for recurrent stroke or for all events (i.e., recurrent stroke, myocardial infarction, or death) were significantly higher in stroke patients with aCL than in those without aCL (240 ,263).

In a study of 139 patients with SLE, IgM aCL (present either in the past or as a persistent finding) was the only aCL isotype to be negatively associated with survival (225). A history of thromboembolic events had a strong negative association with survival, but thromboembolic events were not among the common causes of death. Further, the negative association of IgM aCL with survival was not related to the clinical criteria of APS. A second survival study of 667 patients with SLE found that APS led to decreased survival because of some (i.e., thrombocytopenia, arterial

occlusions, and hemolytic anemias), but not all, APS manifestations (264). In a third study, LA was a predictor of mortality (because of vascular occlusions) in SLE (265). Further longitudinal cohort studies may clarify the predictive value of aPL antibodies for morbidity and mortality in SLE.

Classification Criteria for Antiphospholipid Antibody Syndrome

Classification criteria for APS were proposed at the International Conference in Sapporo, Japan (266) (Table 65-6). The Sapporo criteria, in their simplest form, require either a thrombotic manifestation (venous, arterial, or vasculopathy) or pregnancy morbidity, in the setting of mild- to moderate-titer aCL or LA.

These new classification criteria differ from previous criteria in three major ways. First, vasculopathy now is included as a thrombotic manifestation. This is an important clarification, because some patients with the catastrophic presentation of APS do not have a defined thrombosis in one named vessel, but present instead with vasculopathy, usually widespread. Second, the pregnancy criterion has been revised extensively. One late fetal loss, for example, now is sufficient for the diagnosis of APS. Much more controversial is the inclusion of severe preeclampsia or placental insufficiency as part of the pregnancy morbidity criterion, because not all studies have confirmed an association of aPL antibodies with these outcomes (267). Third, thrombocytopenia is no longer a "stand alone" clinical criterion for APS.

Table 65-6: Criteria for Classification of the Antiphospholipid Antibody Syndrome*

Clinical	Laboratory
Vascular thrombosis One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by imaging or Doppler studies or histopathology, with the exception of superficial venous thrombosis. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.	Anticardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titer, on two or more occasions, at least 6 weeks apart, measured by a standard enzyme linked immunosorbent assay for $ß_2$-glycoprotein I-dependent anticardiolipin antibodies.
Pregnancy morbidity a. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th weeks of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or b. One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or eclampsia, or severe placental insufficiency or c. Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic, or hormonal abnormalities and paternal and maternal chromosomal causes excluded.	Lupus anticoagulant present in plasma on two or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostatsis

Patients with the syndrome should have at least one clinical plus one laboratory finding during their disease. The aPL test must be positive on at least two occasions more than 3 months apart.

From Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999;42:1309–1311.

Even the Sapporo classification criteria fail to address some important clinical manifestations of APS. Currently, there is no way to classify nonthrombotic neurologic manifestations, such as chorea and transverse myelitis, or to classify cardiac valvular vegetations.

The Sapporo classification criteria have shown good sensitivity, specificity, and positive and negative predictive value in a validation study (268). However, only 61% of SLE patients with secondary APS were classified correctly.

At the Sydney APS conference, revisions were made to the Sapporo classification criteria. In the laboratory criteria, anti-$ß_2$GPI IgG and IgM isotypes were added. Repeat positive assays are now required over a 3-month period, instead of the previous 6-week rule. Clinical criteria occurring greater than 5 years before the positive assays can no longer be classified as APS. Older patients must have other causes of thrombosis ruled out. Clear definitions of placental insufficiency are now given (269).

SLE is the disease overwhelmingly associated with secondary APS. Patients who have “lupus-like disease” may not fit either primary or secondary classification well (270). The distinction between primary (i.e., without associated connective tissue disease) (271 ,272 ,273) versus secondary (i.e., with associated connective tissue disease, usually SLE) forms of APS is controversial. Some authors have argued, and quite convincingly, that no distinction need be made (55 ,274), because neither the clinical features nor the aPL antibody specificities differ in the primary and the secondary forms. In one series representing three European referral centers, equal numbers of patients with primary and secondary APS were seen. The clinical features were similar, but cardiac valvular disease, hemolytic anemia, hypocomplementemia, and neutropenia were more common in those with secondary APS (275).

Some overlap of primary and secondary APS is inevitable, and this may result from a shared genetic predisposition (276 ,277). A few patients with primary APS may evolve into secondary APS over time (278 ,279 ,280). In a recent study, 8% developed SLE and 5% lupus-like disease (243). Progression to secondary APS may be more frequent in young women (281).

Differential Diagnosis of Hypercoagulability

Major causes of genetic hypercoagulability include Factor V Leiden, the prothrombin mutation, protein C, protein S, and antithrombin III deficiency. Elevated levels of homocysteine can predispose to arterial thrombosis and to atherosclerosis.

Major acquired causes of hypercoagulability include pregnancy and the postpartum period, oral contraceptives and estrogen replacement therapy, nephrotic syndrome, diabetes mellitus, hyperlipidemia, obesity, postoperative state, vasculitis, and malignancy (282).

A different approach to hypercoagulability is to consider acute versus chronic presentations (APS can present in both ways) of thrombotic angiopathies (283). Acute presentations include hemolytic uremic syndrome/thrombotic thrombocytopenia purpura, allograft rejection, drugs (cyclosporin, FK506, and OKT3), chemotherapeutic agents (mitomycin, cis-platin, and bleomycin), hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome (which can be associated with APS), malignant nephrosclerosis, systemic sclerosis, radiation nephritis, and HIV. Chronic presentations include healing hemolytic uremic syndrome, radiation nephropathy, transplantation nephropathy, dysfibrinogenemias, and polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes (POEMS) syndrome.

Prevalence of Antiphospholipid Antibody Syndrome

Retrospective or cross-sectional studies allow only an estimate of the prevalence of APS in patients who have aPL antibodies. Patients with a clinical manifestation of APS, but who are negative for aPL at the time of the study may have had aPL at the time of the event. In an editorial, Hughes (284) quoted an APS frequency of 35% in SLE. McNeil et al. (285) concluded that the frequency of thrombosis was 42% in aPL-positive patients with SLE and 31% in all patients with aPL. The review of Love and Santora listed 30% (286). These figures, however, may reflect selection bias, will be affected by length of follow-up, and are not adjusted for age, gender, or ethnicity background.

Frequency of Antiphospholipid Antibody Syndrome in Venous Thrombosis (Non-SLE)

In a study of 100 patients with verified venous thrombosis, 24 had aCL antibody, and four had LA (287). In a second study, 15% had aPL antibodies (288). In a third study, 30% had aPL (289). A lesser prevalence of 6% also has been reported (290). In the large Spanish multicenter study on thrombophilia (EMET) consisting of 2,132 patients with venous thrombosis, only 4% had aPL antibodies (291). Given new knowledge of the importance of factor V Leiden mutation as a cause of resistance to activated protein C, new and comprehensive studies of the frequency of various genetic or acquired predispositions to deep venous thrombosis in the general population are needed. No association of aPL antibodies has been found in upper extremity deep venous thrombosis (292).

In patients with pulmonary emboli, 10% have had aPL antibodies (293). APL antibodies are most frequently found in chronic thromboembolism (64%) (294).

Frequency of Antiphospholipid Antibody Syndrome in Arterial Thrombosis (Non-SLE)

Stroke

The most common arterial thrombotic presentation of APS is stroke. The frequency of aPL in unselected patients with thrombotic stroke has ranged from 5% to 29% (295 ,296 ,297 ,298 ,299 ,300 ,301 ,302). Some studies (298 ,301 ,340 ,343), but not all (295 ,299), have found the frequency of aPL in stroke patients to be significantly higher than that in controls.

The frequency of aPL in younger stroke patients (<50 years of age) appears to be higher (303 ,304 ,305 ,306 ,307 ,308 ,309). In studies that included aCL antibody assays, the frequency of aPL has ranged from 18% to 46%. Two studies, which used only LA assays (304 ,305), reported much lower frequencies, (2% to 4%). It is possible that aCL antibody is more prevalent than LA in stroke patients, but the use of insensitive LA assays also may explain the low reported frequencies. The Stroke Prevention in Young Women study, a population based case-control study, found that either anticardiolipin or lupus anticoagulant positivity increased the odds of stroke to 1.87 (237).

Coronary Artery Disease

Myocardial infarction in aPL antibody-positive patients without atherosclerosis represents a “pure” thrombotic event (310 ,311 ,312). The role of aPL antibodies in coronary

artery disease (or atherosclerotic disease in general), however, remains highly controversial. Carotid intima media thickness was increased in connective tissue disease patients with aPL antibodies (versus controls) (313).

Certainly, there is a scientific basis for interest in this potential association (314 ,315). The importance of oxidized LDL in the induction of atherosclerosis and cross-reaction between aPL and oxidized LDL antibodies suggest a potential mechanism for the progression of atherosclerosis (316 ,317). Patients with aPL antibodies have decreased paraoxonase activity, leading to increased LDL oxidation (318). Using neural network models, anti-oxLDL, aCL, and anti-β_2GPI (in decreasing order) were associated with atherosclerosis (319).

In contrast, some studies have shown that aPL antibodies might be protective against atherosclerosis. In LDL receptor–deficient mice, the passive administration of aPL antibodies reduces atherosclerosis (320). In patients with SLE, those with aPL antibodies have lower amounts of plaque and coronary calcium (321).

Several studies have found an association of aCL with myocardial infarction (321 ,322 ,323 ,324), either with graft occlusion (323 ,325) or in coronary artery disease (326 ,327). In contrast, many large studies have failed to find either an association with coronary atherosclerotic disease or with a higher rate of subsequent adverse events (328 ,329 ,330 ,331 ,332 ,333 ,334). In a nested, case-control study of middle-aged dyslipidemic men participating in the Helsinki Heart Study (112), aCL was significantly higher in those who had suffered a myocardial infarction or cardiac death. In the highest quartile of aCL, the relative risk for myocardial infarction was 2.0 (95% confidence interval, 1.1 to 3.5), independent of confounding factors. An association of aCL with antibodies to oxidized LDL was also found in this study. Anticardiolipin was predictive of myocardial infarction in the Honolulu Heart Program (238).

Association of Lupus Anticoagulant versus Anticardiolipin Antibody with Risk of Antiphospholipid Antibody Syndrome

In eight studies that used different, but equally valid, LA assays, the LA test was a more specific associate of thrombosis in patients with lupus than was aCL (34 ,96 ,335 ,336 ,337 ,338 ,339 ,340 ,341). In patients with lupus and aCL, the simultaneous demonstration of LA also increases the specificity for APS manifestations (130). In a study that used the PTT (which my group has shown will fail to detect 50% of LAs), however, aCL was found to be a better predictor of fetal distress (231). Very few studies of fetal loss have taken into account that rising factor VIII levels during pregnancy will affect many of the commonly used assays for LA, including the aPTT. In this setting, the RVVT remains a valid assay (342).

A second, although related, question is if patients with both antibodies are at greater risk for APS. In two studies, patients with both antibodies had an increased risk of thrombosis over those with LA alone (337 ,343). Patients with both have been found to have more arterial thrombosis (344) and recurrent pregnancy losses (345). In contrast, Triplett et al. (69) did not find an increased risk of thrombosis with both autoantibodies.

In general, the greatest risk is associated with LA (versus aCL), higher titer aCL, IgG isotype of aCL, and persistence of either antibody for 6 months or longer (346 ,347).

Multifactorial Nature of Risk Factors for Thrombosis in Systemic Lupus Erythematosus

The fluctuating titers of aPL antibodies in patients with SLE, associated with disease activity or treatment, suggests that the thrombotic risk engendered by these antibodies might not be constant over time. Additionally, it is important to recognize that patients with lupus can have multiple other risk factors for thrombosis. Hasselaar et al. (16) found reduced concentrations of antithrombin III, plasminogen, free protein S, and protein C in some patients. Mayumi et al. (348) found raised concentrations of fibrinopeptide 20A, and thromboxane B2 in some patients with lupus and LA.

In our group's prospective study of patients with lupus, we have emphasized the role of factors other than aPL antibodies in predicting future thrombotic events. Serologic markers of disease activity (both a low serum C3 level and elevated antidouble-stranded DNA), hypertension, and hypercholesterolemia all are significantly associated with future thrombotic events (35 ,349).

Clinical Presentation

Dermatologic Manifestations

The important role of the dermatologist in recognizing APS has been emphasized by Alegre et al. (350). In their series, 41% of patients had a skin lesion as the first sign of disease. Multisystem thrombotic events developed in 40% of their patients with cutaneous manifestations of APS. Livedo reticularis, in particular, predicts multisystem thrombosis (351). Frances et al. found that livedo reticularis was significantly associated with cerebral or ocular ischemic arterial events, seizures, all arterial events, cardiac valve abnormalities, and systemic hypertension (352).

Livedo reticularis is the hallmark dermatologic manifestation of APS (271 ,351 ,352 ,353 ,354 ,355 ,356). Its association with thrombosis was recognized before APS was described, first by Champion and Rook (357) in a patient with angina, claudication, and cerebral thrombosis, and then by Sneddon (358), who described six patients with livedo reticularis and cerebral ischemia. The name Sneddon syndrome continues to be used to describe the clinical syndrome of livedo reticularis and transient ischemic attacks, strokes, or other cerebral ischemia (359). Livedo reticularis also occurs in infectious illnesses, including syphilis and tuberculosis; other immunologic diseases, including polyarteritis nodosa and cryoglobulinemia; and in cholesterol crystal embolization (360). In a mild form, it is a normal variant in young women (361). Patients with

lupus may have both cryoglobulins and aPL antibodies, thus further confusing the differential diagnosis (362).

The pathology of the livedo reticularis in Sneddon syndrome is an endarteritis obliterans without vasculitis (357 ,358 ,363). Some skin biopsy specimens have shown endothelial proliferation of deep dermal vessels, and some have been normal (364 ,365 ,366). Sneddon syndrome differs from APS in that the clinical presentation is one of livedo reticularis and predominantly cerebral arterial thromboses rather than recurrent pregnancy loss, venous thrombosis, and thrombocytopenia. In fact, there are patients with Sneddon syndrome who do not have aPL antibodies (360). However, many patients with Sneddon syndrome do have aPL antibodies, such as one patient (367) who had central retinal artery occlusion, cerebral ischemia, livedo reticularis, and aCL antibody.

The relationship between aPL antibodies and vasculitis remains controversial. Most skin biopsies of livedo reticularis or other cutaneous manifestations of APS show bland, noninflammatory pathology, but reports of vasculitis exist (368). In some cases, this may reflect the coexistence of two different disease processes in a patient with lupus; in other cases, there appears to be a causal link. In the study by Weinstein et al. (355), patients with livedo reticularis had histologically proven vasculitis at sites that were unrelated to the livedo reticularis. Livedoid vasculitis has been associated with central nervous system involvement in lupus (369). When vasculitis occurs in livedo reticularis, it can be associated with ulceration (370 ,371).

A second, but less common, cutaneous manifestation of APS is leg ulceration, often resembling pyoderma gangrenosum (271 ,350 ,372 ,373 ,374 ,375 ,376 ,377 ,378 ,379 ,380 ,381). There appears to be a spectrum of pathology, with vascular thrombosis, capillary proliferation, endarteritis obliterans, and lymphocytic infiltration all playing a role (382).

Distal cutaneous ischemia, presenting as erythematous and purplish macules on fingers or toes, is another cutaneous manifestation of APS (379). Severe cutaneous necrosis also is reported (350 ,383 ,384 ,385 ,386 ,387 ,388). Cutaneous necrosis may be multifactorial, as in one patient with both protein S deficiency and aPL antibodies (389). Cutaneous necrosis occurred in one patient treated with LMW heparin (390). Cutaneous necrosis can be the initial manifestation of APS (391).

Both superficial thrombophlebitis (350 ,356 ,392 ,393) and thrombosis of dermal vessels have been reported (350 ,379). In the series of Alegre et al. (350), 34% of patients with LA had thrombophlebitis, and in this retrospective series, it was the most common cutaneous manifestation.

Davies and Triplett (394) have described a patient with LA and aPL antibodies who developed the blue-toe syndrome when given corticosteroids for thrombocytopenia. Blue-toe syndrome is more classically associated with microembolism of fibrin-platelet debris or cholesterol crystals from proximal atherosclerotic lesions (395), or with warfarin, with destabilization of coagulation over ulcerated atherosclerotic plaques (395).

Subungual splinter hemorrhages also have been reported in patients with aPL antibodies by several groups (396 ,397 ,398 ,399). Nailfold capillary changes with hemorrhages and hemosiderin deposits can be seen (400).

Venous Thrombosis

Deep venous thrombosis remains the most classic site of thrombosis in patients with LA. In the review by Lechner and Pabinger-Fasching (139), deep venous thrombosis occurred in 64% of 25 patients with thrombosis and in 71.2% of 80 cases from the literature. This prevalence far exceeded that of arterial thrombosis: 25% of patients had cerebral thrombosis, and 16% had peripheral arterial thrombosis. Only four patients had both venous and arterial thrombosis. Venous thrombosis in other distributions certainly occurred, including the retinal and renal veins, but was less common. Superior mesenteric vein (401), renal vein (402 ,435), splenic vein (403), and hepatic venous thrombosis (404) also have been reported.

A special clinical concern in patients with aPL antibodies and venous thrombosis is the pattern of recurrent thrombosis (405). In the series of six patients reported by Asherson et al. (405), five of the six second events were deep venous thrombosis, but one was a myocardial infarction. All occurred 6 to 12 weeks after warfarin was stopped. Long delays between events are not unusual, however, such as the patient who started with a deep venous thrombosis of the leg at age 24, only to develop Budd-Chiari syndrome 6 years later (406).

Neurologic Syndromes

After deep venous thrombosis, cerebral events are the most commonly encountered thrombotic events in patients with APS. Levine and Welch (407) have reviewed the spectrum of neurologic disease in APS, which includes focal cerebral ischemia, ocular ischemia, transverse myelopathy, complicated migraines, chorea, seizures (usually secondary to cerebral ischemic events), apnea, multi-infarct dementia, ischemic encephalopathy, pseudotumor cerebri, and transient global amnesia. Recently it has been recognized that EEGs are frequently abnormal, with all APS patients having abnormalities and 71% of aPL-positive patients in one series (408).

Neurologic disease in patients with aPL antibodies who are selected from hospital clinics is not rare. Twenty-five of 80 patients with elevated aCL in one study had neurologic disease, with four patterns being found: (1) encephalopathy, (2) multiple cerebral infarctions, (3) migraine-like headaches, and (4) visual abnormalities, including amaurosis fugax and ischemic optic neuropathy (409). In a study of 48 patients with aPL antibodies and both cerebral and visual problems, the most common presentations were transient cerebral ischemia (12 patients) or cerebral infarction (23 patients) (410). Severe vascular headache also was seen in 16 patients, as was visual disturbance (11 patients), seizures (5 patients), vascular dementia (3 patients), transient global amnesia (3 patients), and cerebral venous thrombosis (2 patients).

Methodologic issues abound in cross-sectional studies. One issue is persistence of aPL antibodies. In a study of 120 patients with transient ischemic attack or stroke, 19 of 21 with aCL showed persistence, but only 9 of 20 with a LA demonstrated by dilute prothrombin time (411).

Transient Ischemic Attacks

Landi et al. (412) reported two young women with LA and transient ischemic attacks who had normal cerebral arteriograms. Levine et al. (410) also found that patients with transient neurologic dysfunction tended to have normal cerebral angiograms; however, two of their patients with transient ischemic attacks or recurrent amaurosis fugax had ipsilateral carotid artery stenosis of greater than 75%. Therefore, it is not clear from available reports whether cerebral angiograms should be done in all young patients with transient ischemic attacks and aPL antibodies. Although the typical patient is younger than would be expected for the presentation of atherosclerotic neurologic disease, many patients have had other risk factors, including hypertension, diabetes, and smoking (412 ,413).

Cerebral Infarction

APS and Sneddon syndrome overlap in important ways. In one study, patients with Sneddon syndrome who did not have aPL antibodies were more likely to have large livedo racemosa. Those with aPL antibodies were more likely to have seizures, mitral regurgitation, and thrombocytopenia (414).

Cerebral infarcts in APS can occur in any territory and have been found in both the anterior and posterior circulation. They generally involve the superficial cerebrum, although deep infarcts also are seen (410). In a series of 15 patients, 10 had anterior circulation infarcts, 5 had posterior circulation infarcts, and one had epilepsy (415). Magnetic resonance imaging (MRI) is consistently more sensitive than computed tomography in detecting infarcts in patients with APS (410). Some strokes in patients with aPL antibodies are embolic, with mitral valve vegetations (i.e., Libman-Sacks endocarditis) and intracardiac clot being two possible sources (416).

Strokes in APS often are multiple, recurrent, and can lead to a multi-infarct dementia, as reported by Asherson et al. (417) in 9 of their 35 patients with cerebrovascular disease (418 ,419 ,420). Encephalopathy also has been reported in patients with APS, and in some patients, it may occur before true infarction can be demonstrated on brain scan (421). Molad et al. (422) found that focal white matter brain lesions on MRI were more common in patients with systemic lupus and the LA. Cerebral venous thrombosis in two patients with LA has also been reported (423).

Cerebral atrophy has been noted in several studies and case reports of patients with APS. It is assumed that the mechanism is cerebral infarction (424 ,425). Cerebral hypoperfusion also has been reported in APS (426).

Multiple Sclerosis

The differentiation of neurologic APS from multiple sclerosis is not always possible. In one study, patients with multiple sclerosis had a higher severity score of white matter lesions, whereas patients with APS had higher scores in the putamen (427).

Transverse Myelopathy

Lupoid sclerosis is a syndrome in SLE in which neurologic events such as optic neuritis and transverse myelitis occur, resembling multiple sclerosis (428). All four patients with transverse myelitis in the series of 500 patients with SLE reported by Lavalle et al. (429) had aCL antibody. Four other patients with lupus, transverse myelitis, and aCL antibody have been reported (430 ,431 ,432 ,433), as has anterior spinal artery syndrome (434). In a British series of SLE patients, 73% with transverse myelitis had aPL antibodies (435). Not all series have found an association of aPL antibodies with myelitis, including a recent study (436).

Ocular Symptoms

In addition to amaurosis fugax, multiple other ocular syndromes have been reported in patients with aPL antibodies, including retinal artery occlusion (367), ischemic optic neuropathy (437), occlusive vascular retinopathy (438 ,439), and retinal vein occlusion (440 ,441). A recent case-control study suggested an association with aCL, but not LA, for retinal vasculopathy (442).

Migraine

Severe, recurrent migraine episodes sometimes are reported in patients with aPL antibodies who later go on to have a cerebral ischemic event (410 ,416). In one study, six of 16 patients with migrainous cerebral infarction had aPL antibodies (443 ,444). However, other studies have failed to confirm any association of aPL antibodies with migraine (445).

Apnea

One patient with lupus and two episodes of apnea as well as other evidence of brainstem dysfunction was found to have aCL antibody (446). She also had cerebrospinal fluid oligoclonal bands.

Chorea

Chorea, whether in patients with or without SLE, can be associated with aPL antibodies (447 ,448 ,449 ,450 ,451). In a review of 12 patients with chorea, six had lupus like disease and six had SLE (452). All of the patients in this series were women, but men and children also have been reported (453). In the Asherson et al. series (452), 8 of the 12 patients with chorea had features of APS, including thrombocytopenia, recurrent

pregnancy loss, and thrombosis, and most had aPL antibodies. Four patients with aPL antibodies developed cerebral infarcts, and three others had transient ischemic attacks. One patient developed dementia at the time that chorea appeared. Cardioembolic caudate infarction was the cause of chorea in one patient with LA (454).

In the largest case series of 50 patients with APS and chorea, other manifestations of APS were frequent, including 28% with stroke, 24% with venous thrombosis, 18% with pregnancy loss, 6% with peripheral arterial disease, 4% with myocardial infarction, and 44% with thrombocytopenia. Multiple treatment regimens were used, with the majority of patients responding to corticosteroids or haloperidol (455).

Cognitive Function

Experimental murine APS includes cognitive function abnormalities, such as hyperactivity, in its neurologic presentations (456).

Two major studies have suggested that persistence of aPL antibodies is associated with cognitive dysfunction in humans. In one study, persistent IgG aCL was associated with problems in speed of attention and concentration (457). In the second study, both persistent IgG aCL and IgA aCL were associated with cognitive dysfunction, in the areas of psychomotor speed and conceptual reasoning/executive ability, respectively (458).

Cardiac Manifestations

Cardiac manifestations of APS have been reviewed (459).

Angina and Myocardial Infarction

Both angina and myocardial infarction have been reported in association with aPL antibodies, although these appear to be less frequent than neurologic arterial events in APS (460). Coronary artery disease secondary to premature atherosclerosis is a well-recognized complication of corticosteroid therapy in patients with lupus (461 ,462), but myocardial infarction without sufficient risk factors (463) and without atherosclerosis on coronary arteriography (460) is the usual presentation in young patients with aPL antibodies. Often, thrombus is demonstrated as the cause of the ischemia (464 ,465).

Valvular Disease

Multiple groups have found an increased prevalence of valve vegetations and mitral regurgitation in patients with aPL antibodies (310 ,466 ,467 ,468 ,469 ,470 ,471 ,472). In patients with cerebral events and aPL antibodies, cardiac valvular vegetations are a potential source of emboli (416 ,466 ,473). APL antibodies were found in three of 15 patients with cyanotic congenital heart disease, and two of these three had thrombotic episodes and false-positive VDRL (474).

Intracardiac Thrombus

Intracardiac thrombus has been described in several patients with aPL antibodies (475 ,476 ,477 ,478 ,479).

Coronary Vasculopathy

A patient has been reported with coronary artery vasculopathy in the setting of aPL antibodies (480). Vasculopathy secondary to aPL antibodies is a well-recognized clinical presentation of APS (481 ,482 ,483).

Atherosclerosis

In a small series of patients, an association of atherosclerosis of the lower limbs with aPL antibodies was reported (484). Three of these patients also had myocardial infarction.

Pregnancy Manifestations

Pregnancy Loss

In a review of 110 women with multiple obstetric complications, 5% had aCL (485).

The most specific association of aPL antibodies is with late (midtrimester) pregnancy loss (486 ,487 ,488 ,489).

An association with early first-trimester losses has been much more difficult to confirm. In a case-control study of 93 women with first-trimester losses versus 190 controls, there was no association with aPL antibodies (490). However, in a prospective study of 325 pregnancies, aCL and antiphosphatidylserine (but not anti-β_2GPI) predicted pregnancy loss (83).

Pregnancy-Associated Thromboembolism

Both genetic causes of hypercoagulability and aPL antibodies are associated with pregnancy thromboembolism (491 ,492).

Preeclampsia

Some studies have found an association of aPL antibodies with severe preeclampsia (78 ,79 ,493 ,494 ,495 ,496 ,497 ,498) but others have failed to confirm this (499 ,500 ,501). HELLP syndrome has been reported in several case reports (502 ,503 ,504).

Intrauterine Growth Retardation

Most studies have not found an association of aPL antibodies with intrauterine growth retardation (493 ,500 ,505 ,506 ,507).

Infertility

Several studies have suggested an increase in aPL antibodies in infertility and in vitro fertilization (IVF) failures, but methodologic problems with study design hamper interpretation of these studies (508).

Pulmonary Manifestations

Pulmonary Emboli

Pulmonary emboli are a frequent complication of deep venous thrombosis in patients with APS (139). When thoracic imaging was done in 88 patients with APS, 10% had pulmonary emboli (509).

Pulmonary Hypertension

Pulmonary hypertension has been reported in several patients with aPL antibodies (510 ,511). In most, the mechanism has been pulmonary emboli (512 ,513), although in situ thrombosis remains a possibility (514 ,515).

Pulmonary Capillaritis with Hemorrhage

Pulmonary capillaritis is an unusual APS presentation usually manifested clinically by hemorrhage (516). Pulmonary hemorrhage is a recognized pulmonary manifestation of APS (517) and in one case was associated with microvascular pulmonary thrombosis, not capillaritis, which responded to methylprednisolone (518).

Pulmonary Alveolitis

There is one report of fibrosing alveolitis in an APS patient (519).

Pulmonary Vasculopathy

A noninflammatory vasculopathy can occur in the lungs with APS (520).

Pulmonary Artery Thrombosis

Pulmonary artery thrombosis has been reported (514 ,544), including a postpartum case (521 ,551).

Adult Respiratory-Distress Syndrome

Multiple cases of adult respiratory distress syndrome in patients with APS have been reported (522 ,523).

Renal Manifestations

Glomerular Thrombi and Renal Insufficiency

APS of the kidney is associated with hypertension, elevation of serum creatinine, and progression of histiologic lesions on repeat biopsy (524). Kant et al. (525) described two patients with lupus and LA who had thrombi in the glomerular capillaries and prominent vascular disease in the renal biopsy specimen. This work was extended in a second study of

23 biopsy specimens from 14 patients with LA (526). Thrombi were found in 14 of 18 specimens with LA, which was increased significantly over the rate in biopsy specimens without LA. In a series of 16 cases of primary APS, small-vessel occlusive lesions and focal cortical atrophy were found (527).

Renal biopsy findings in 12 women with LA, four of who had systemic lupus, showed a pattern of narrowing of the arteries because of recanalizing thrombi and cellular intimal proliferation (528). Renal function was impaired severely in four of these patients.

Hyalinosis of arterioles, fibrin thrombi of arterioles, and intimal proliferation in small- or medium-sized arteries was found on renal biopsy in four patients with APS (529). One patient with endothelial swelling of the glomerular capillary wall and intimal proliferation with focal luminal occlusion on renal biopsy had a deterioration in renal function when she was switched from coumarin to aspirin (530).

Accelerated Hypertension

Accelerated hypertension was reported in a 14-year-old girl with LA who had ischemic changes on renal biopsy (531). Asherson (532) has emphasized the importance of hypertension as a feature of the catastrophic aPL syndrome, which is a presentation of APS with multiorgan failure and prominent vasculopathy.

Renal Artery Thrombosis

Renal artery thrombosis leading to renovascular hypertension has been reported in a 13-year-old girl with aPL antibodies (533).

Nephrotic Syndrome

Pérez-VÄsquez et al. (534) have found a negative association between nephrotic syndrome and APS in patients with lupus. This may be partially explained by urinary loss of IgG (534). In dialysis patients, maintenance of graft access is more difficult in aPL-positive patients.

Renal Transplant

APS may contribute to early graft loss (535) and other renal transplant morbidity (536) in SLE.

Endocrine Manifestations

Adrenal Failure

Adrenal failure now has been reported in multiple patients with aPL antibodies (522 ,537 ,538). Multiple mechanisms may contribute, but hemorrhage or hemorrhagic infarction is the most common (539). Most of the adrenal hemorrhages reported so far have been spontaneous (453 ,540 ,541 ,542 ,543 ,544 ,545), but some have been in the setting of anticoagulation (544 ,546 ,547 ,548). Another typical presentation appears to be postoperative or exertion-related hemorrhage (392 ,544 ,549 ,550 ,551 ,552). Adrenal infarction after cessation of warfarin therapy also has been reported as a cause of adrenal failure (546).

Gastrointestinal Manifestations

Portal and Hepatic Vein Thrombosis

aPL antibodies are one of the major predisposing factors to both portal and hepatic vein thrombosis. In one study, 11% with portal vein thrombosis and 19% with hepatic vein thrombosis had an aPL antibody (553). In a second study of 23 patients with portal vein thrombosis, 17% had IgG aCL, 4% had IgM aCL, and one patient had a lupus anticoagulant (554). Hepatic vessel thrombosis was reported in two patients with APS after liver transplant (555).

Other Arterial Manifestations

Arterial thrombi in virtually every known territory have been reported in patients with aPL antibodies. Of particular note are aortic syndromes (556 ,557 ,558) and digital or extremity gangrene (559 ,560). Acute extremity arterial insufficiency has been reported secondary to emboli after pyelography (561).

Hematologic Manifestations

Thrombocytopenia

Thrombocytopenia is so well recognized as a manifestation of APS that it is was one of the former major criteria for the syndrome (29 ,562). Some LA antibodies bind and can induce a morphologic change in platelets (563 ,564). Similarly, aCL antibodies have been shown to bind to platelets (471). Thrombocytopenia in both patients with autoimmune disease (565) and patients with chronic immune thrombocytopenia purpura (566) is associated with aCL antibody. Patients with SLE and with primary aPL antibody syndrome make antibodies that react with a 50- to 70-kd internal platelet protein (567).

Some investigators (180), but not all (179), have found that the presence of aPL antibodies in patients with thrombocytopenia is associated with fetal loss, thrombosis, and bleeding. Thrombocytopenia was more common in APS patients who had arterial thrombosis in one study (568). Although 7 of 27 patients with immune thrombocytopenia purpura had aPL antibodies, this did not separate this subgroup in clinically meaningful ways (569).

Hemolytic Anemia

Both aCL (especially IgM) and LA are associated with the positive Coombs test and with hemolytic anemia (36 ,37 ,570 ,571 ,572).

Bone Marrow Necrosis

Rare cases of bone marrow necrosis have been reported secondary to APS (573 ,574).

Hemorrhage

Patients with the lupus anticoagulant directed against prothrombin, on rare occasions, may develop clinically important hypoprothrombinemia leading to hemorrhage. This complication appears to occur more often in children (101 ,575).

Vascular Access

Multiple thromboses of vascular access have been reported in patients with APS. In one study, this was associated with antibodies to bovine thrombin (used by surgeons) (576).

Musculoskeletal

An association of aPL antibodies with avascular necrosis of bone was not found in four large series of patients with SLE (159 ,577 ,578). In four other series, however, avascular necrosis was more frequent in patients with SLE and aPL antibodies (578 ,579 ,580 ,581). In one case report, the catastrophic form of APS presented as multiple sites of avascular necrosis (582). Avascular necrosis also was found in a single vertebral body in an APS patient (583). Asymptomatic avascular necrosis occurs in 2% of primary APS patients (584). Generally, patients with avascular necrosis have a multiplicity of thrombophilic and hypofibrinolytic abnormalities (585).

Neonatal Manifestations

Transplacental transfer of aPL antibodies can lead to APS manifestations in neonates. There is one report of umbilical cord thrombosis (586), several reports of stroke (587 ,588), seizures (589), multiple thromboses (590) and fatal aortic thrombosis (591).

Other consequences of placental insufficiency as a result of APS include growth restriction (486), although this has not been confirmed in all studies (591 ,592 ,593), and severe preeclampsia (494), also not confirmed in all studies (594).

Drug-Induced Lupus Anticoagulant

Several commonly used medications, including phenothiazines, procainamide, hydralazine, and anti-TNF biologics can induce not just lupus but also drug-induced aPL antibodies. Many of the drug-induced LAs are IgM rather than IgG and, for some time, were regarded as benign. However, drug-induced LAs rarely may be associated with the thrombotic events that characterize APS (69 ,595).

Treatment

Newly Diagnosed, Asymptomatic Patients with aPL Antibodies

Asymptomatic patients with aPL antibodies either may remain untreated (596) or be treated with low-dose aspirin. Reduction of other risk factors for thrombosis, including smoking cessation and avoidance of oral contraceptives, should be undertaken as well. There are no prospective studies proving that low dose aspirin will prevent future thrombosis, however. In fact, the Physician's Health Study did not find aspirin to be effective in preventing future deep venous thrombosis or pulmonary emboli in male physicians (236). Erkan et al. have shown, in a retrospective chart review of women with pregnancy-related APS, that low dose aspirin was associated with a reduction in later thrombosis (597). Aspirin does inhibit the activation of endothelial cells by aPL antibodies (598). In a decision analysis, aspirin was favored as the best prophylactic therapy, with an eleven month gain in survival in a typical patient (599). However, as many as 25% of the general population is resistant to aspirin.

In patients with SLE and aPL antibodies, our prospective cohort study has shown that the use of hydroxychloroquine

reduces the odds ratio for future thrombosis (244 ,600). The mechanism is likely multifactorial, because hydroxychloroquine lowers titers of aPL antibodies, controls disease activity (601) and may have a beneficial rheologic effect. In an animal model, hydroxychloroquine reduces thrombus size (21). Additionally, hydroxychloroquine reverses platelet activation induced by aPL antibodies (602). Quinacrine, another antimalarial drug, reduces anticardiolipin titers (603).

Management of Acute Arterial Thrombosis

Treatment of an acute arterial event in a patient with APS must balance aggressive therapy to recanulate the vessel with the risk of hemorrhagic complications or of further thrombosis resulting from instrumentation of the vessel. Thrombolytics (604) and angioplasty (605 ,606) have been used successfully in individual cases. Heparin remains the usual therapy in most cases, however. Heparin inhibits the binding of $ß_2$GPI to phospholipids and promotes plasmin-mediated inactivation of $ß_2$GPI (607). However, in a murine model of APS pregnancy loss, the therapeutic role of heparin is not anticoagulation, but anti-inflammatory, by inhibiting complement activation (608).

Management of Acute/Chronic Venous Thrombosis

Heparin or thrombolytics is the usual therapy for acute deep venous thrombosis or pulmonary embolus. Thromboendarter-ectomy has been performed successfully in chronic pulmonary thromboembolism (609).

Long-Term Management after a Thrombotic Event

The high frequency of recurrent thrombotic events in APS has been demonstrated conclusively (254 ,262). Case reports or small series have suggested the utility of warfarin as a long-term treatment (278 ,412 ,416 ,417 ,610 ,611), and three large studies have addressed this issue in detail. Rosove and Brewer (254) reported that intensive warfarin with an international normalized ratio (INR) of three or greater was the most effective antithrombotic treatment. Khamashta et al. (255) reached a similar conclusion. One potential explanation for why patients with APS might need a higher-than-usual intensity of anticoagulation is if the INR underestimates the anticoagulant effect of warfarin when the patient has LA (612). Derksen et al. (258), in a study that was limited to venous thrombosis in APS, found the probability of no recurrence to be 100% with oral anticoagulation versus 22% with no treatment. The price to be paid for the intensive anticoagulation, however, is the increased risk of bleeding. If low-dose aspirin is added to the regimen, this will further increase the bleeding risk.

These case series did not address several important clinical issues. First, the need for high-intensity warfarin was not demonstrated prospectively, or shown to be necessary for both arterial and venous thrombosis. Venous and arterial events were not separated in two of the case series (254 ,255). It often is assumed that recurrent thrombotic events occur in the same distribution, arterial or venous, and that APS can be divided into these two subgroups in terms of treatment. We have disagreed personally with this stance, because about 25% of our patients with recurrent thrombosis had initially venous thromboembolism and then “crossed over” with recurrent thrombosis on the arterial side of the circulation, or vice versa.

It is important to acknowledge that there are studies suggesting low-intensity warfarin may be sufficient to prevent recurrent venous thromboembolism (613 ,614 ,615), although these studies did not specifically address APS. Furthermore, there are studies (256 ,257 ,259) including a prospective one (239) showing that an INR below three, even below two, may be sufficient to prevent recurrent venous thromboembolism in aPL antibody syndrome.

Second, patients whose thromboembolic event occurred with an identified precipitant, especially pregnancy or oral contraceptive use, are not separately analyzed. If a known precipitant can be removed, does the patient still require long-term anticoagulation?

Third, the case series do not adequately address the addition of low-dose aspirin to warfarin. Is this helpful in those who present with arterial, rather than venous, thromboembolism?

Fourth, the potential beneficial role of hydroxychloroquine (which patients with secondary APS might be taking for SLE manifestations) on titers of aPL antibodies (244) and thrombosis prophylaxis has not been a focus of the major case series or clinical trials.

Fifth, the special management concerns in the profoundly thrombocytopenic patient are not emphasized. We have advised caution in patients with a platelet count below 50,000. The platelet count should be raised above 50,000 (using intravenous immunoglobulin, prednisone, danocrine, etc.) and then warfarin can be begun cautiously, aiming for the “lower end” of the therapeutic range.

Ultimately, case series cannot be the standard by which treatment is decided. A pivotal clinical trial of APS patients compared usual intensity warfarin with high intensity warfarin in patients with thrombosis due to primary or secondary APS. Efficacy was equal in both arms (616). Clinical trials comparing warfarin, low molecular weight heparin, aspirin, warfarin/aspirin, and aspirin/hydroxychloroquine still are required.

Management of Stroke

An analysis of aPL antibodies at the baseline visit of the WARSS trial (warfarin versus aspirin after a first stroke) was surprising for several reasons (617). First, the aPL positive patients did not have a worse prognosis. Second, there was no difference in outcome of recurrent stroke or vascular events in the two arms. This suggests that aspirin, 325 mg, might be an acceptable therapy for stroke with aPL antibodies (618). An important caveat is that patients with aPL in the WARSS trial did not meet either the Sapporo (persistence of

antibodies for 6 weeks) or Sydney (persistence for 3 months) classification criteria for APS.

Long-Term Anticoagulation

A decision analysis concluded that long-term anticoagulation was the optimum management in APS (619). There still is no consensus on the recommendation that patients with APS thrombosis receive long-term anticoagulation with warfarin (620). For example, in one study, 51% of patients with APS had no recurrence of thrombosis with no warfarin (621). The roles of low-dose corticosteroid therapy, immunosuppressive drugs, anti-platelet drugs other than aspirin, and intravenous immunoglobulin (622) in the long-term treatment of APS are unknown.

Patients with Thrombocytopenia and Antiphospholipid Antibody Syndrome

Thrombocytopenia does not protect patients with APS from either thrombosis or pregnancy loss. The patient with APS and both thrombosis and thrombocytopenia remains a special treatment challenge. In one report, heparin-induced thrombocytopenia developed in 56% of LA-positive patients who had pulmonary hypertension, further confusing the issue (293). Our group has recommended using prednisone, danocrine, intravenous immunoglobulin, or other therapies for thrombocytopenia to keep the platelet count above 50,000/mL if therapy with heparin or warfarin is contemplated. There is a report of low dose aspirin correcting thrombocytopenia, but this seems to be the exception (623). Careful introduction of warfarin (with less-intensive anticoagulation, such as an INR of 2 to 2.5) can then be considered. Splenectomy may be an option for patients with refractory life-threatening thrombocytopenia (624 ,625), but we have seen recurrences postsplenectomy.

Treatment of Cardiac Valvular Disease

Valvular vegetations occur most frequently on the mitral or aortic valve. They represent a major source of embolic transient ischemic attacks and strokes. Studies differ on the efficacy of treatment with anticoagulation alone. In a series of 13 patients, warfarin and antiplatelet therapy had no effect (626). Thus, corticosteroids may have a role in addition to anticoagulation (627).

Treatment for Transverse Myelitis and Chorea

Transverse myelitis and chorea may represent largely nonthrombotic manifestations of aPL antibodies. However, some patients have true cord or basal ganglia infarcts on MRI. Acute therapy usually is with intravenous methylprednisolone “pulse” therapy, 1,000 mg daily for 3 days, followed by high-dose corticosteroids with or without immunosuppressives (628). The need for immunosuppression has been emphasized in a recent series (435). The addition of anticoagulation must be made on a case-by-case basis, taking into account the presence of any thrombosis on MRI or history of thrombosis elsewhere.

Treatment for Pregnancy Loss

The preferred treatment for pregnancy loss has been heparin in prophylactic doses and aspirin (629 ,630), although a third clinical trial found aspirin to be equally beneficial (631). Low molecular weight heparin appears to be equivalent in benefit to unfractionated heparin (632).

Treatment for Catastrophic APS

Although rare, catastrophic APS has a high mortality (50%) in most series. Precipitating factors are found in 22%, including infections, drugs, surgery, and cessation of warfarin (273). We have also found oral contraceptives and pregnancy to be precipitants. Thrombocytopenia has been reported in 68% (273). Treatment includes heparin, to prevent additional thrombosis; corticosteroids to treat the “cytokine storm” and to prevent further production of aPL antibodies; and plasmapheresis or intravenous immunoglobulin to remove circulating aPL antibodies. Plasmapheresis appears to improve survival in case series (273), although it increases the risk of severe infections (633). Intravenous immunoglobulin may be able to substitute for plasmapheresis, which is a difficult procedure in an unstable patient. Treatment of catastrophic APS has been reviewed by Asherson (532).

Other Therapies

B Cell Tolerance

The ideal prophylactic treatment would be to prevent production of aPL antibodies, by the reintroduction of tolerance. The technology of B cell tolerance has reached fruition in LJP394, a B cell toleragen for anti-dsDNA. LJP394 has been shown to reduce anti-dsDNA in patients with SLE (634), but failed to prevent lupus nephritis flares in two clinical trials (635).

The great benefit of this approach in APS would be its ability to induce specific autoantibody tolerance, whereas sparing normal immune function. To induce tolerance, B cells are exposed to an antibody-binding epitope that leads to cross-linking of surface receptors. In the absence of T cell help, B cell anergy and apoptosis then occur.

Iverson and colleagues have identified a peptide epitope that binds anti-β_2GPI–dependent aCL antibodies and covalently linked it to an organic platform. Multivalent presentation of this peptide epitope on the organic platform reduced antibody production in an immunized mouse model (636). A bioconjugate B cell toleragen for domain I of β_2GPI led to a 89% to 96% reduction in anti-β_2GPI in an animal study (637).

High-Dose Cyclophosphamide, with or without Stem-Cell Rescue

A more radical attempt to induce tolerance is high dose cyclophosphamide with autologous stem cell rescue. One 19-year-old woman with SLE and secondary APS later developed refractory Evans syndrome and underwent stem cell transplantation. Her conditioning regimen consisted of cyclophosphamide, anti-T lymphocyte globulin, and prednisone, followed by transplantation with autologous $CD34^+$ stem cells and progenitor cells. Eight months after the procedure, her aPL antibody assays, both aCL and lupus anticoagulant, remained negative (638).

Our group has raised concerns about the durability of the response to high-dose cyclophosphamide with or without stem-cell rescue: when long-term follow-up is available, relapses are common (639 ,640). Although long-term clinical remissions occur (639), most patients continue to have aPL antibodies in our series. However, Gladstone et al. have reported on several SLE patients who become aPL negative after high-dose cyclophosphamide (641).

Intravenous Immunoglobulin

Commercial preparations of intravenous immunoglobulin (IVIG) have been found to contain both aPL and anti-DNA autoantibodies in addition to antiidiotypic antibodies, but the autoantibodies have not been found to be pathogenic (642). In fact, in murine models of APS, IVIG has been beneficial (643). This beneficial effect of IVIG is likely mediated through multiple mechanisms, including antiidiotypes, IL-3 secretion (644), and saturation of the IgG transport receptor (645).

IVIG remains of interest in APS for the following multiple reasons: it binds aPL antibodies, down-regulates their production, and can raise the platelet count in thrombocytopenic patients. About 30% of women with APS and pregnancy loss fail treatment with heparin and aspirin, and require consideration of alternative treatments, such as IVIG.

However, the successful use of IVIG has been in case reports and small series, leading to the potential of publication bias. It often is combined with heparin, aspirin, and even prednisone, making it more difficult to assess the added benefit of IVIG. Multiple regimens have been used daily, although the two most common are 400 mg/kg for 5 days monthly or 1 g/kg/day for 2 days monthly (646). In a pilot clinical trial, the addition of IVIG did not improve pregnancy outcome in APS (647 ,654). IVIG, because it is usually low risk, likely will continue to be used in the treatment of pregnant patients failing conventional therapy (648). However, IVIG has been associated with thrombotic events (649 ,650).

It is even more difficult to assess the role of IVIG in infertility. Multiple groups have shown an increase in aPL antibodies in women with unexplained infertility and in women with recurrent IVF failures, but negative series also exist (651). In a large case-control study, heparin and aspirin improved IVF results. The addition of IVIG in women who had failed two consecutive IVF attempts with heparin and aspirin improved the birth rate to 41% from 17%, if antiphosphatidylserine or antiphosphatidylethanolamine were present (652). These results require confirmation from other groups, however, before IVIG can be recommended routinely to women with IVF failure and aPL antibodies.

Anti-TNF

In a murine model of APS pregnancy loss, TNF-α is involved downstream of complement activation, and anti-TNF strategies are beneficial (653). However, anti-TNF biologics, used for rheumatoid arthritis, can induce aPL antibodies (654), possibly because of infections (655).

Statins

One statin, fluvostatin, has been shown to reduce aPL-induced thrombosis and endothelial cell activation in an animal model (656). It inhibits the up-regulation of tissue factor by aPL (657). Experience in humans is needed, however, because statins can cause drug-induced lupus.

Rituximab

Rituximab, a B cell depleting monoclonal antibody used in the treatment of non-Hodgkins lymphoma, has reduced or normalized aPL antibody titers in several case reports (658 ,659).

Future Therapies

The recognition that complement activation is a requirement for aPL-mediated thrombosis (21) and pregnancy loss (20) may lead to studies of biologics that block complement (660 ,661 ,662).

Peptide Therapies

In murine models of APS, several promising novel therapeutic approaches have been studied. Peptides that react specifically with anti-$ß_2$GPI monoclonal antibodies were identified and found to inhibit endothelial cell activation and expression of adhesion molecules. These peptides prevented experimental APS in BALB/c mice (663).

Oral Tolerance

A novel approach in murine APS is to induce oral tolerance to low-dose $ß_2$GPI. If $ß_2$GPI was given orally before priming the BALB/c mice with $ß_2$GPI, it completely prevented experimental APS. However, it had less effect if given 70 days postimmunization. The induction of suppression was $ß_2$GPI specific, but also mediated by TGF-ß (664).

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Chapter 66
Additional Therapies Used in the Management of Lupus

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Previous chapters have discussed therapies for lupus erythematosus including general measures, local applications, nonsteroidal antiinflammatory drugs, antimalarials, corticosteroids, and traditional immunosuppressive agents. Occasionally, specific subsets of lupus require additional approaches, and some patients with refractory disease benefit from innovative management. This chapter reviews these approaches.

Medications

Antileprosy Drugs

Dapsone

Dapsone, or 4,4-diaminodiphenylsulphone, interferes with folate metabolism and inhibits para-aminobenzoic acid. It also blocks the alternate pathway of complement activation and neutrophil cytotoxicity (1), and it inhibits superoxide and hydroxyl generation in patients with rheumatoid arthritis (2 ,3). Its use is limited by its toxicity, which includes sulfhemoglobinemia and methemoglobinemia, a dose-related hemolytic anemia, a “dapsone hypersensitivity syndrome,” and aplastic anemia (4 ,5). In a case reported during 1978, it was said to benefit urticaria associated with systemic lupus erythematosus (SLE) (6). Small series (5 ,7 ,8 ,9 ,10 ,11 ,12 ,13 ,14 ,15 ,16) have reported that dapsone can ameliorate vasculitis, bullae, urticaria, oral ulcerations, thrombocytopenia, lupus panniculitis, and subacute cutaneous lupus. Dapsone may be steroid-sparing, and it can be effective in chloroquine-resistant patients.

Dubois' group found dapsone to be helpful in three of seven patients, but three developed a significant anemia (17). Coburn and Shuster (18) reported that 9 of 11 patients with discoid LE improved on 100 mg daily. Lindskov and Reymann (19) gave dapsone to 33 patients with chronic-cutaneous LE—8 had excellent and 8 had fair results, but 17 (52%) had no response. Dapsone occasionally can make rashes worse (20 ,21), perhaps because of its sulfa component.

All patients treated with dapsone should have their baseline glucose-6-phosphate dehydrogenase levels determined; the drug should not be given to individuals with low levels. Complete blood counts should be performed every 2 weeks for the first 3 months and then every 2 months thereafter. Dapsone should be started at a dose of 25 mg twice daily and eventually raised to 100 mg daily. Dapsone also interacts with all oxidant drugs, such as phenacetin and macrodantin. Concurrent administration of 800 U of vitamin E daily may decrease the degree of dapsone-induced hemolysis (22). Delayed hypersensitivity reactions can occur (23).

I believe that dapsone has a place in the treatment of cutaneous (especially bullous and lupus profundus) and musculoskeletal lupus, especially when steroids are ineffective or contraindicated. Hematologic toxicity is high, however, and the drug should be used infrequently and cautiously.

Thalidomide

Thalidomide has become one of the newer options for refractory cutaneous lupus, which carries significant concerns and promise.

Mechanism of Action

Also known as -N-phythalimidoglutarimide, thalidomide is a highly teratogenic drug with antileprosy and antilupus effects. It has no influence on the complement system, but it can stabilize lysosomal membranes, reduce tumor necrosis factor-α (TNF-α) activity, antagonize prostaglandin, inhibit neutrophil chemotaxis and angiogenesis, and alter cellular and humeral immunity (23 ,24 ,25 ,26). Thalidomide inhibits ultraviolet B (UVB)-induced mouse keratinocyte apoptosis in both TNF-α–independent and independent pathways (27), as well as UVB-induced erythema (28).

Clinical Studies

Barba Rubio et al. (29 ,30) pioneered thalidomide's use in discoid LE in Mexico during the mid-1970s. Improvement begins within weeks on doses between 100 and 400 mg daily (31). Knop et al. (32) reported that in 46 patients with antimalarial-resistant discoid LE given 400 mg/day and followed for up to 2 years, 90% had complete or marked regression. Also, 71% relapsed on its discontinuation and improved

when it was restarted, and 25% developed a polyneuritis. The drug also can heal antimalarial-resistant lesions (33). Forty-four percent of 16 patients at Graham Hughes' London unit had complete cutaneous remissions with thalidomide; another 37% had a partial response. Twenty-seven percent developed a peripheral neuropathy (34 ,35). Twenty of 23 Brazilians with chloroquine-resistant chronic-cutaneous lupus had clearing of lesions with thalidomide; 52% experienced drowsiness and 22% abdominal distension (24). Twenty-nine patients had a 74% complete response at the Wake Forest (36), 66% of 27 patients in Leeds given 100 mg a day or less without neurotoxicity (37), and a Greek group also found similar efficacy without neurotoxicity with lower dosing (38).

Similar favorable responses and toxicity have been noted by others in smaller groups of patients (25 ,31 ,39 ,40 ,41 ,42), and in hypertrophic lupus (43) and lupus profundus (44). Lymphocyte counts tend to increase whereas inflammatory parameters and serologies are usually not altered with its use in lupus (45).

Adverse Reactions

Side effects of thalidomide include teratogenicity, fatigue, dizziness, weight gain, constipation, amenorrhea, dry mouth, and a polyneuropathy that is associated with chronic administration. It is sensory, axonal neuropathy, which does not always resolve with the drug's discontinuation (46 ,47). Other important side effects include amenorrhea and pustuloderma, and thrombosis (48 ,49). Higher doses (above 200 mg a day) are associated with a thrombotic risk (50 ,51 ,52). It is available in the United States from physicians who have registered with the New Jersey-based Celgene Corporation and comply with stringent requirements.

Conclusions

Thalidomide is a useful, second-line treatment for severe, cutaneous disease refractory to standard treatment. Caution should be exercised in using doses of more than 100 mg a day and it thalidomide should be conceived as a bridge therapy to clear the skin so that other modalities can ultimately be used chronically. Thalidomide should never be used in women who are pregnant or contemplating pregnancy.

Clofazimine

In 1976, Krivanek et al. (53) reported resolution of discoid LE in all of nine patients treated with 100 to 300 mg/day of clofazimine (Lamprene, Novartis) for 3 months, but a follow-up paper (54) found it to be effective only in early, acute-onset lesions. The drug has antileprosy, antibacterial, and antimalarial activity. It is sequestered in macrophages, stabilizes lysosomal enzymes, and stimulates the production of reactive oxidants (55). Two other favorable studies have appeared (56 ,57), but Dubois' group had no responses among eight patients treated (17). Long-term use of clofazimine in LE can result in cutaneous pigment deposits (58).

Other Novel Immune Suppressives

Chapter 61 reviews most immune-suppressive agents that at least occasionally are used to manage SLE. A few additional ones deserve mention here.

Antimetabolites

Cytarabine helped three patients with refractory cutaneous lupus (59). Fludarabine is a purine antimetabolite that helped one lupus patient (60), but was associated with a graft-versus-host reaction in another (61). Eleven patients were studied at the National Institutes of Health (NIH) but the study was terminated early because of a high rate of bone marrow suppression (62 ,63). The nucleoside analog, 2-chlordeoxyadenosine (2-CdA, cladribine) was given to 12 patients with proliferative nephritis as an infusion. Fifty percent had substantial improvement at the NIH (64), but disappointing results in two others (65).

Immunophylles: Tacrolimus, Pinecrolimus, Rapamycin

Immunophylles block IL-2 cell stimulated T cell proliferation. Chapters 57 and 61 discuss cyclosporin, topical tacrolimus, and pinecrolimus.

Seventeen Hong Kong patients followed by Mok et al. with diffuse proliferative lupus nephritis did as well as cyclophosphamide after 3 months in a preliminary report (66). Three of four patients who were resistant to cyclosporin or cyclophosphamide improved somewhat with tacrolimus (Prograf, FK-506) (67 ,68). It was well tolerated in six cases of pediatric lupus (69). Several of our renal transplant patients have tolerated rapamycin (sirolimus, Rapamune, Wyeth Ayerst), but no studies of its use in lupus have been published (70).

Mizoribine

Mizoribine is an oral immune suppressive similar to azathioprine, which is available in Japan. A nucleoside of the imidazole class, in several studies, doses of 5 to 10 mg/kg/day have been suggested to be effective for lupus nephritis, children, and as a steroid sparing vehicle, but no controlled trials have been published (71 ,72 ,73 ,74 ,75). It has also been studied in rheumatoid arthritis.

Gold

In the 1940s and 1950s, gold frequently was used to treat LE. It was thought to be beneficial but toxic (76 ,77 ,78 ,79). Reports of this period antedated criteria for defining SLE and rheumatoid arthritis as well as the introduction of lupus serologies. As a result, the *Physicians' Desk Reference* listed SLE as a contraindication for using gold sodium thiomalate.

In 1983, 16 patients with SLE but without renal involvement were given oral gold at the University of California, San Diego (80). They had fevers, fatigue, arthritis, serositis, vasculitis, rashes, and mouth ulcers. Gold was of modest benefit, with significant improvement noted only in physician assessment and steroid dose reduction. A British group treated 22 patients with biopsy-documented cutaneous lupus with oral gold for up to 1 year (81). Of these, 12 had dramatic clearing of lesions, and 5 others demonstrated definite improvement. In another report (82), 7 of 12 patients who received aurothioglucose injections had improved arthritis and decreased steroid requirements. Some still are concerned that gold can flare SLE (83), and further studies are needed to define its potential role.

Vitamin A, Beta Carotene, and Retinoids

Beta carotene, vitamin A, and retinoids are related compounds that may have antilupus actions because of their sun-blocking and antioxidant activities. Skin tests with vitamin A have revealed an increased hyperreactivity in patients with SLE and their relatives compared with controls (84), and its oral administration in SLE enhances natural killer-cell activity and mitogenic responsiveness (85).

Beta carotene has been used to treat polymorphous light eruption, erythrohepatic protoporphyria, and discoid LE. Of seven patients with cutaneous lupus, six improved in two reports (86 ,87), but Dubois and Patterson (88) found beneficial results in only 1 of 26 patients.

Retinoids inhibit collagenase, prostaglandin E2, and rheumatoid synovial proliferation; interfere with intracellular-binding proteins; and interact with kinases, such as cyclic AMP (89 ,90). Additionally, epidermal antibodies can be altered, and an effect on epidermal cell differentiation may be observed (91). Three retinoids have been evaluated in cutaneous lupus: (1) isotretinoin (13-cis-retinoic acid; Accutane, Roche Laboratories); (2) etretinate (Tegison, Roche Laboratories); and (3) the aromatic retinoid acitretin (Soriatane, Roche Laboratories). When given in doses of 40 mg twice daily, isotretinoin induced complete resolution of lesions in eight of ten patients, including several with refractory, subacute-cutaneous lupus (91). In another open trial, it was effective in 20 of 24 patients (31). Other case reports and a literature review have confirmed these findings (92 ,93 ,94 ,95 ,96).

I have had similar success, but unless the patient is kept on a maintenance dose of 10 to 40 mg daily, recurrences are common. Isotretinoin can cause arthralgias and skeletal hyperostoses (97). Etretinate also may ameliorate cutaneous lesions (98), but extraspinal tendon and ligamentous calcifications have resulted from therapy (99). The drug is no longer is available in the United States. An aromatic retinoid, acitretin (Soriatene), is still available in the United States. In one paper (100), seven favorable reports were reviewed, and 15 of 20 patients studied had complete clearing of all lesions. This included five of six patients with refractory, subacute-cutaneous lupus. A randomized, double-blind study found that 46 of patients treated with acitretin improved (101). The recommended dose is 20 to 50 mg a day. The teratogenicity of the retinoids is a major concern in treating females of childbearing age. Topical retinoids with sunscreens also may be useful (102).

In summary, retinoid derivatives are useful for subacute cutaneous lupus and may ameliorate antimalarial resistant cutaneous disease.

Miscellaneous Hormonal Interventions

Chapter 63 discusses the use of contraceptive and other menses-altering or menses-regulating hormones.

Danazol

Danazol (Danocrine, Sanofi) is an impeded androgen whose effects in SLE are unclear. It may decrease Fc receptor expression and platelet-associated IgG, can reverse protein S deficiency (103), and also may have a hormonal downregulating action. Danazol displaces steroids by binding to steroid-binding globulin, which frees the latter compound. Its most promising use so far is for the treatment of idiopathic thrombocytopenia, in which a 67% response rate and steroid-sparing effects are observed, and after an initial response, low doses can be administered as maintenance therapy (104 ,105).

Hematologic Manifestaions

Idiopathic thrombocytopenia caused by SLE responds well in some patients to between 400 and 800 mg/day of therapy (106 ,107 ,108 ,109 ,110). Cervera et al. (111) noted that all of 16 patients given danazol achieved a complete remission when it was started at 200 mg/day and increased stepwise up to 1,200 mg until benefit or toxicity was observed. Danazol in combination with corticosteroids or hydroxychloroquine resulted in a 64% response among 18 French ITP patients (112). Several reports also have documented its efficacy in cases of SLE with autoimmune hemolytic anemia (110 ,113 ,114 ,115 ,116). Danazol has been used in case reports of red cell aplasias and cytopenias (117 ,118).

Other Manifestations and Toxicities

Additionally, danazol has been reported to help patients with persistent, premenstrual LE flares (119). In combination with cyproterone acetate, 11 patients with SLE had fewer exacerbations, and persistent, disabling mouth ulcers disappeared in three (120). Danazol may be effective in discoid LE (121); of 21 patients given danazol in a controlled trial with corticosteroids, all had fewer flares, lower steroid requirements, and higher hemoglobin levels, platelet counts, and C4 complement levels than 20 patients taking steroids alone. Of the 21 patients in the danazol group, however, eight withdrew because of hepatotoxicity, gastric symptoms, or asthenia (122).

Occasionally, danazol can worsen SLE (123), and it has been associated with the development of hepatocellular carcinoma in one patient (124) and with hyperglucagonemia in another (125).

Danazol is useful for ITP and possibly hemolytic anemis in SLE has no role in the management of life-threatening, nonhematologic manifestations of lupus.

Testosterones

In 1948, Lamb gave androgens to five patients with lupus, but without significant improvement (126). In 1950, Dubois et al. and Fromer (127 ,128) treated several female patients with massive doses of testosterone, both orally and intramuscularly, using as much as 500 to 1,000 mg/day for as long as 5 weeks without benefit.

After a 30-year hiatus, interest in androgen therapy resurfaced. Lahita and Kunkel (129) treated four men and four women with 19-nortestosterone (Nandrolene) for 2 months to 2 years. The condition of the men grew worse, but some women improved. Minimal masculinization was noted, sedimentation rates and anti-DNA levels decreased slightly, and hemoglobin and white counts both improved. Swaak et al. (130) found that modest improvements were outweighed by hirsutism and voice alterations among 36 patients in a placebo-controlled trial, and Hazelton et al. (131) observed no clinical change in ten patients treated with the drug. A Soviet androgen preparation, Sustanon-250, has been purported to decrease disease activity (132 ,133 ,134). Our group studied a testosterone patch being developed by Proctor and Gamble to 60 women as part of a double-blind, placebo controlled trial with SLE. No change in disease activity or sexual dysfunction was noted (135).

Dehydroepiandrosterone

Dehydroepiandrosterone (DHEA) is a steroid precursor of androgens, and to a lesser extent estrogens. Produced in the adrenal gland, its levels decline with age. DHEA increases IL-2, soluble-adhesion molecules, and interferon (IFN) while downregulating interleukin-4 (IL-4), IL-5 and IL-6 (136 ,137 ,138). It increases growth-hormone levels, may improve bone density, fatigue, libido, and cognitive dysfunction (139 ,140). Although available over the counter as a “dietary supplement,” a quality control review of 16 preparations showed that 0% to 150% of what was claimed on the label was actually in the product (141).

Early studies at Stanford University showed that in doses of 100 to 200 mg a day, favorable effects were reported in mild-to-moderate lupus in an open-label study (142), double-blind trial (143), and at long-term follow-up (144 ,145). In patients with severe SLE, bone density improved but disease activity changes were not statistically significant (146). Several pivotal trials were ultimately performed. A double-blind, randomized, placebo-controlled trial of 191 female lupus patients suggested that it was steroid sparing in individuals with a SLEDAI score greater than 2 (147). In another trial, 381 patients given 200 mg daily or placebo noted significant improvements in myalgias, oral stomatitis, and serum C3 complement (148). In a Tawanese study, 120 women randomized to DHEA versus placebo showed decreased flare rates and improved patient global assessment. IL-10 synthesis was suppressed (149 ,150). The drug was well tolerated with mild acne and hirsutism being common but rarely requiring drug discontinuation. Suggestions that the drug might improve bone mineralization in steroid dependent lupus patients led to a controlled trial that failed to reach its primary endpoint (151 ,152 ,153). The FDA did not approve DHEA for lupus as it objected to a post hoc analysis by the pharmaceutical company, and noted that the drug did not improve sedimentation rate, SLEDAI scores, or anti-DNA. Concomitant fibromyalgia was not assessed. In my practice, I use compounded DHEA in patients with fatigue and cognitive impairment as an adjunct in some individuals in doses of 50 to 100 mg a day.

Tamoxifen, Bromocriptine, and Growth Hormone

A double-blind, cross-over trial treating 11 patients with SLE using tamoxifen, an antiestrogen, demonstrated no benefits from the drug (154). Prolactin appears to have proinflammatory effects, and interest has centered on the use of prolactin suppression with bromocriptine in SLE (see Chapter 64). In one report, recombinant growth hormone reactivated a previously quiescent nephritis (155).

Gamma Globulin and Intravenous Immune Globulin

Intravenous immune globulin (IVIG) delays the clearance of antibody-coated autologous red blood cells, competitively inhibits reticuloendothelial Fc receptor blockade, has anti-idiotypic antibody activity, modulates the release and function of proinflammatory cytokines and adhesion molecule expression, and decreases pokeweed mitogen-induced B cell differentiation (156 ,157 ,158 ,159 ,160). Hypogammaglobulinemia with recurrent infections is a rare event in SLE (see Chapters 45 and 46), and use of intramuscular gamma globulin to prevent infection in lupus is not uncommon, even though no controlled studies have documented its efficacy (161 ,162 ,163).

Intravenous gamma globulin first was used in a case of lupus nephritis in 1982 (164), and its use has increased since then (165). It may be acutely helpful for autoimmune thrombocytopenia secondary to SLE (166 ,167 ,168 ,169) and for the neonatal thrombocytopenia that is seen in children of mothers with SLE (170). Gamma globulin is thought to be useful for serious disease exacerbations (171 ,172 ,173), such as in central nervous system (CNS) lupus (174 ,175), pericarditis (176), cardiac dysfunction (177), acquired factor VIII deficiency (178 ,179), pancytopenia (180 ,181), refractory cutaneous lupus (182 ,183 ,184), myelofibrosis (185), nephritis (186 ,187 ,188 ,189 ,190), polyneuritis (191), hypoprothrombinemia with the lupus anticoagulant (192), pulmonary hemorrhage (193), and for preventing recurrent fetal loss in patients with the antiphospholipid syndrome (194–200, see Chapter 54). In a controlled study, low molecular weight heparin was superior to IVIg (200). Seventeen of 20 Israeli

lupus patients with a variety of clinical manifestations responded to therapy (201). In patients with mild disease, little response to gamma globulin has been noted (202).

The use of gamma globulin for the treatment of lupus nephritis is controversial (203). The drug is expensive and potentially dangerous. The reader should appreciate that it often is ineffective (204) or temporarily effective (205). It can flare disease activity (206), induce acute renal failure (207 ,208), myocardial infarction (209), aseptic meningitis (210), and vasculitic rashes (211) among other symptoms. Low or absent IgA (seen in 5% with SLE) is a relative contraindication to its administration.

Summary

IVIG is a first-line therapy for idiopathic thrombocytopenic purpura, IgG subclass deficiency, and chronic inflammatory demyelinating polyneuropathy associated with SLE. It may be useful in other serious manifestions of SLE as a second line therapeutic approach in refractory cases.

Levamisole

The T cell immunostimulant drug levamisole, now widely used to manage colon cancer, first was noted to be effective for SLE in 1975, in a case report of an ANA-negative patient (212). This was followed by uncontrolled studies with conflicting results (213 ,214 ,215 ,216 ,217 ,218 ,219 ,220 ,221). The only controlled trial of levamisole was performed by Hadidi et al. (222). In their study, 26 patients with SLE who had been inadequately controlled by up to 30 mg/day of prednisone were given either 150 mg of levamisole or a placebo weekly for 6 months. Most patients had to have their steroid doses increased, and no improvements were observed.

Antilymphocyte and Antithymocyte Globulin

Because antilymphocyte globulin is immunosuppressive, it has been tried experimentally in a number of patients with SLE and is part of some ongoing stem cell protocols. Treatment usually has been combined with steroids and other agents. Fever as well as local and hematologic reactions have been frequent. Results generally are equivocal (223 ,224 ,225). In the largest and only controlled study (226), nine patients given antilymphocyte globulin, azathioprine, and prednisone did no better than those in a prednisone-only treated group. Pancytopenia was reversed in one patient given anti-thymocyte globulin (227).

Antibiotics

Chloramphenicol and its analogues inhibit antibody production by interfering with nucleic acid synthesis or (228). An analogue of chloramphenicol, thiamphenicol, was given to six patients with lupus nephritis (229). Following a 16-day course of 2 g daily, four patients had increased complement and decreased anti-DNA levels, lower antinuclear antibody titers, and disappearance of glomerular-bound gamma globulin. Sustained remissions function lasted for 9 months to 3 years following a single course of therapy. In 1979, Richmond (230) gave thiamphenicol to 13 patients with lupus nephritis for 2 weeks (231) and then reviewed the drug's record of inhibiting cell-mediated immune reactions and prolonging rat renal allograft survival. Symptomatic improvement was noted in only two patients, but six had serologic improvement.

Penicillin (232 ,233), sulfonamides (234 ,235 ,236), tetracycline (237), and streptomycin (238 ,239) also have been purported to help in SLE. The usefulness of these approaches is doubtful, however, and no controlled studies are available.

Interferon and Other Antiviral Agents

Interferon (IFN)-α induces the formation of anti-DNA and antinuclear antibodies (240). Numerous reports document the induction of SLE in patients who receive the drug for a variety of reasons (241 ,242 ,243 ,244 ,245 ,246 ,247 ,248 ,249 ,250), as well as IFN-ß-1a (251). IFN-γ administered for presumed rheumatoid arthritis induced multisystemic SLE in two patients (252 ,253), and 13 cases of its induction after therapy for hepatitis C have been reviewed (254). Schapira reviewed 24 cases of interferon-induced Raynaud (255). Surprisingly, IFN-α (especially intralesional) has been effective in managing patients with refractory discoid and subacute cutaneous lupus (256 ,257 ,258 ,259).

In one case report (260), isoprinosine given to a patient with lupus for a viral infection produced improvement in disease activity.

Thymosin and Thymectomy

Because the thymus gland is an important lymphoid organ in which lymphocytes differentiate, proliferate, and mature, experimental thymectomy has been undertaken to treat SLE, but with uniformly negative results (261 ,262 ,263 ,264 ,265 ,266). The administration of thymosin, or thymic hormone, represents the opposite approach. It increases T-lymphocyte counts, improves lymphocyte responsiveness, and decreases null-cell counts in vitro (267 ,268 ,269 ,270). Thymulin inhibits cytokine response in SLE (271). Factor V thymosin was given to four patients with SLE, and improvement was claimed in three, with no adverse reactions observed (272). Unfortunately, improvement was not defined or described, nor was the degree of disease activity in these patients stated. Thymus factor X was thought to be useful in a poorly documented report (273).

Vasodilators as Disease Modifying Agents

Prostaglandin E1 (PGE1) is a vasodilator that can suppress the effector systems of inflammation and both enhance and diminish cellular and immune responses (274). Several case reports have associated PGE1 infusion with improved renal function in lupus nephritis and decreased levels of

circulating immune complexes (275 ,276 ,277), as well as improving cerebral function and blood flow (278 ,279 ,280). Pentoxyfylline inhibits TNF-α gene transcription and may improve refractory nephrotic syndrome in high doses (800–1,600 mg) (279). Renal function also improved in a double-blind, crossover study of ten patients given a thromboxane inhibitor (281) as well as an open-label one (282 ,283), and the angiotensin-converting enzyme (ACE) inhibitor captopril (284) may be capable of reducing proteinuria. Chapters 35 and 36 discuss the use of vasodilators for pulmonary hypertension, severe Raynaud, and digital gangrene.

Rheumatoid Arthritis Biologics: TNF-α Blockers and Anakinra

Although TNF-α blockers have shown promise in animal models of SLE, their use in SLE has been complicated by the development of antinuclear antibody, anti-dsDNA and anticardioloipin antibody in 10% to 40% of lupus patients, especially those receiving chimeric preparations such as infliximab. This is infrequently clinically relevant, but numerous cases of drug-induced lupus have been reported with these agents (285 ,286 ,287 ,288 ,289). On the other hand, many patients with rheumatoid-like arthritis with SLE who do not have autoantibodies other than antinuclear antibody have had anecdotal good responses to TNF-α blockers. Recently, Smolen's group has shown that 6 lupus patients demonstrated clinical improvement with infliximab in patients with both renal and musculoskeletal disease (290) (see Chapter 44). The author believes that if the patient has a prominent inflammatory arthritis with a negative anti-dsDNA, TNF blockers can be carefully employed in selected patients with SLE.

Anakinra was well tolerated in 3 SLE patients with modest effects (291).

Drugs to Avoid: D-penicillamine, Minocycline, Sulfasalazine

d-Penicillamine, minocycline, and sulfasalazine clearly are effective for rheumatoid arthritis, and d-penicillamine may have antiscleroderma actions. These drugs have been given to patients with SLE who were thought to have rheumatoid arthritis or scleroderma. Because sulfa drugs and tetracyclines may exacerbate lupus (and can be photosensitizing) and d-penicillamine can induce lupus, extreme caution is advised if SLE is suspected (292 ,293 ,294) (see Chapter 44). The issue with sulfasalazine is more problematic because three reports among 15 patients have suggested that the drug is modestly effective for chronic-cutaneous (but not systemic) lupus (295 ,296 ,297). Several reports of drug induced lupus have appeared (298 ,299 ,300 ,301). It has been suggested that responders who are less prone to light-sensitive reactions tend to be rapid acetylators (295). I advise against using this drug in view of numerous reports of its exacerbating or inducing SLE (301) (see Chapter 44).

Should Radiation Therapy Be Avoided?

Although total lymphoid irradiation has been used to manage SLE (see Chapter 58), anecdotal reports of disease flares in patients undergoing radiation therapy for cancers are widespread. A review of the literature reveals cases of preexisting cutaneous lupus flared by radiation therapy (302 ,303 ,304) and one of widespread pelvic necrosis (305). On the other hand, a definitive 1993 matched-controlled, prospective evaluation of 61 patients with collagen vascular disorders failed to find an increased incidence of reactions compared with the autoimmune group (306). A smaller survey of six lupus patients supports this (307). Benk et al. reported that radiation therapy is often inappropriately denied to lupus patients, who uniformly tolerated treatments well at the University of Toronto (308).

Scleroderma patients seem to tolerate radiation therapy poorly with accelerated cutaneous and systemic fibrosis (309 ,310 ,311), and radiation issues with rheumatoid arthritis and other autoimmune diseases have been reviewed.

In summary, unless a patient has lupus with a scleroderma crossover, radiation therapy is very infrequently associated with any complications. I advise my patients who need radiation therapy to undergo it and have not had any problems.

Miscellaneous Agents

The use of novel immune suppressives such as lobenzarit, 15-deoxyspergualin, and the 5-lipoxygenase inhibitor zileuton (312 ,313 ,314) appear to be safe and have shown antilupus effects in small, open-labeled human trials. Heparin has been purported to help lupus psychosis (315).

The Russian literature contains numerous reports of the beneficial effects of methylxanthines, splenin, lysozyme, and prospidin (316 ,317 ,318 ,319). Other drugs and modalities that have been tried include tuberculin (320); arsenic (321); heliotherapy (322); paraaminobenzoic acid (323 ,324); colchicine (325); aminoglutethimide (326); hemotherapy (327); transfer factor (328); auriculo acupuncture (329); phenytoin (330); hyperbaric oxygen (331), topical DNCB (332), the complement inhibitor nafamostat mesylate (333); and sodium diethyldithiocarbamate (334). Routine splenectomy has been advised in patients without significant hematologic complications (335).

Chapter 62 discusses complementary, herbal, and vitamin therapies.

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Chapter 67

Adjunctive Measures and Issues: Allergies, Antibiotics, Vaccines and Disability

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Some of the most common queries that lupologists are asked to deal with relate to adjunctive issues. They do not concern treating lupus per se; rather, they relate to handling circumstances that have the potential to impact the disease. This chapter explores the following commonly encountered problems: (1) Do patients with lupus have more allergies than others, and in any case, should they receive allergy shots? (2) Should patients with lupus receive antibiotic prophylaxis before special procedures, and are there any antibiotics that warrant extra caution? (3) What about immunizations in general? (4) When and how are lupus patients disabled?

Do Patients with Lupus Have More Allergies (ATOPY) Than Healthy Individuals?

In 1976, Goldman et al. (1) first documented that patients with lupus had a significantly increased incidence of allergic rhinitis and drug allergy, although IgE levels were not different from those in healthy subjects. In 1985, a Spanish group (2) used Goldman et al.'s methodology and confirmed their data among 63 patients with systemic lupus erythematosus (SLE) compared to 51 autoimmune individuals, and 133 healthy volunteers. Of patients with SLE, 73% had evidence for urticaria, rhinitis, pharyngitis, conjunctivitis, asthma, or allergy to foods, drugs, and insect stings, compared with 37% of others. Sequeira et al. (3) found that 132 patients with SLE had significantly greater drug allergies, skin allergies, and insect allergies than 66 individuals with non-SLE disorders. Interestingly, their family members also had more allergies than the control group. Fifty-six percent of 44 Israeli patients with lupus had self-reported allergies (4). A series of papers in the last 10 years is at complete variance with these earlier conclusions. Sekigawa et al. concluded that 52 lupus patients had less allergies than 52 matched controls (5 ,6), confirming an abstract that atopic disorders are decreased in SLE (7), and a study among 101 lupus patients that pollen allergies are no different than in 130 healthy volunteers (8). Also life-threatening angioedema is extremely rare in SLE (9).

Elkayam et al. associated SLE flares in atopic patients with higher IgE levels (10), and 36 children of 26 Japanese mothers with SLE had higher IgE levels (11). However, a case-controlled study of 49 lupus patients in England found no increased risk of IgE mediated/associated allergic disorders (12). Two additional reports have confirmed elevated IgE levels in SLE patients (13 ,14), and two other studies suggest that this may reflect IgE-ANA and increased autoantibody formation (15 ,16).

Why Might Patients with Lupus Have More Allergies?

The issue of causation has been addressed on several levels. Diumenjo et al. (2) hypothesized three mechanisms: (1) a higher level of hypersensitivity to exogenous antigens, (2) anaphylactoid products of complement activation, or (3) cytotropic autoantibodies. Gruber et al. (17) found IgM-anti-IgE (in 27%) and IgG-anti-IgE (in 34%) antibodies among 67 patients with SLE. Significant correlations were observed with articular involvement, with lymphadenopathy, and anti-DNA antibodies as compared to those without the antibodies. IgE levels are increased in SLE, correlate with disease activity and not necessarily allergy (1 ,18 ,19). Urticaria and hives may or may not represent allergic responses to exogenous antigens. Sekigawa et al. has hypothesized that higher IgE levels noted in male SLE patients may reflect stronger hyperresponsiveness because of DNA hypomethylation that is not related to the influence of sex hormones (13 ,20).

Should Allergic Patients with Systemic Lupus Erythematosus Receive Allergy Shots (Immunotherapy)?

Immunotherapy (i.e., desensitization protocols) was introduced early in this century to help allergy sufferers, and it became clear that giving immunotherapy to otherwise healthy individuals carried a risk of nonspecific formation of

autoantibodies. In a study at Louisiana State University, 40% of patients with allergic rhinitis (one half of whom were receiving immunotherapy) had a positive antinuclear antibody (ANA), compared with 11% who had miscellaneous medical diseases and 10% of healthy controls. This observation did not extend to rheumatoid factor, anticytoplasmic antibodies, or anti-DNA (21). Among asthmatics, 7 of 50 (four of whom were receiving immunotherapy) had an ANA of 1:40 or greater, compared with none of 35 patients with miscellaneous medical diseases (22). Phanuphak and Kohler (23) related the onset of polyarteritis nodosa with immunotherapy in six patients. On this basis of studies such as these, as well as of personal anecdotal experiences that autoimmune patients flared their disease after receiving allergy shots, the World Health Organization (WHO) Working Group of the International Union of Immunological Sciences formally recommended in 1989 that patients with autoimmune disease not receive immunotherapy (24 ,25). Tanac et al, studied 63 asthmatic children. Of those who underwent immunotherapy, 17.5% developed autoantibodies, but none were symptomatic (26). Table 67.1 summarizes these considerations.

Table 67-1: Lupus, Allergies, and Allergic Reactions

1. There is probably no increased prevalence of atopy and allergies in lupus patients.
2. Lupus patients have higher IgE and IgE associated autoantibodies, but this is not clinically relevant.
3. Lupus patients are more sensitive to sulfa based antibiotics, which can flare the disease, and this is rarely cross-reactive with sulfa-based nonantibiotic preparations.
4. Allergy immunotherapy needs to be individualized; it can lead to disease flares and increased autoantibody formation in a minority with SLE.

In our own practice, about one half of our lupus-allergy patients have at least a mild flare of symptoms after having allergy shots, and we prefer alternative therapies to desensitization protocols.

Lupus and Antibiotic Allergies

In 1992, Petri and Albritton (27) published an exhaustive case-control study of 221 patients with SLE, their 186 best friends, and 178 relatives from the Johns Hopkins Lupus Cohort. As expected, sulfa antibiotic lupus flares (in 21%) and reactions (defined as rash, hives, fever, asthma, nausea, or lupus flare, and noted in 31%) were the most pronounced. Significantly increased reactions also were found among those given penicillin/cephalosporins, tetracyclines, and erythromycins. A letter to the editor responding to Petri and Albritton's survey confirmed these findings (28). Of 250 drug reactions reported in one center among patients with SLE, 30 were to sulfonamides, 25 to penicillin, 20 to cephalosporin, 19 to tetracyclines, and 18 to erythromycin. This represented 57% of 340 patients with lupus, compared with 14% of their 303 chronic arthritis clinic patients. Forty-seven reactions were to nonsteroidal anti-inflammatory agents (NSAIDs). Another survey reported an odds ratio of 2.6 for medication allergies and 1.8 for hives in a case-control study of 195 patients with SLE (29). Pope et al. queried 249 patients, 145 of whom had SLE. Over half with lupus were sulfa allergic (p = 0.003) (30). This was confirmed by Cooper et al. among 265 recently diagnosed patients in the Carolinas who had an adjusted odds ratio of sulfa allergy at 2.8 (31).

Are There Any Antibiotics that Lupus Patients Should Avoid?

Because patients with SLE tend to develop more infections than healthy people, the choice of optimal antibiotics is a frequently encountered problem. Sulfonamides (and, to a lesser extent, tetracyclines) are noted for their sun-sensitizing properties, which can flare rashes, occasionally induce fevers, and exacerbate the disease in general (27 ,28 ,29 ,32 ,33 ,34 ,35 ,36 ,37 ,38 ,39 ,40). Sulfa antibiotics are only rarely cross-reactive with sulfonamide nonantibiotics (nonarylamine sulfonamides). Thus patients who need diuretics, diabetic oral agents or nonsteroidals that contain sulfa rarely encounter a problem (41).

Minocycline can induce a lupuslike reaction, autoimmune hepatitis and a positive p-ANCA directed against myeloperoxidase (42 ,43 ,44 ,45). Among 27,688 acne patients aged 15 to 29, individuals taking minocycline had a 8.5-fold increased risk for developing a lupus reaction (46). Hess recently reviewed some of the speculative mechanisms by which this could occur (47). The above-mentioned antibiotics commonly are prescribed for young women by their dermatologists (for acne) and gynecologists (for urinary-tract infection), who frequently are unaware that treating a patient with lupus warrants special antibiotic considerations. The previous section details the studies that document this (27 ,28 ,29).

On a practical level, we tend to divide antibiotic reactions into those that cause skin rashes or urticaria alone (penicillins/cephalosporins, erythromycins) and those that produce disease flares as well. Sulfa-based antibiotics are well-known provocateurs of disease activity and should be used with extreme caution; preferably, they should be avoided. Although uncommon, ciprofloxacin and its relatives can induce a vasculitic reaction (48), arthralgias (49), and flares of SLE (50).

Should Lupus Patients Receive Antibiotic Prophylaxis?

Although bacterial endocarditis is rare, an established consensus recommends that antibiotic prophylaxis be given to high-risk patients before dental and certain surgical procedures (51). Miller et al. found that 18.5% of 361 lupus outpatients had a heart murmur, but only 13 (3%) had a

significant valvular abnormality warranting antibiotic prophylaxis (52). However, patients with SLE, especially those with antiphospholipid antibodies, are at an increased risk for developing cardiac vegetations. These vegetations usually are asymptomatic, and they are noted on two-dimensional echocardiograms approximately 30% of the time (53) (see Chapter 32). Because as many as 40% of patients with lupus have at least one antiphospholipid antibody, and because mitral valve prolapse is more prevalent in patients with SLE we recommend that all of our immune-suppressed patients with lupus receive antibiotic prophylaxis before dental procedures (54). The drug of choice is amoxicillin, although erythromycin, penicillins, and cephalosporins are acceptable alternatives in ampicillin-sensitive patients.

Should Patients with Lupus Be Immunized?

The issue of immunization is both controversial and misunderstood. This section attempts to clarify the misunderstandings and to summarize the salient points, including the following:

- Infrequent reports have claimed that immunizations induce systemic lupus (55 ,56 ,57 ,58 ,59 ,60 ,61 ,62 ,63). Older et al. and Shoenfeld et al.'s 1999 and 2000 literature reviews report 24 cases of SLE following vaccination: 10 after hepatitis B, 3 typhoid/paratyphoid, 8 “combinations”, 2 anthrax, and 1 tetanus (64 ,65).
- Patients with SLE tolerate most immunizations well, and adverse reactions are uncommon (66). Disease flares, however, may be slightly more frequent than the incidence of spontaneous flares (67).
- Immunizations are less effective in patients with SLE who are on high doses of corticosteroids. In one survey, protective levels of antibody after immunization were achieved in 90% receiving tetanus toxoid, 88% with *Haemophilus influenza* type B, and slightly more than half receiving pneumococcus (68). Antibody responses depend on the concentration of antigen, HLA type, and concurrent medication. Patients with lupus might make more anti-DNA and other autoantibodies after repeated immunizations (69).
- Immunizations with killed vaccine (e.g., pneumococcus, influenza, tetanus) generally are regarded as safe, but the safety of live vaccines (e.g., polio, mumps, Flu Mist, yellow fever, measles, rubella) has not been established in patients with SLE who are on high doses of steroids or cytotoxics. When somebody living with a severely immune-compromised patient with lupus receives a live vaccine, the patient should avoid secretory contact with them for 2 weeks.
- The putative mechanisms by which vaccine associated lupus can arise include: rabbits or mice immunized with protein or oligopeptides that are lupus autoantigens can develop clinical lupus, killed microbial components of the vaccine can produce an adjuvant reaction, and molecular mimicry (reviewed in (70)).

What About Specific Immunizations?

In 1980, Jarrett et al. (71) reported on their experience administering pneumococcal vaccine to patients with SLE. Mean antibody levels at both 1 and 12 months following vaccination were significantly lower than those in control patients in this and other groups (72 ,73 ,74). However, in follow-up studies (75 ,76), the persistence of pneumococcal antibodies in immunized patients was found to be protective for a mean of 3 years. In a double-blind, controlled study, Klippel et al. (77) obtained similar results. Concurrent immunosuppression did not affect response (78).

In similar studies of *influenza vaccination* in patients with SLE, Williams et al. (79) noted no disease flares but decreased antibody titers compared with a control group in a double-blind trial. Three other studies have reported similar findings (80 ,81) whereas two others found no increased rate or disease flares or decreased effectiveness (82 ,83) Herron et al. (84) observed that antibody responses are especially decreased in steroid-treated patients; of 20 immunized patients, one experienced a serious flare after injection. Influenza vaccines rarely induce a systemic vasculitis (85). Mitchell et al. (86) attempted to study the kinetics of specific anti-influenzal antibody production by cultured lymphocytes following immunization, but in vitro responses (which have been reported to be decreased) (87 ,88) did not correlate with in vivo changes. Louie et al. reported that four of 11 immunized patients did not develop significant levels of antibodies and one developed new-onset proliferative nephritis (89). Abu-Shakra reported the short-lived formation of IgG or IgM cardiolipin antibodies in 9 of 24 lupus patients after influenza vaccination (90).

Abe and Homma (91) administered *tetanus toxoid* to 200 subjects with SLE and a similar number of controls. No difference in antibody titer existed between these groups following both primary and secondary immunizations, but a subgroup had lower antibody responses. In one study, the time of appearance of the antibodies and serum titers were normal in patients with SLE (92), but others found lower responses (93) and documented a restricted IgG1 response (94). Nies et al. (95) studied antitetanus toxoid antibody synthesis after booster immunization in SLE and a control group. The patients with SLE had decreased prebooster antibody levels, and one third had a blunted antibody response. It was shown that the blunted response results from poor B-cell responsiveness and is not related to T helper or suppressor function. In an ominous follow-up report (69), this group found increased anti-DNA production in vitro following keyhole limpet hemocyanin immunization. This was especially true after secondary immunization and might represent a risk from the repeated immunization of patients with SLE, although there is no evidence that the anti-DNA contains pathogenic subsets.

Severe exacerbations of SLE have been reported after *hepatitis B vaccines* (62 ,96 ,97), and the vaccine has been thought to induce lupus (59 ,60 ,61 ,63 ,98 ,99). Fifteen lupus patients had a lower antibody index compared with

14 normal controls, and in one unit, all SLE pediatric dialysis patients failed to seroconvert after hepatitis B vaccination (100 ,101). Self-reported associations are higher. Forty-two patients responded to a query in a Lupus Foundation of America newsletter claiming the onset of lupus within weeks of hepatitis B vaccine (99).

Additional reports have examined responses to other microbes. Antibody responses after immunization with flagellin derived from *Salmonella* Adelaide, Proteus 0X-2, and *Rickettsia rickettsi* were normal (92 ,102), decreased with antistreptolysin O, *Escherichia coli,* and *Shigella* sp. (102 ,104), and both increased and decreased with *Brucella* sp. (103 ,105).

In summary, we recommend that patients with SLE should receive all necessary immunizations. Children of patients with SLE who are immunocompromised and receive live-virus vaccines should avoid secretory contact with their parent for approximately 1 to 2 weeks, or a rheumatologist should be consulted. They also should be consulted before giving routine, but not necessary, immunizations (e.g., influenza) to patients on steroids or to those with active disease. In 1996, the British Society of Rheumatology Clinical Affairs Committee issued guidelines stating that (a) patients on 40 mg of prednisolone a day should have vaccinations postponed until at least 3 months after immune-suppressive treatment has been stopped, or doses lowered to 20 mg a day, and (b) though hepatitis B vaccination is usually safe, further study of its role in autoimmunity was recommended (106).

The Economic Impact of SLE and Disability Issues

Health resource utilization in SLE has only recently been addressed. A three-country study suggested that the mean cumulative costs per patient over a 4-year period in Canada, the United States, and the United Kingdom were $13,509, $22,724, and $15,537, respectively. Thus, increased expenditures in the United States did not assure superior health outcomes (107). The same group noted that direct costs were one third and indirect two thirds of the total (108). Most of the indirect costs related to disability.

Being able to work has a significant impact on our economy and productivity, and most lupus patients who wish to work are employed. Lupus patients often find employment difficult due to fatigue, inflammation, swollen joints, medications, cognitive dysfunction, cardiopulmonary compromise, seizures precluding having a driver's license, or difficulty coping with the disease. Cutaneous lupus limits outdoor employment; Raynaud mandates a warmer workplace. Lupus patients often function best in a flexible environment where they do not have to “clock in or out” and their job is more task or project oriented. They should be able to pace themselves with several rest periods during the day.

Many lupus patients find it difficult to obtain disability insurance. Historically, Social Security disability with Medicare benefits has been reserved for patients with active, organ-threatening disease even though many of them are working without any obvious impairment.

A small number of studies have addressed the employability of lupus patients. Out out of Petri's 916 patients in the Hopkins cohort, 197 were receiving disability payments (109). They tended to have increased Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage scores. Two early surveys suggested that disability correlated with disease activity, marriage or family responsibilities, and musculoskeletal complaints (110 ,111). The advent of the Stanford Health Assessment Questionnaire (HAQ), clinical activity, and damage indices resulted in three recent disability surveys with differing results. In Saskatchewan, Canada, only 14% of 160 SLE patients were receiving disability payments, which did not correlate with HAQ scores and suggested only mild functional impairment (112). HAQ scores do not measure psychological impact or deal with concurrent fibromyalgia. Partridge et al.'s university-based group from five centers noted that 40% of 159 newly diagnosed SLE patients had quit work within 3.4 years (113). The predictors of early work disability were not having greater than a high-school education, receiving Medicaid, being without health insurance, having a job requiring physical strength, an income below poverty level, or greater disease activity at diagnosis. Half were African American. Murphy et al. similarly noted that 52% of 46 patients at the University of Pennsylvania (most were African American) had applied for disability as a result of fatigue, musculoskeletal, or neuropsychiatric symptoms (114). The musculoskeletal objective findings were minimal. In our experience, most community-based SLE patients with a high-school diploma are employed. If fibromyalgia is excluded, most disability is found among those with serious organ-threatening disease, neuropsychiatric impairments, and avascular necrosis (115).

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Chapter 68

Issues in Drug Development in SLE: Clinical Trial Design, Outcome Measures, and Biomarkers

Vibeke Strand

Introduction

No new treatments for SLE have been approved in over 40 years. The heterogeneous population combined with the multisystemic and variable disease course has made it extremely difficult to develop and gain regulatory approval for new treatments. The difficulty defining outcome measures that function well in randomized controlled trials (RCTs) has slowed clinical testing of new therapies, while the absence of a regulatory precedent, against which new treatments may be compared, has made it difficult to draw meaningful conclusions from existing data.

This chapter will review how to assess outcome in RCTs in SLE, lessons learned and future directions. The U.S. Food and Drug Administration (FDA) has recently released the *Guidance for Industry Systemic Lupus Erythematosus—Developing Drugs for Treatment* to help develop a roadmap for development of promising new therapies. Its recommendations are discussed, as well as the use of biomarkers as early endpoints to assess efficacy in RCTs, and to prospectively identify a patient population with active disease who may best respond to a new therapeutic intervention. Although there are no newly approved therapies at this time, there are many efforts underway to facilitate their development.

Domains to Assess in SLE RCTS: OMERACT Recommendations

The international consensus effort in rheumatology: “outcome measures in rheumatology clinical trials” (OMERACT) recommended in 1998 that five domains be assessed in all SLE trials: disease activity, damage, health related quality of life (HRQOL), adverse events, and economic impact (1 ,2). No recommendations have been made regarding how the domains should be measured in RCTs, and SLE specific instruments have largely been developed based on data from longitudinal observational studies and may not perform as well in clinical trials (Table 68-1).

Measures of Disease Activity

Disease Activity Indices

Six disease activity indices (DAIs) have been developed; Chapter 47 discusses these. They are the: British Isles lupus assessment group index (BILAG), systemic lupus erythematosus disease activity index (SLEDAI) and safety of estrogen in lupus erythematosus national assessment trial version of SLEDAI (SELENA-SLEDAI), systemic lupus activity measure (SLAM), European consensus lupus activity measurement index (ECLAM), lupus activity index (LAI) and SLE index score (SIS). The SLEDAI/SELENA-SLEDAI/SLEDAI 2000, SLAM/SLAM-R, BILAG, ECLAM, and LAI have all been shown to be sensitive to change and validated against each other (1 ,3 ,4 ,5 ,6). Ideally, DAIs facilitate comparisons across patients with differing organ system involvement.

Although validated against each other, the DAIs do not function interchangeably, because of differences in their methodologic development, items included and weighting of disease manifestations (7). Although there is good evidence for validity, discrimination, and feasibility in cohort studies, few DAIs have been used or validated in RCTs, and no agreement exists as to which indices perform best (8 ,9). SLEDAI preferentially weights involvement in the CNS and renal organ systems, whereas SLAM scores fatigue and other patient-reported symptoms. An early version of the SLEDAI was first used in a placebo-controlled withdrawal trial supporting the efficacy of hydroxychloroquine in SLE (10). Both SLEDAI and SLAM have been updated: SELENA-SLEDAI/SLEDAI 2K, and SLAM-R, in part because of difficulties with their use in clinical trials (11 ,12). The most detailed of the instruments is the BILAG, which assesses each involved organ system in detail, resulting in a score that recommends when treatment should be instituted or changed. It has also been regularly updated (13 ,14).

Because SLE can have a relapsing, remitting course, DAIs identify disease activity, which may or may not imply severity, outcome, or damage (1 ,15). Although SLEDAI scores at initial presentation have been shown to correlate with

mortality, organ damage and development of coronary artery disease, these outcomes may not occur within the limited time frames pragmatically assessed in RCTs (16 ,17). Physician assessments may not correlate with patient assessments of disease activity or severity—renal involvement is frequently asymptomatic until it is severe or long-standing, or active cerebritis may interfere with a patient's understanding and reporting of disease manifestations (18). Thus, DAIs may not accurately reflect outcome in relatively short-term RCTs, and should be supported by secondary measures.

Table 68-1: OMERACT Domains and Measurement Tools

Domain	Measurement Tool
1. Disease activity	Disease activity indices: SLEDAI/SELENA-SLEDAI/SLEDAI 2K, SLAM/SLAM-R, BILAG, ECLAM, LAI and SIS Renal flares SLE flares
2. Damage	SLICC/ACR damage index (SDI) End-stage renal disease (ESRD) Renal biopsy
3. Health-related quality of life (HRQOL)	SF-36 SLE specific measures under development
4. Adverse events	Organ damage Hospitalization Death
5. Cost or economic impact	Direct and indirect costs Health utilities; EQ 5D, HUI, SF-6D

Additionally, without sufficient training, even scoring within the same index can vary dramatically. Currently, it is recommended to use a computer program to generate scores for all five DAIs from a single-entry form, although pragmatically it has not been possible to collect all components of each index within a single program (13 ,19). Efforts to develop standardized reporting will allow researchers to utilize their index of choice and facilitate clinical research efforts and exchange of information within the scientific and medical community.

Renal Flares

Measures including renal flare (either proteinuric or nephritic based on changes in urinary protein and sediment) and/or treatment failure, e.g., lack of improvement in renal function, have been successfully utilized as endpoint measures in the NIH series and recent trials with abetimus sodium (20 ,21 ,22). These outcomes have been shown to be valid in RCTs, especially if confirmed by a separate data adjudication committee (23 ,24).

Although results from the NIH trials have informed clinical practice for several decades, application and confirmation of the clinical significance of similar definitions to current, multicenter RCTs are problematic. Site-specific spun urine sediments cannot be accurately verified at a central lab after shipment, nor is it pragmatically possible to confirm reported findings using a local lab. Changes in 24-hour urine protein levels per se are not useful, unless considered in the context of accurate creatinine clearance (CCr) determinations, which may be influenced by administration of/or changes in ACE inhibitors, NSAIDs, or COX-2 selective inhibitors. Although iothalamate glomerular filtration rates (GFR) most accurately reflect changes in renal function, they are expensive, performed differently across sites and remain unavailable at many other sites, further challenging their practical utilization in multicenter RCTs. Recent publications, reflecting experience in RCTs and observational cohorts, demonstrate that urinary protein/creatinine ratios predict clinically meaningful changes and when to perform 24-hour CCr and protein determinations—indicating they are a pragmatic and appropriate substitute, given the frequency of inaccurate sample collections (25).

Another concern with using renal flare as the sole measure of disease activity is that rarely do renal manifestations represent isolated organ system involvement. In one series, when isolated hematuria (>5 RBC/hpf) was observed, 81% of SLE patients had nonrenal disease manifestations, 48% other renal abnormalities, 53% decreased complement levels, or increased anti-dsDNA Abs. In patients with isolated pyuria (<5 WBC/hpf without infection), 82% had nonrenal manifestations, 58% other renal abnormalities, and 57% either decreased complement or increased anti-dsDNA Abs (26). It is therefore important to assess all organ systems in a RCT in SLE, as patients may not develop the same pattern of involvement with each disease exacerbation. An added complexity is that patients with active disease may have improvement in one organ system yet develop new or worsening manifestations of SLE in another system. Thus, defining improvement in disease activity can be very complex.

SLE Flares

The Fortin definition of “Major SLE Flare”—initiation or increase in immunosuppressive or high dose corticosteroid therapy, hospitalization, or death because of SLE—is easily applied and may not require adjudication when utilized in an RCT (27 ,28). Recent trials have prospectively defined “mild/moderate” and “severe” SLE flares, by increases in SELENA SLEDAI greater than or equal to 3 or SLEDAI-2K greater than or equal to 4 (11 ,29 ,30 ,31 ,32 ,33). The BILAG DAI identifies disease flares as “A” for major flare and “B” for moderate flare, and improvement from an A to a C or D in these absolute states may function well as an endpoint in a clinical trial, provided it is linked with disease activity indices and patient reported outcomes reflecting overall “no deterioration,” e.g., stabilization or improvement (34).

Other definitions for disease flares—renal, major SLE, or other—have evolved over time. All may function equally well in a RCT, but must be defined a priori. Accepted, standardized definitions for disease and organ-specific SLE flares would help to compare results across trials in differing protocol populations.

Measures of Damage

SLICC/ACR Damage Index

The SLICC/ACR damage index (SDI) measures organ system damage that has been present for at least 6 months, because of the disease or its treatment (35 ,36 ,37). It is designed to identify irreversible damage, and should not be assessed more frequently than every 6 months. It has successfully demonstrated that early damage is associated with high dose corticosteroid use and is a predictor of mortality (38 ,39). Nonetheless, experiences in several recent RCTs with the SDI indicate that deterioration, as well as improvement, may be identified with both placebo and active treatments—whether a result of difficulties in interpretation and training, patient heterogeneity, or inter-intra observer variability. The SDI should be used as a component of outcome where improvement in disease activity must also be considered in the context of no worsening in any organ system. It may be particularly useful as a means of stratifying patients at trial entry because increased damage is prognostic of a worse outcome.

Other Measures of Damage

End-stage renal disease (ESRD) is a valid measurement of damage, but usually occurs too infrequently to be practical as an endpoint in RCTs of 6 to 18 months duration. Importantly, progression to ESRD is variable: in a variety of RCTs, it was identified in 6% to 29% of patients over 3 to as long as 20 (mean 8.36) years of observation (40 ,41 ,42 ,43 ,44 ,45 ,46). Importantly, less than 10% of patients studied developed ESRD within 3 to 5 years, the maximal length of time it is pragmatically feasible to observe patients following enrollment in a clinical trial. Useful predictors of progression to ESRD include: lack of response of proteinuria to initial treatment, high serum Cr and/or high disease activity or chronicity by renal biopsy, racial differences, male gender, age, severe infection, hypertension and duration of disease at baseline (40 ,43 ,47 ,48 ,49). Doubling of serum Cr has been found to be highly correlated with development of ESRD (50). A more pragmatic definition for use in RCTs may be an approximately 50% increase from baseline in serum Cr (51).

In a cohort study of 87 patients with SLE nephritis, the delay between diagnosis of renal disease and renal biopsy was a significant predictor at the time of first biopsy for subsequent renal insufficiency and death (52). Biopsy definitions of activity and chronicity help to define the need to add or change therapy, but have neither been utilized nor are accepted as regulatory endpoints in RCTs (53 ,54). In part, this may be a result of the potential morbidity of the procedure and its limitations, including sampling errors; as well as the difficulty mandating clinical practice within a clinical trial setting.

Measures of Health Related Quality of Life (HRQOL)

Valid, objective measures of HRQOL are clinically useful, as patient and physician perceptions of disease activity and global health may differ (55 ,56). HRQOL has been found to be lower and uniquely affected in patients with SLE compared with normal age- and gender-matched controls, as well as patients with other chronic diseases. Furthermore, decrements in HRQOL correlate with increased disease activity and organ system damage (57 ,58 ,59).

Assessment of HRQOL is difficult to capture on a large scale, as mean changes across treatment groups in RCTs may not be statistically significant, yet clinically meaningful to individual patients. Furthermore, mean or median improvements in a treatment group that are statistically significant compared with placebo may not necessarily be clinically meaningful or readily understood. An individualized patient reported measure of improvement, stabilization, or deterioration in HRQOL can be a valuable addition to disease assessment and management.

Measures of physical function, including the Health Assessment Questionnaire (HAQ), modified HAQ (MHAQ), Arthritis Impact Measurement Scale (AIMS), and Krupp Fatigue Severity Scale (KFSS) measure physical function and/or fatigue, but do not assess HRQOL across multiple domains in SLE (1). The generic Medical Outcomes Survey Short Form-36 (SF-36) has best reflected the impact of SLE in observational cohort studies (60 ,61 ,62 ,63 ,64). It accounts for fatigue and physical function, including discretionary activities such as walking a mile or shopping. Thumboo et al. showed SF-36 to have high internal consistency, with acceptable test-retest reliability, except role-physical, with mean differences in repetitive test scores of less than 2 points in five of eight domains. SF-36 scores weakly correlated with BILAG scores and SDI (damage) scores, suggesting divergent construct validity of the SF-36—meaning it offered an independent assessment of the impact of SLE (65).

The SF-36 has been widely tested, translated in many languages and culturally back translated and extensively validated in a variety of chronic diseases including rheumatoid arthritis, osteoarthritis, psoriatic arthritis and systemic sclerosis (66 ,67). As country specific normative values are available, this allows comparison of HRQOL in SLE with other chronic diseases within a given society. It has been recommended to assess HRQOL in patients with SLE since 1995 (68). Improvements in SF-36 scores correlate with decreases in disease activity and damage (69). Decrements in SF-36 scores reflect ESRD and immunosuppressive use (70). Together with a patient global assessment of disease activity by Visual Analog Scale (VAS), the SF-36 performs well as a patient reported measure of HRQOL in SLE, where both physical and mental domains are frequently affected by active disease.

Adverse Events

Adverse events are reported in a standardized manner in RCTs and may include organ damage, hospitalization, or death—attributable to treatment and/or the disease. Demonstration of clinical utility of a therapy necessarily requires assessment of its risk/benefit profile. Requiring that a patient must not have developed a severe or irreversible

adverse event before assessing clinical response may facilitate such an evaluation (71).

Economic Costs

The direct cost of resources utilized because of disease, therapy, or adverse event needs to be evaluated in any new treatment of SLE. There are also defined indirect costs: time lost from work, work in the home, childcare costs, loss in productivity, and so on that affect the patient (59). Treatment and medical costs, as well as personal and productivity losses, need to be accounted for in a systematic manner. Recently, a study of 485 patients in Canada, the United States, and the United Kingdom found mean cumulative costs per patient over 4 years were $15,845, $20,244, and $17,647 in the three respective countries. Canadian and British patients utilized 20% and 13% less resources than U.S. patients, but experienced similar health outcomes (72). Merely spending more money treating SLE does not guarantee superior health outcome. Furthermore, indirect costs are at least as large as, if not larger than, the direct costs of SLE (73). Reducing the economic impact of SLE requires treatments that reduce inability to work both within and outside the home, as well as the direct costs of the disease and its treatment.

Lessons Learned: It Is Difficult to Assess Outcome in SLE RCTS Disease Activity Indices

In a recent RCT, SLAM and SLEDAI did not similarly identify patients with active disease nor demonstrate similar improvements or deterioration in the same patients (71). SLAM scores range from 0–86 (SLAM-R 0–8 1), active disease is defined by a score more than 7 (4). SELENA/SLEDAI scores range from 0 to 105, rarely do patients have scores more than 20; scores approximately 2 have been shown to reflect inactive disease (74). Changes in SELENA/SLEDAI/SLEDAI 2K scores have been used to define “flare”: flare = an increase more than or equal to 3 or more than or equal to 4, “improvement” = a reduction more than 3, “persistently active disease” = a change of +/- 3, and “remission” = a score of 0 (11 ,29 ,30 ,31 ,32 ,33).

The adjusted mean SLEDAI (AMS) was developed as a way to summarize disease activity over time (16 ,17). It is equivalent to the area under the curve of SLEDAI-2K over time divided by time interval and is able to capture the multiple facets of a patient's profile over any time period. Used with a time-dependent covariate survival regression, change in AMS and length of time from visit to visit is accounted for, versus fixed variables, such as gender or SLEDAI-2K at presentation. AMS can reflect disease activity over time as an important predictor of major outcomes in patients with SLE, but has never been utilized in an RCT (16 ,17). As with all AUC analyses, it is difficult to interpret whether small statistically significant changes are clinically meaningful or that an overall positive result despite a fluctuating course of improvements and deteriorations is a desired outcome.

Only one disease activity score, BILAG, specifically developed to identify the need to institute, increase, maintain or discontinue current treatment based on clinical signs and symptoms—may perform best of the DAIs to “protocolize” management of patients in a clinical trial—that change of therapy would only be allowed when indicated by BILAG. If used in this way to manage patients on a per-visit basis, its use as a primary outcome measure in a clinical trial may be confounded. To date, there is no experience with using it as a primary outcome measure in a regulatory sanctioned RCT, nor as an “area under the curve” analysis of disease activity.

Definitions of “Flare”

Over time a body of evidence has developed that shows the number of major and/or minor SLE flares over 12 months can be predicted in selected populations, which can be used as a meaningful measure of clinical benefit. Decreased number of flares with active treatment implies prevention—the amount of time required to demonstrate this as clinically meaningful remains unclear.

In the anti-CD40L mAb RCT reported by Kalunian et al., the SLE flare index identified flares over 12 months in 65% of patients receiving placebo; 10% of these were considered severe (75). In a series of 250 patients, Gordon and Isenberg identified 26% with BILAG “B” and 10% with “A,” or major SLE flares, over a 12-month period (33). Not dissimilarly, in the SELENA RCTs, in 351 and 183 randomized patients: 51% to 64% and 89% to 76% flared; in 5% to 8% and 8% to 9% these were severe (30 ,32). As demonstrated in both abetimus sodium protocols, a predefined definition of “renal flare” was shown to be reproducible, adjudicated, and confirmed by a separate “renal events committee” (23). In patients with elevated anti dsDNA antibodies, and a prior history of SLE nephritis, renal flares were reported in 3% and 4% receiving active therapy, and 21% and 20% placebo (76). In comparison, analyses applying the Fortin, or “generic” definition of major SLE flare identified flare rates of 24% with active therapy in both studies, compared with 42% and 31% with placebo (24). The lower rate of major SLE flares in the placebo group in the second study may be attributed to a variety of causes, including differing patient populations and background immunosuppressive use, but the identical renal and/or major SLE flare rates with active therapy in both protocols are striking. In both the SELENA and abetimus sodium RCTs, it was shown that patients with a history of renal disease or stable active disease have higher rates of major disease flares.

Definitions of Renal Damage

A positive response to treatment of SLE nephritis may be defined by stabilized or improved renal function. Several clinical measures of response and/or remission have been

used in RCTs and are summarized by Schiffenbauer et al. (21 ,22). Positive responses have included: approximately 50% decline in hematuria with less than 5 RBC/hpf, nonnephrotic proteinuria less than 1 g/day or nephritic proteinuria less than 3 g/d with an approximately 50% decrease, and no cellular casts (Table 68-2 and 68-3).

In a RCT comparing mycophenolate mofetil (MMF) with azathioprine (AZA) in patients with diffuse proliferative lupus nephritis by Chan et al., definitions of complete and partial remission functioned well, with long-term responses in 90% of each group in a follow up publication (77 ,78). Only 4 patients, all randomized to receive CTX-AZA, developed ESRD or death. Contreras et al. compared MMF with AZA or CTX for maintained remission following induction therapy with CTX or AZA and found significant differences between treatment groups (51). In the open label comparison of MMF with CTX for induction therapy, Ginzler et al. employed the following definitions: Complete remission on initial regimen: +/- 10% of normal Cr, inactive urinary sediment and proteinuria less than 500 mg/24 hour; partial remission: less than or equal to 50% improvement of baseline values, without 10% worsening in any parameter; and treatment failure in patients failing to show greater than or equal to 30% improvement in greater than or equal to 1 renal parameter at 12 weeks who were offered cross-over to the alternate therapy (79). Although specific definitions of response and deterioration in renal function varied, all successfully identified clinically meaningful lack of improvement or worsening, which occurred in a small number of patients over protocol observation periods of 12 to 18 months.

Table 68-2: Severe SLE Flares≫ over 12 Months: Data from Longitudinal Observational Studies [LOS] and Randomized Controlled Trials [RCTs]

Study	Flare Definition	[n]	Flare Rates
IDEC 131[75] anti CD40L RCT	SLE Flare Index	n = 94	65% any flare 10% severe flare
Isenberg [34] Gordon: LOS	BILAG	n = 250	62% “A or B” flare 10.4% severe, “A” flare
LJP RCTs [23,76]	“Fortin definition”[28]	n = 250	35% major flare
Buyon [32]	SELENA SLEDAI	n = 351	51–64% any flare
HRT RCT			4.9–8.1% severe flare
Petri [30]	SELENA SLEDAI	n = 183	69–76% any flare
OC RCT			8.4–8.7% severe flare
Sanchez [31]	Δ in SLEDAI	n = 162	84–95% any flare
Guerrero			3.0–9.0% severe flare
RANGE:	“Any flare” mild, mod or severe: “severe flare”: Relative risk for “severe flare”: stable active disease: “Major or severe flare” in patients w/ history of renal disease:	49–95% over 12 months 3–10% over 12 months 2.87–3.50 [p <0.05] history of renal disease: 2.2 [p = NS] [30,32] 31–42%, mean 35% over 12 months [23,76]	

Other Definitions of Damage: Bone Mineral Density

Patients with SLE receiving glucocorticoids show bone mineral density (BMD) loss over time because of disease activity as well as corticosteroid treatment. In a RCT in SLE patients with mild to moderate disease receiving glucocorticoids, prasterone (dehydroepiandrosterone [DHEA]) treatment significantly increased BMD at both lumbar spine and total hip (80). Bone mineral density can also be used as a measure of damage due to SLE and/or its treatment.

What Have We Learned from HRQOL Assessments?

Use of SF-36 physical and mental component (PCS and MCS) summary scores can lead to a falsely negative result when real changes in HRQOL have occurred. The summary scores minimize changes, especially in SLE where decrements and improvements in multiple domains, mental as well as physical, may frequently occur simultaneously. In an observational study of patients with depression, use of the PCS was insensitive to change whereas scoring each domain reflected improvements with treatment (81). Examining changes across all eight domains, rather than the summary PCS and MCS scores, may better reflect treatment associated improvements.

Table 68-3: Examples and Definitions of Endpoints in Some Renal Trials

Renal flare
Illei et al.: an episode of increased activity of SLE nephritis; classified as either proteinuric (defined as an increase in protein uria >2 g/day with a stable serum creatinine level and inactive urinary sediment, no cellular casts and <10 red blood cells (RBCs)/high powered field (hpf)) or nephritic (defined as mild or severe; mild: defined as reappearance of cellular casts or >10 RBCs/hpf with an increase in proteinuria <2 g/day and with a stable serum creatinine level; severe: defined as reappearance of cellular casts or >10 RBCs/hpf with an increase in serum creatinine 30% over the level at the time of complete response regardless of the level of proteinuria).
Alarcon-Segovia et al.: that it be attributed to SLE by the treating physician and/or medical monitor and one or more of the following three criteria were met:
A. a reproducible increase from baseline in 24 hour urine protein;
B. a reproducible increase from baseline in serum creatinine of >20% or at least 0.3 mg/dL, whichever was greater, accompanied by proteinuria (>1,000 mg/24 hours), hematuria (>4 RBC/hpf) and/or red cell casts; or
C. new reproducible hematuria (>11–20 RBC/hpf) or a reproducible increase in hematuria by two grades compared to baseline associated with >25% dysmorphic red blood cells of glomerular origin, exclusive of menses, accompanied by either an 800-mg increase in 24-hour protein or new appearance of RBC casts.
Treatment Failure
Houssiau et al.: defined as one of the following three features:
D. For patients with baseline serum creatinine levels >1.3 mg/dL and <2.6 mg/dL: absence of a primary response

1. serum creatinine levels >1.3 at 6 months;
2. for patients with baseline serum creatinine >2.6 mg/dL: serum creatinine levels that did not improve by 50% at 6 months;
3. for patients with nephritic syndrome but without renal impairment at baseline; persistence of nephritic syndrome at 6 months.

E. Glucocorticoid resistant flare. Doubling of serum creatinine over the lowest value reached at any time during follow up and confirmed on two consecutive visits 1 month apart
Complete Renal Remission
Chan et al.: proteinuria <0.3 g/24 hours, with normal urinary sediment, normal serum albumin and serum creatinine and creatinine clearance values within 15% of baseline values.

Reported HRQOL, by SF-36, improves significantly in patients with sustained reductions in anti-dsDNA Abs, whether receiving active treatment or placebo (82). Furthermore, active treatment-related improvement is maintained, even in those who develop renal or major SLE flares (83). In these trials, patient global assessments of disease activity paralleled reported SF-36 scores, showing improvement when HRQOL was maintained or improved. Interestingly, patient perceptions of improvement and deterioration were not symmetrical (82).

The minimum clinically important difference (MCID) or degree of improvement in patient reported outcomes perceptible to them, on an individual basis, is considered to represent clinically meaningful change. Improvements of 33% to 36% over baseline (or 18% >placebo) are thought to be clinically important (84 ,85). In rheumatoid arthritis, and osteoarthritis, improvements of 5 to 10 points in individual domain scores of SF-36 and 2.5 to 5 points in PCS and MCS summary scores can be considered to represent MCID (86 ,87 ,88 ,89 ,90). Although these definitions are relevant only on an individual basis, when median changes within a treatment group exceed MCID, the majority of patients are reporting clinically meaningful changes. Another analysis that has been helpful in RA has been comparison of the percentage of patients with improvement which meets or exceeds MCID (91).

In the phase 3 abetimus sodium trial, definitions of MCID in SF-36 domains and summary scores were based on a 15-point global change scale, after Guyatt et al., corresponding to improvement by a score of 6: “a little better” and worsening by a score of 10: “a little worse” (92). MCID was determined to range from 6.7 to 11.4 points in domain scores and 3.4 to 3.9 in PCS scores, consistent with literature reported values in RA and OA and other chronic diseases (82). Of interest, clinically important worsening ranged from -14.7 to 1.7 for domains and -2.1 and -0.8 for MCS and PCS, respectively—demonstrating that MCID was not symmetrical. Although it has yet to be determined how significant changes in patient-reported HRQOL should be predefined in a RCT, definitions of clinically meaningful improvement, which meet or exceed MCID, or stabilization (“lack of deterioration”) may offer another useful means to support the efficacy of a new therapy.

Disease-specific HRQOL instruments are currently under development in SLE and include:

- A symptom checklist published in quality of life research in 2003/2004 (93)
- An instrument developed by Leong et al. published in *Rheumatology* in 2005 (94)
- The SLE-QOL: an instrument from UK researchers, reported in abstract form at the 2004 International Society of Quality of Life Research meeting (95 ,96)

Use of both generic and disease-specific measures of HRQOL are complementary and important in RCTs; as well as supporting economic analyses. Generic instruments facilitate comparisons across diseases but may not measure all domains of interest to an SLE patient; disease-specific instruments reflect the more important aspects of HRQOL and are likely more sensitive to change.

Responder Analyses

A well-constructed SLE responder index would merge the information from the various DAIs, SDI (damage), and HRQOL measures into a single definition that would identify a patient as either a “responder” or “nonresponder.” Responder indices are presented on a per-patient basis, facilitate comparisons across treatments, are easy to use and report, and gain statistical power with repeated use. But a responder index without prospective, objective validation in RCTs is not reliable as a measure. Several responder analyses have been proposed in recent trials but without supportive data from prior RCTs to indicate their validity.

The response index for lupus erythematosus (RIFLE) was first proposed for use in a placebo RCT evaluating anti-CD40L, where the active treatment failed to differentiate from placebo by SELENA SLEDAI or other secondary measures: BILAG, physician and patient global disease activity, time to flare, KFSS and SF-36 (75 ,97). An initial placebo RCT examined whether prasterone would allow reduction of corticosteroid use in steroid-dependent patients with SLE. “Responders” were defined as those receiving approximately 7.5 mg/day prednisone for a least 2 months including the last observation over the 7- to 9-month treatment period (74). In those with active disease, defined as SLEDAI scores greater than 2 (omitting scoring for anti-dsDNA Abs and complement levels), a significant number of patients receiving active therapy were classified as responders compared with placebo. In a second prasterone trial, a “responder” definition proposed in collaboration with the FDA required that patients could not have developed new adverse organ system manifestations, either because of treatment or their underlying disease, based on SDI (damage) (71). If these criteria were met, then patients were assessed for “improvement and/or stabilization” by two physician-assessed DAIs: SLEDAI and SLAM, and two patient-reported measures: global disease activity and KFSS (fatigue). In the large subset of patients with active disease at baseline, statistically more patients receiving active treatment were responders than with placebo.

Another important point is that improvement and/or stabilization, e.g., “lack of deterioration” should be considered a response, especially in SLE with a relapsing and remitting course, characterized by heterogeneous and unpredictable organ system involvement. Statistical definitions of no change or improvement must account for test-retest variability in these measures, which can be large. For example, differences in screening and baseline values for SLEDAI, SLAM, KFSS, and patient global assessed 7 to 14 days apart in patients with “stable” disease and no change in treatment ranged from 0.5 for SLEDAI and KFSS to 1 .0 for SLAM and 10 mm for patient global assessment by VAS (71). And “lack of deterioration” implies that patients cannot have significant worsening during the trial, even over a short period of time (e.g., >30 days, requiring change in therapy).

Systemic Lupus Erythematosus (SLE) Guidance Document

The U.S. FDA recently released a draft *Guidance for Industry Systemic Lupus Erythematosus—Developing Drugs for Treatment*, discussing the development of new therapies for SLE designed to offer useful guidelines for clinical trial development (54). It was developed after several meetings of the Arthritis Advisory Committee discussing trial design in SLE. Written in the absence of a regulatory precedent, it is meant to be advisory, to help develop a roadmap for promising new therapies. It is published in the Federal Register and posted online, where comments are invited: http://www.fda.gov/cder/guidance/index.htm. The document defines treatment, as well as disease-induced damage and queries whether the SDI (damage) could identify all treatment associated toxicities, noting that distinguishing between disease and treatment associated damage would greatly help understand the safety of new therapies. It suggests the definition of “major SLE flare” to be useful and states that serologies and/or surrogate markers may function as supportive evidence of efficacy, although none as yet have been validated. It adds patient global assessment of disease activity to the list of recommended outcome measures and suggests that fatigue and HRQOL are important measures to assess.

Claims for Approval from the FDA *Draft* Guidance Document

The FDA SLE Guidance Document outlines five proposed claims for treatment, presented below, with selected pertinent comments.

- ‘1. Reduction in Disease Activity

  Because the BILAG evaluates patients based on the need for additional treatment, the clinical interpretation of a change in score is apparent. For other indices, deciding whether changes in score are clinically

meaningful may be more complicated. If a disease activity measure other than the BILAG is chosen, confirmation of a positive result with two different DAIs would be important to confirm the findings.

- 2. Treatment of Lupus Involving a Specifically Identified Organ

  For products being proposed for use in the manner of a specified short course of treatment leading to induction of a sustained remission, studies of 3 to 6 months' duration may be acceptable with longer-term follow-up for safety and durability of response. For products being proposed for chronic use, studies as short as 1 year may be considered.

- 3&4. Complete Clinical Response/Remission

  Complete absence of disease activity at all sites for at least 6 consecutive months. This response is termed complete clinical response if the subjects continue to receive SLE-directed therapies. Remission occurs if subjects were receiving no ongoing therapy for their SLE. A trial in support of the claim of complete clinical response should be at least 12 months in duration and demonstrate an increase in the proportion of subjects in whom a disease activity measure achieves zero.

- 5. Reduction in Flares

  Proposals for clinical trials using renal and/or major SLE flare as an endpoint should: (1) provide a clear and accepted definition for flare, and data supporting the choice of that definition; (2) provide evidence that reducing flare incidence by that specific definition of flare would be expected to translate into a clinical benefit to the patient; and (3) assess the durability of the clinical benefit. A successful clinical trial could be defined as an increase in the time-to-flare or as a decrease in the number or severity of flares over the course of a 1-year trial.”

Trial Design and Analysis

Although the major precedent for SLE trials remains the combined NIH experience in nephritis, it is difficult to design RCTs for organ specific damage as most patients have multisystem disease. Thus trials designed to focus only on selected organ system involvement must also assess global health/HRQOL.

Protocol mandated treatment regimens with early rescue—a predefined endpoint of “treatment failure”—are preferred to individual patient management within a protocol. Definitions for when to end a trial because of unforeseen adverse effects or other patient safety concerns must be predetermined.

In designing trials with time-to-flare analysis as an endpoint, all patients should be exposed to the same duration of treatment, which can be difficult to justify if treatment benefit is evident in one group but not another. Stopping studies when a sufficient number of events have accrued may be risky; not knowing their distribution between active and placebo treatment.

Patients cannot be denied standard of care. Therefore, new medications must be tested in an add-on or head-to-head comparison design. Placebo controls are additionally difficult to use except in the presence of background therapy, which leads to confounding polypharmacy. Pharmacokinetic and pharmacodynamic interactions between existing and additional treatments require documentation. An accepted standard of care within the protocol needs to be clearly delineated for the protection of patients.

Studies to Show Superior Safety or Comparable Efficacy

These trials aim to provide the superiority of a new treatment over an existing one. Promising therapies may be compared to accepted therapy, to show superiority or noninferiority. If the goal is only comparable efficacy, then a nonequivalence design may be utilized, which will usually allow a smaller sample size.

Currently only hydroxychloroquine, aspirin, and prednisone are approved for use in SLE. Although not specifically labeled for treatment of SLE, cyclophosphamide is considered standard of care for nephritis. Treatment regimens omitting cyclophosphamide are gaining rapid acceptance and may be used as the active comparator—azathioprine has been widely utilized in Europe, as confirmed in the EURO LUPUS trial, and several RCTs have shown promising results with mycophenolate mofetil (51 ,77 ,78 ,79 ,98).

Alternatively, if superior efficacy to accepted standard of care can be demonstrated for a new therapy, priority review may be considered under the US Code of Federal Regulations (CFR), which govern the regulatory role of the FDA: 21 CFR 314, subpart H or 21 CFR 601, subpart E (99 ,100). These regulations provide that the agent must treat a serious or life-threatening disease, provides meaningful therapeutic benefit over existing treatments, and then require further studies post approval to verify the clinical benefit. This precedent was established with therapies for AIDS, and failure to show benefit in phase 4 may lead to expedited withdrawal. It can be very difficult to demonstrate statistical superiority of a treatment in an active controlled trial, especially when only limited RCT data are available to support the efficacy of active comparators in SLE, and only three agents are currently labeled for use. Demonstration of superiority may therefore require a combined analysis of safety as well as efficacy.

It is also important for trials to be sufficiently powered to demonstrate that if a new product is not inferior to an accepted therapy that it has at least as good or improved safety profile. The two recently published SELENA-SLEDAI RCTs demonstrated the noninferiority of hormone replacement therapy and oral contraceptives to placebo by the number of disease flares in patients with SLE (30 ,32).

Similarly, it is appropriate for steroid-sparing agents to demonstrate not only that reduction in steroid use is statistically significant, but that these reductions are clinically meaningful as well as reflecting an improved safety profile.

Past definitions of clinically meaningful changes include sustained reductions in daily prednisone dose to approximately 7.5 mg—tapering regimens have never been agreed upon or strictly adhered to in an RCT. As with DAIs developed by Delphi process, consensus proposed tapering regimens may be difficult to adhere to when managing a heterogeneous protocol population (101). To demonstrate an improved safety profile is even more challenging as it is difficult to know how to adequately power trials to show statistical differences in safety.

Phase 2 Trials

Phase 2 trials are used to explore the dosing, activity, and toxicity of prospective treatments. Safety of patients needs to be of concern and possible interactions with background treatments need to be evaluated. Outcome measures in phase 2 trials should be carefully evaluated for validity and sensitivity for use in phase 3 trials, as information about the new therapy is most likely incomplete at this point in its development. It is important to note that small phase 2 trials in SLE have substantial risk for being underpowered, given the heterogeneity of the disease population, organ system involvement, requirement for background therapy, and so on.

Phase 3 Trials

Concomitant medications pose a complication for RCT designs in SLE. Acceptable baseline levels of medication and acceptable treatment changes should be defined. Rescue medications and patient withdrawal from the study must also be considered. All RCTs must be adequately blinded to ensure lack of bias. Extension studies should be considered for long term evaluation, but in general, most trials should be at least 12 months long in duration.

Candidate Biomarkers/Surrogate Markers as Endpoints

Biomarkers or “early markers” indicate when a therapeutic agent has been successfully delivered, and has the desired pharmacodynamic effect, which may ultimately reflect potential clinical benefit. Surrogate markers substitute for a clinically meaningful endpoint, and are laboratory measurements or physical signs which have been shown to directly correlate with, and predict clinical outcome.

Biomarkers are an appealing idea for early detection of disease and treatment evaluation, and there is scientific interest in exploring when biomarkers may substitute for clinical endpoints in phase 3 trials, especially in a challenging indication such as SLE. At an NIH workshop held in 1998, participants agreed this primarily applied to situations where trials using clinical endpoints were not feasible, as in the case of ESRD or mortality (102). That no RCT has successfully led to regulatory approval of a new treatment for SLE again raises the question of whether the therapy or the outcome measures failed in these trials.

Table 68-4: Promising Biomarkers in SLE

Class	Examples
Autoantibodies	Anti-dsDNA Abs
Complement and complement split products	C3, C4, CH_{50} Erythrocyte surface CR1/C4d ratios Reticulocyte surface C4d levels
Alterations in circulating B and plasma	CD27 high-plasma cells,
Cell subsets	CD19+, CD20+ B cell subsets
Interferon-α “signature”	IFN-α gene products
Other cytokines	sIL-2R, sTNF-R, IL-6, and IL-10

As there are no currently accepted biomarkers for SLE clinical trials, to use the term “surrogate markers” is premature. In other areas of clinical evaluation, experience has shown that identifying biomarkers and surrogate markers is very difficult and requires a large combined experience of many RCTs (9). Nonetheless, “early markers” of disease activity based on the biologic effects of a therapy should and can be used in SLE trials, and have been extensively reviewed by Illei and Lipsky (103 ,104). Such markers can be particularly useful in phase 2 studies, prior to definitive demonstration of efficacy. Ongoing efforts are underway to identify SLE biomarkers, supported in part by NIH NIAMS, as well to validate and standardize their means of identification (105).

Approval may be based on a validated surrogate endpoint. If the surrogate is not validated, but appears to be reasonably likely to predict a clinical benefit, accelerated approval may be considered under 21 CFR 314, subpart H or 21 CFR 601, subpart E (99 ,100). In this case, approval would be contingent upon a phase 4 study to verify the clinical benefit. Supporting the proposition that the surrogate is reasonably likely to predict clinical benefit is essential to this approach.

Although there are no regulatory precedents at this time, there are biomarkers, which may be suitable to suggest to the FDA for inclusion as examples to support a subpart H or E discussion and are presented below. Importantly, biomarkers may also serve to prospectively identify a patient population with active disease who may best respond to a new therapeutic intervention (Table 68-4).

Anti-dsDNA Antibodies

Anti-dsDNA Abs are the leading biomarker candidate based on published evidence that increasing anti-dsDNA Abs levels may predict disease flares in at least a subset of patients with SLE. Prospective studies have shown anti-dsDNA Abs often rise well before a major SLE flare and decrease at the time of or following a flare (106). Several studies suggest that increases in anti-dsDNA Abs can be used to initiate

pre-emptive treatment to reduce renal flares. Swaak et al. followed 143 SLE patients for as long as 6 years demonstrating that twofold increases in antidsDNA Abs predicted 22 major disease exacerbations (21 renal, 12 nonrenal) and no flares in those with stable anti-dsDNA Ab levels (107). ter Borg et al. followed 72 SLE patients showing 24 of 33 exacerbations were predicted by significant increases in anti-dsDNA Abs levels 8 to 10 weeks prior to flare, a more sensitive measure than complement 3 or 4 levels for predicting exacerbations (108). In two subsequent series, anti-dsDNA Abs were measured monthly; patients with predefined increases (with or without accompanying symptoms) were randomized to conventional treatment or prospective addition of 30 mg prednisone or MMF daily (109 ,110). In both reports, clinical relapses at 6 months were significantly less in patients receiving expectant therapy. Using anti-dsDNA Abs as an indication to treat, only 2 of 22 with active treatment relapsed, versus 20 of 24 with placebo. These observations are consistent with series of “clinically quiescent, serologically active” patients described by Gladman et al., and recently by Buyon et al., where expectant treatment resulted in fewer disease exacerbations (111 ,112).

In an open label series of patients receiving the anti-CD40L mAb BG9588, reductions in anti-dsDNA Ab levels were associated with decreases in identified anti-dsDNA Ab-producing B cells, increased complement 3 levels, and decreased hematuria (113 ,114). In two placebo RCTs in 487 SLE patients, Linnik et al. confirmed that decreases in anti-dsDNA Abs correlated with increases in complement 3 levels and less risk of renal flare over time (76). Fifty percent reductions in anti-dsDNA Ab levels were associated with 52% (95% confidence interval [95% CI], 26%–68%, nominal p <0.0007) and 53% reductions (95% CI, 33%–69%, nominal p <0.0001) in the risk of renal flare in the two trials, respectively. As discussed previously, sustained reductions in anti-dsDNA Abs were associated with improvement in patient reported HRQOL in these trials whether in patients receiving active therapy or placebo superimposed on background therapy. As anti-dsDNA Abs may mediate at least some of the brain damage observed in neuropsychiatric SLE; this mechanism may help to explain these findings (115).

The data support the use of anti-dsDNA Ab titers as a biomarker, viewed in the context of corresponding improvements in complement 3 levels and HRQOL and fewer SLE and renal flares in patients with sustained reductions, but only in the subset of patients with these autoantibodies (116). To be used as a valid and reliable biomarker, standards need to be developed for measuring anti-dsDNA Abs, including when sampling should occur, the relationship between increases in Ab levels and clinical flares, as well as definitions utilized to identify prospective changes. Utilizing a central lab and the correct assay method is critical. For example, the Farr assay is preferable to other ELISA based tests to determine anti-dsDNA Abs, despite its expense, technical requirements, and use of radioactive material (117).

Complement and Complement Split Products

Erythrocyte CR1/C4d Ratio

Complement consumption is a hallmark of active SLE, although historically assays have been insensitive to change until complement synthesis is outstripped by activation with persistent immune complex formation and deposition. Manzi and Ahearn et al. have shown that ineffective clearance of complement-bearing immune complexes by erythrocytes expressing complement receptor 1 (CR1) leads to persistently decreased erythrocyte CR1 and elevated complement 4 split product, C4d, levels with high diagnostic sensitivity and specificity for SLE (118 ,119). In pilot, cross-sectional studies, two color-flow cytometric analyses of erythrocyte C4d (E-C4d) and E-CR1 levels show little change in normal individuals over time; abnormally high E-C4d and low E-CR1 levels are 81% sensitive and 91% specific for SLE compared with healthy individuals, and within an individual SLE patient fluctuate over time and appear to correlate with disease activity (120). Because erythrocytes have a lifespan of approximately 120 days, E-C4d levels likely reflect cumulative complement activation and disease activity over that range of time; reticulocyte bound C4d (R-C4d) may serve as a better reflection of current or impending disease flares and are elevated in patients with SLE compared with normals and other diseases; fluctuations correlated with disease activity as measured by SLEDAI and SLAM. Once this assay is commercially available for pragmatic use in RCTs it may prove to be a very promising biomarker.

Although no RCTs have been performed in SLE with specific complement inhibitors, promising data have been reported with a mAb to complement 5 (eculizumab) in paroxysmal nocturnal hemoglobinuria, demonstrating excellent correlations between improvement in complement levels, measures of hemolysis, and decreased transfusion requirements (121).

Alterations in Circulating B and Plasma Cell Subsets

Although T cells and dendritic cells play well-documented roles in SLE, a central role for B cell hyperactivity has been recently clarified by several open label series with promising therapeutic agents. Specific alterations in circulating B and plasma cell subsets in patients with active SLE and normalization after treatment may serve as useful biomarkers. CD27 is expressed on memory B cells, and a subset of plasma cells, which have been shown to be greatly expanded in peripheral blood of patients with active SLE (122). Flow cytometric analysis of CD27hi plasma cells may be a reliable assessment of disease activity (123).

Circulating T and B cells in patients with active SLE spontaneously express CD40L or CD1 54, suggesting that activated lymphocytes are being released from germinal centers (GCs), which facilitate autoantibody production. Grammer et al. studied CD1 9+ peripheral B cells before

and after treatment with humanized anti-CD40L mAb (BG9588, 5c8) (124). CD38 plasmablasts not found in normal individuals were evident in the circulation, which disappeared following treatment, as did CD38hiIgD^{+} and CD38^{+}IgD^{-} B cell subsets. Disappearance of the bright CD38 plasma cells was associated with decreases in anti-dsDNA Abs, proteinuria, and SLEDAI scores.

Tocilizumab

In a pilot open-label study of tocilizumab, a humanized anti-IL-6 receptor mAb in patients with active SLE, ESR levels fell, and disease activity improved (125). Abnormal circulating CD19lowIgD- plasma cells decreased with treatment: IgD+ B cells, functionally anergic, disappeared and increased levels of naïve CD19hiIgD^{+}CD27^{-}CD21^{+}CD23^{-} B cells normalized, with associated increases in CD19hiIgD^{+} CD27^{-}CD21^{+}CD23^{+} B cells.

Rituximab

Rituximab, a chimeric anti-CD20 mAb, depletes circulating B cells, with more rapid and complete depletion in SLE patients with a specific FcγIIIR polymorphism (126 ,127 ,128). In patients who were “good depletors,” remaining B cells expressed a homogenous memory phenotype, whereas a heterogeneous population of naïve and memory B cells, as well as a high percentage of pre-GC CD38 cells, remained in “poor depletors.” In several recent open-label series, B cell depletion with rituximab led to a sustained clinical response with some reports of impressive disease improvement and variable effects on anti-dsDNA Abs (129 ,130 ,131).

Epratuzumab: Epratuzumab is a humanized mAb to the CD22 surface antigen on B cells that negatively regulates BcR signal transduction and B cell homing, which has been shown effective in more than 300 patients with NHL. An open-label series in 14 SLE patients with BILAG “B” activity in one or more organ systems was positive, demonstrating B cell depletion without immunogenicity (132).

B Lymphocyte Stimulator (BLyS) Antagonists

BLyS is a vital B cell growth factor released by activated monocytes and macrophages, which binds to B cell receptors and promotes antibody production (133). A variety of antagonists to BLyS are in development, including anti-BLyS mAb (belimumab). Preclinical and clinical results to date show that belimumab reduces circulating CD20^{+} B cells, activated B cell subsets and immunoglobulin levels. An observational study of 244 SLE patients over 2 years found 35% of patients had elevated BLyS at one or more time points which statistically correlated with SLEDAI scores, IgG levels and anti-dsDNA Abs (134). A phase 1 study using belimumab in SLE demonstrated selective depletion of B cells with no overt toxicity (135).

Together, the body of data following administration of promising therapeutics, targeting different cell surface antigens, growth factors and cytokines, suggest that alterations in circulating B- and plasma cell subsets will function as useful biomarkers both to ascertain successful delivery of the agent and to correlate with improvements in clinical status.

Interferon-a “Signature”

IFN-α gene expression is increased in SLE and correlates with both disease activity and severity in a subset of patients (136 ,137). Plasmacytoid dendritic cells, a source of type I IFNs, are decreased in SLE plasma but elevated in involved skin and kidney. Oligonucleotide microarray profiling of peripheral blood mononuclear cells in patients with active and inactive SLE has revealed a distinct IFN-α gene product profile or “signature,” which seems to correlate with active disease manifestations and reflects response to treatment with high dose glucocorticoids (138 ,139). Patients with high levels of IFN-α gene products had a higher prevalence of renal disease and SDI (damage) with low C3 levels and Sm and RNP autoantibodies rather than anti dsDNA Abs (140).

As DNA in anti-dsDNA complexes and apoptotic debris activate IFN-α via Toll-like receptors, it is unclear whether the interferon “signature” of induced gene products is responsible for activation of disease or a pathologic marker of disease activity (141). A variety of agents, including mAbs to IFN-α and IFN-α/ßR, mAbs to FcγIIa, IL- 10 and IL- 18, and mAbs to BDCA2 on plasmacytoid dendritic cells inhibit ongoing IFN-α production and are being explored as therapeutic options in SLE (142). Identification of an increased IFN-α “signature” may represent a promising biomarker to identify patients with impending flares, or active SLE, as well as responses to treatment.

Other Cytokines

Unregulated cytokine production, including TNF-α, IL-6, IL-18, and IFNs are overexpressed systemically and locally and play a large role in the inflammatory response in SLE that leads to irreversible organ damage. IL-6 plays a critical role in the B cell hyperactivity and immunopathology of human SLE, and may have a direct role in mediating tissue damage (143). Serum IL-6 levels do not predict disease activity in patients with SLE, although urinary IL-6 may identify active nephritis (144). Increased serum IL-10 and IL-6 levels have been repeatedly shown in patients with active SLE, as well as first-degree relatives (145). Serum IL-10 levels correlated with anti-dsDNA Ab titers, with a weak correlation between circulating IL-10 levels and disease activity as measured by SLEDAI but not SLAM (146). IL-6 and IL-10 levels may play a role as biomarkers in the future; inhibitory agents including monoclonal antibodies targeting their effects will likely better clarify their actions.

Several series have shown that increases in soluble IL-2 receptor (sIL-2R) levels occur during flares of SLE and

decrease with treatment and clinical improvement, especially in patients with nephritis (147 ,148). Circulating levels of soluble p55 and p75 TNF-α receptors have been reported to be increased in patients with active SLE and may also serve as potential biomarkers of disease activity. An open label series administering the anti-TNF mAb, infliximab, led to improvement in arthritis and proteinuria, although was associated with increased anti-dsDNA Abs in a few patients who received re-treatment (149 ,150 ,151).

In summary, the variety of symptomatology and cyclical nature of SLE indicate that consistent biomarkers across patient populations may be difficult to validate. Sensitive and reproducible measures of anti-dsDNA Abs and/or complement activation/depletion, presence of circulating abnormal B cell subsets including preplasma and plasma cells and/or elevated levels of IFN-α, IL-6, and IL-10, or their gene products may serve as biomarkers of active disease as well as potential targets for therapeutic intervention. A variety of multiplexed assays are now available which efficiently analyze hundreds or thousands of samples simultaneously, which should significantly further efforts to identify promising biomarkers (152).

Lessons Learned and Future Directions

Designing successful RCTs for SLE is quite difficult. Specifically, outcome measures are complicated to define and reproduce in such a variable disease. Because of the cyclic nature of the disease it can be difficult to assess clinical endpoints over fixed time intervals. Often in SLE, there are poor correlations between patient and physician assessments of disease activity. Responder analyses do not function well if they are used before validation in early RCTs. Repeatedly in SLE, it has been difficult to replicate findings from one promising trial in a larger confirmatory trial—changes in medical practice at both an individual and group level may confound treatment effects. Objective outcome measures are well defined in renal and hematologic disease, and proposed for other manifestations, but few SLE patients have isolated organ system involvement at any one time. And it is critical to always assess patient reported outcomes.

Attainment of predefined endpoints may require prolonged therapy. If a benefit is conferred over short term or extension trials, a control group becomes unethical, even with background therapy. Yet, there is a need for a control group, although background therapy—even if stable for a period of time prior to enrollment—may well confound treatment effects. Research designs must include definitions for increasing, tapering, or stopping of glucocorticoid doses, as well as criteria for removing patients from the trial if their treatment requires prohibited medications or other nonprotocolized management. Thus trial designs must anticipate treatment of emergent disease manifestations and changes in disease course. Definitions of increased disease activity, flare, and allowed concomitant treatment must be established before trial initiation and strictly adhered to. Protocol mandated treatment regimens should therefore offer early rescue when a predefined endpoint of treatment failure is reached, rather than allowing individualized patient management within a protocol.

Patient selection criteria must also take into account demographics and disease activity and/or damage levels that contribute significantly to the heterogeneity of the SLE population. Although it is appealing to try and define specific criteria to identify a more homogeneous and responsive population, these have so far eluded us. Hopefully data from ongoing and future RCTs will help to inform these efforts, including the use of promising biomarkers.

Although there is a limited body of evidence derived from RCTs, early markers of treatment response have correlated with longer term clinical outcomes and may facilitate future treatment development. Some potential candidates have been discussed in this chapter. If they are biomarkers, it is crucial to use validated methods and a central lab to assure consistency and reproducibility of the assays. Importantly, biomarkers may prospectively identify a patient population with active disease who may best respond to a new therapeutic intervention.

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Chapter 69

Prognosis, Mortality, and Morbidity in Systemic Lupus Erythematosus

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Murray B. Urowitz

The concept of the clinical spectrum of systemic lupus erythematosus (SLE) has evolved over the last 50 years. Before identification of the LE-cell phenomenon in 1948, the diagnosis of this condition was difficult. In 1971, the preliminary criteria for the classification of SLE, based on knowledge of the disease at the time, were described (1), but they included few laboratory tests. These criteria were revised in 1982 (2) and again in 1997 (3) to include serologic tests that had become more generally available. Many patients who had been labeled as having lupus in earlier studies would not meet the current criteria for diagnosis of the disease; on the other hand, mild cases of SLE in the past would have been overlooked. A recent study documented that the application of the 1997 criteria would have excluded two patients from a longitudinal cohort study because of the removal of the LE cell preparation, while the addition of the anticardiolipin antibody to the criteria set would not have affected the inclusion of patients had the LE cell test remained (4). Therefore, early survival studies included only those patients with severe SLE who could be diagnosed by biopsy or at autopsy; thus, long-term survival was unusual. After the introduction of corticosteroids in 1948, the reported survival times in SLE improved significantly. All of these factors have conspired to produce an improved survival in SLE over time. This chapter examines survival in SLE, predictors for mortality, and causes of death. Other important prognostic outcomes that contribute to morbidity in patients with SLE also are discussed.

Survival Analysis

Early studies of survival in patients with SLE reported that no more than 50% survived the first 3 years of their illness (5 ,6). Merrell and Shulman (7) introduced the life-table method to describe survival in SLE and defined the time of diagnosis of SLE as the start point for calculating disease duration. In their classic 1955 study (7), data extrapolated from 99 cases of SLE predicted that 78% of these patients would be alive 1 year after diagnosis, 67% after 2 years, and 51% after 4 years. Patients who were diagnosed within 2 years of the onset of symptoms had a poorer survival. Subsequent studies demonstrated better survival in the 1960s than in the previous decade (8 ,9 ,10 ,11). Dubois' group pioneered large-scale survival studies with reports in 1956 and 1963 (12 ,13) that included 163 and 520 patients, respectively. In the 1956 survey, the 5-year survival rate was 40%. Of the 60 patients from the presteroid era, or those who were inadequately treated, 50 succumbed within 2 years after diagnosis. The duration of disease from diagnosis to death was divided by Dubois into three periods of observation: (1) 1950 to 1955, (2) 1956 to 1962, and (3) 1963 to 1973. This was not an artificial division; 1956 marked the first time that central nervous system (CNS) lupus was treated with high doses of steroids, and 1963 marked the general availability of procedures such as the use of potent nonmercurial diuretics and peritoneal dialysis. The median duration of disease (until death) in the group treated before 1955 was less than 2 years, and by 1973, it had increased to 8.5 years (14).

In 1966, Leonhardt (15) studied 54 Swedish patients with SLE and obtained survival curves from the time of diagnosis of about 90% at 1 year and 70% at 5 years whereas matched normal individuals had 97% and 94% 5-year and 10-year survivals, respectively. Estes and Christian (16) used Merrell and Shulman's methods on 150 patients who were seen between 1962 and 1970, 90 of whom received steroid therapy. The 5-year and 10-year survival rates were 77% and 59%, respectively. Urowitz et al. (17) reported a 5-year survival rate of 75%, whereas the 10- and 15-year survival rates dropped to 63% and 53%, respectively. In a subsequent evaluation by Lee et al. (18), the first- and second-year survival rates were estimated at 93.1%, and by 5 years, the survival rate decreased only to 91.2%.

Tremendous improvement in survival was evident throughout the 1970s. Urman and Rothfield (19) evaluated the survivorship of 156 patients who were treated at the University of Connecticut between 1968 and 1976, and they compared it with that of 209 of Rothfield's New York City patients who were treated from 1957 to 1968. Although the validity of comparing two such totally disparate groups has been questioned, the 5-year survival rates were 93% and 70%, respectively. Over 90% received steroids, but less than 1% were treated with immunosuppressive drugs. Improved survival was attributed to better disease understanding, newer antibiotics, use of C3 and anti-DNA to monitor activity, and judicious adjustment of steroid

doses. The prevalence of CNS disease decreased significantly between the two groups.

In 1981, Wallace et al. (5 ,20) evaluated the course of 609 patients who had been followed in Dubois' private practice since 1950. The overall 5-, 10-, and 15-year survival rates were 88%, 79%, and 74%, respectively. Overall survival improved only in those who were diagnosed since 1970. Patients older than 50 years of age had a benign course, and children did well (100% 10-year survival) only if renal disease was not present. At least 100 of the 609 patients were off all medication and in complete remission for at least 5 years. These findings are consistent with those in a report by our group (21) of several patients with severe multisystem disease who were off all medications and were asymptomatic a mean of 75 months later.

Ginzler et al. (22) reported the results of a nine-center study of 1,103 patients in 1982. The 5- and 10-year survival rates were 86% and 76%, respectively. (Note the similarity to the Wallace data.) No improvement in survival was noted in the patients entered between 1965 and 1970 compared with those entered between 1971 and 1976. One-half of the patients received public funding, and they had a significantly lower survival rate. Overall, whites lived longer than African Americans, but this difference was not noted in the privately funded patients.

Other investigators have reported better survival rates in patients with SLE than those found in the Wallace and Ginzler studies. At the Johns Hopkins Hospital, 140 subjects had 94% and 82% 5- and 10-year survivals, respectively (23). A British group (24) observed 98% 5-year survival among 50 patients who were followed for 29 months. Fries and Holman (25) reported that more than 90% of their 193 patients survived for 10 years. Jonsson et al. (26) identified 133 patients with SLE out of 158,572 Swedish individuals. Only nine deaths occurred in these patients, and the 5-year survival rate was 95%. In India, however, patients with SLE have not fared as well, with survival rates of 68% at 5 years and only 50% at 10 years (27). In a large, hospital-based Danish study (28 ,29), 39 deaths occurred between 1965 and 1983, and an 80% 10-year survival rate was reported, which did not change when patients were divided into those diagnosed before and those diagnosed after 1973. Similarly, a Dutch group (30) that followed 110 patients between 1970 and 1988 noted only 14 deaths, with survival rates of 92% and 87% at 5 and 10 years, respectively. Reveille et al. (31) examined survivorship in 389 patients who were seen at the University of Alabama hospitals between 1975 and 1984. The 89 deaths in this group provided 89% and 84% 5- and 10-year survival rates, respectively. In another southeastern U.S. study of largely publicly funded patients, Studenski et al. (32) reported the outcomes of 411 patients who were observed between 1969 and 1983 at Duke University hospitals in North Carolina. Only patients who were seen within 2 years of diagnosis were considered. The 81 deaths in this group allowed 84% 5-year and 82% 10-year Kaplan-Meier survival curves to be devised. A subsequent analysis of the Duke University cohort followed between 1969 and 1983 revealed slightly lower survival rates of 82%, 71%, and 63% for 5-, 10-, and 15-year survival (33).

In the nineties, there was continued improvement in the survival rates of patients with SLE in the Western world. In a study of 66 patients from Finland (34), the 10- and 15-year survival rates were 91% and 81%, respectively. Pistiner et al. (35), reviewing data collected in a single practice in Los Angeles, California, reported survival rates of 97% at 5 years, 93% at 10 years, and 83% at 15 years. These authors clearly demonstrate the improved survival in the past decade, because their previous survey (20) demonstrated 88%, 79%, and 74% survival at 5, 10, and 15 years, respectively. Information from the Arthritis, Rheumatism, and Aging Medical Information System (ARAMIS) data bank reveal similar results (36). Data from the University of Toronto Lupus Clinic published in 1995 revealed rates of 93%, 85%, 79%, and 68% for 5-, 10-, 15-, and 20-year survival in a cohort of 665 patients (37). The most recent survival data from the University of Toronto Lupus Clinic provide 5-, 10-, 15-, and 20-year survival rates of 95%, 90%, 84%, and 75%, respectively. Almost identical survival rates were reported by Tucker et al. (38) for a cohort of 165 patients followed at Bloomsbury Rheumatology Unit in London, England. A study of 658 patients from Mexico City provides excellent overall survival rates of 96% at 5 years and 92% at 10 years when measured from the time of first symptom. However, the 5-year survival from entry to the cohort was only 91% (39) (unfortunately, the survival was not calculated from the time of diagnosis and therefore is not comparable to the other reported series). A study of 306 European Spanish patients revealed similar 5-, 10- and 15-year survival rates of 90%, 85% and 80% (40). Among the population of Manitoba, Canada, survival rates at 5, 10, and 15 years were 98%, 96%, and 90% for Caucasians, and 94%, 80%, and 75% for Indians, respectively (41). A multicenter cohort of 513 Danish patients identified survival rates of 91%, 76%, 64%, and 53% for 5, 10, 15, and 20 years, respectively (42). Similar observations for 5- and 10-year survival rates of 92% and 75% were noted for an incident cohort of patients in northern Norway (43). Uramoto et al. (44) compared patients with SLE diagnosed at the Mayo Clinic between 1981 and 1982 to those diagnosed between 1950 and 1979 and found a significant improvement in the survival rates over time. Their patients are older (49 and 46 years at diagnosis) than those included in other cohorts (early thirties). A study from Lund, Sweden demonstrated 93% 5-year survival and 83% 10-year survival (45). The median age at diagnosis for the Swedish cohort was similar to that noted at the Mayo Clinic. Five-year survival rates were similar to the general population, however, the 10-year survival in the SLE cohort was reduced compared to the rate of 96.3% in the general population. A recent study of 245 patients from Barcelona documented a 5-, 10-, and greater than 10-year survival at 97%, 94%,

and 90% respectively (46). Cervera et al. (47) recently reported a 10-year survival of 92% among a cohort of 1,000 European patients with SLE. However, this was based on duration of follow-up and is not comparable to previous studies, as the patients entered this multicenter study with an average disease duration of 101 months. A survival analysis of a German cohort of 338 patients revealed a 97% 5-year survival and 90% 10-year survival (48). Japanese patients with SLE have also demonstrated improved survival in the past 2 decades (49). The 5-year survival rate for patients diagnosed between 1955 and 1969 was 71%, those diagnosed between 1970 and 1979 was 91% and for those diagnosed between 1980 and 1990 it climbed to 96%. A cohort of 178 SLE patients was identified in a population study in northwest Greece between January 1982 and December 2001. Survival in this cohort was excellent with rates of 97% and 90% for 5- and 10-year survival (50). In a multicentered study of 1214 SLE patients from South America (the GLADEL study) the 4-year survival was documented at 95% (51).

The improved survival typical of the Western world has not been noted worldwide. It has been suggested that the survival of patients with SLE from India has not improved at all during the 1980s (52). A comparison of patients with SLE who were registered between 1981 and 1985 with those who were registered between 1986 and 1990 revealed that these two groups were similar in demographics and disease characteristics, and in their mortality rates (72% and 78% at 5 years, respectively). Of note, mortality was higher in the first 2 years of disease. Five- and 10-year survival rates for SLE patients in Singapore during the period of 1970 to 1980 were 70% and 60%, respectively (53). Similar survival rates were noted in Japanese patients with SLE prior to 1980. An additional study from India (54) including patients followed from 1981 to 1993 revealed a cumulative survival of 77% at 5 years and 60% at 10 years. Among black Caribbean patients, poorer survival of only 56% at 5 years has been recorded (55), and a study from Chile (56) showed that 10-year survival of Chilean patients with lupus is somewhat lower than that of patients in several North American centers. In Malaysia, the overall 5- and 10-year survival rates were reported to be 82% and 70%, respectively. The results were lower for Asian Indian patients (70% and 65%, respectively) but were similar in Chinese and Malay patients (83% and 75%, respectively) (57). Similar survival rates of 84% and 75% were noted in cohort of 349 Thai patients (58). A study of Tunisian lupus patients followed between 1987 and 2001 revealed survival rates of 85% at 5 years (59).

In summary, the survival of patients with SLE has improved from 50% at 2 years in 1939 to 5- and 10-year survivals of 70% and 50%, respectively, after the introduction of corticosteroids in the 1950s; to 90% and 80% 5- and 10-year survivals, respectively, in the 1980s; and to almost 70% survival at 20 years in the 1990s (Fig. 69-1 and Table 69-1).

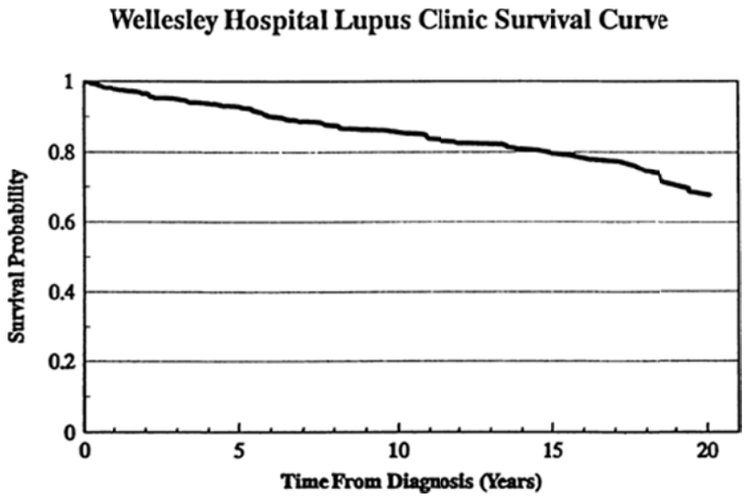

Figure 69-1. Survival in SLE, University of Toronto Lupus Databank. From Abu-Shakra M, Urowitz MB, Gladman DD, et al. Mortality studies in systemic lupus erythematosus. Results from a single centre. I. Causes of death. *J Rheumatol* 1995;22:1259–1264, with permission.

Reasons for Improved Survival in Patients with SLE

It may be concluded that the major contributing factors toward improved survival since 1950 are the availability of dialysis, corticosteroids, and improved antibiotic and antihypertensive agents. Further improvement in subsequent decades may have resulted from earlier diagnosis and the inclusion of milder cases in the more recent studies. Wallace et al. (20) found a mean interval of 4 years from onset to diagnosis in the 1970s; in 1990, this interval was 2 years (34). This is not supported, however, by the patterns of disease that have been seen over the past two decades (5 ,43) or by a recent study from Curaçao (55), in which the delay of diagnosis was only 18 months and the patients fared poorly.

The authors of a Dutch study analyzed the pattern of clinical features in patients with SLE over the past two decades (60). They divided their 110 patients, who were followed prospectively in their lupus clinic, into three groups based on their year of diagnosis. Group A included patients who were diagnosed before 1975, group B included patients diagnosed between 1975 and 1979, and group C included patients diagnosed between 1980 and 1985. There were no differences in the age at diagnosis, the age at onset of symptoms that could be related to SLE, or the interval between onset of symptoms and diagnosis in these groups. Moreover, there were no significant differences in the frequency of clinical features at the time of diagnosis. The number and types of exacerbations in these groups were not different after correction for disease duration. Thus, the authors concluded that the use of American College of Rheumatology (ACR) criteria for the classification of SLE, or the availability of laboratory tests for the diagnosis of the disease, have not led to earlier diagnosis of SLE or a change in its clinical pattern during the past two decades.

A study from Toronto (61) further investigated the reasons for the improved survival noted among patients with SLE

over a 24-year period. These authors had documented the increased survival rates for their patients over that period of time. They divided their patients into three groups, reflecting three periods of admission to their lupus clinic, and compared the survival rates in these groups to those recorded for the general population over the same periods of time. The improved survival in their patients with SLE did not reflect only the general improvement in survival in the population, because the standardized mortality rates decreased over the three periods, from 7.74 in the early 1970s, to 3.02 in the late 1980s and early 1990s. Analysis of variance did not reveal significant differences in the age at diagnosis or at enrollment into the clinic, or in disease activity at presentation as measured by the SLE Disease Activity Index (SLEDAI). Although there was a reduction in deaths because of infection and other morbidity, there were no differences in deaths related to lupus disease activity. The authors concluded that the improved survival in their patients was not the result of earlier diagnosis and/or a milder form of the disease. Because no new medications for SLE were instituted during the period of study, new treatments could not be considered as the reason for improvement. More appropriate use of conventional therapy was a more likely explanation.

Table 69-1: SLE Survival Rates (%) for 5, 10, 15, and 20 Years in Published Series over the Past Five Decades

Author (ref)	Type	No.	Year	Center	5	10	15	20
Merrel & Shulman (7)	Cohort	99	1953	Baltimore	50	—	—	—
Kellum & Hasericke (8)	Cohort	299	1964	Cleveland	69	54	—	—
Urman & Rothfield (19)	Cohort	156	1968	New York	70	63	—	—
Estes & Christian (16)	Cohort	150	1971	New York	77	60	50	—
Urowitz et al. (17)	Cohort	81	1974	Toronto	75	63	53	—
Urman & Rothfield (19)	Cohort	209	1976	Farmington	93	84	—	—
Wallace et al. (20)	Cohort	609	1979	Los Angeles	88	79	74	—
Boey (53)	Cohort	183	1980	Singapore	70	60	—	—
Ginzler et al. (22)	Cohort	1103	1982	Multicenter	86	76	—	—
Jonsson et al. (26)	Pop	86	1985	Sweden	97	—	—	—
Malaviya et al. (27)	Cohort	101	1986	India	68	50	—	—
Gripenberg & Helve (34)	Cohort	66	1988	Finland	—	91	81	—
Swaak et al. (30)	Cohort	110	1989	Holland	92	87	—	—
Reveille et al. (31)	Cohort	389	1990	Alabama	89	83	79	—
Pistiner et al. (35)	Cohort	570	1990	Los Angeles	97	93	83	—
Seleznick & Fries (36)	Cohort	310	1990	Stanford	88	64	—	—
Kumar et al. (52)	Cohort	286	1990	India	78	—	—	—
Wang et al. (57)	Cohort	539	1990	Malaysia	82	70	—	—
Ward et al. (33)	Cohort	408	1991	Durham	82	71	63	—
Nossent (55)	Cohort	68	1993	Curaçao	56	—	—	—
Massardo et al. (56)	Cohort	218	1993	Chile	92	77	66	—
Abu-Shakra et al. (37)	Cohort	665	1993	Toronto	93	85	79	68
Tucker et al. (38)	Cohort	165	1993	London	93	86	78	—
Murali et al. (54)	Cohort	98	1993	India	77	60	—	—
Blanco et al (40)	Cohort	306	1993	Spain	90	85	80	—
Ståhl-Hallengen et al. (45)	Pop	162	1994	Sweden	93	83	—	—
Peshcken & Esdaile (41)	Pop	177	1996	Manitoba Caucasian.	98	96	90	—
		49		Manitoba Indians	94	80	75	—
Jacobsen et al. (42)	Cohort	513	1999	Denmark	91	76	64	53
Alarcon et al. (77)	Cohort	288	2001	US multicentre	86	70	—	—
Nossent (43)	Pop	83	2001	Norway	92	75	—	—
Alamanos (50)	Pop	178	2003	Northwest Green	97	90	—	—
Pons-Estel et al. (51)*	Cohort	1214	2005	South America	95	—	—	—
Gladman & Urowitz**	Cohort	1175	2005	Toronto	95	90	84	75

Pop, population study.
*4-year survival; **Unpublished observations.

In Chile, Massardo et al. (56) also noted that patients whose disease onset was after 1980 fared better than those who were diagnosed earlier, but they did not compare their

patient population with the overall Chilean population. In India, the lack of improvement in survival in their population was thought to result from the general standard of medical care. This lends support to the notion that the improved survival in patients with SLE may have resulted from advances in medical therapy in general, such as improved antibiotics, antihypertensive agents, and the availability of renal dialysis and transplantation, as well as from more judicious use of lupus-specific therapy.

Uramoto et al. (44) demonstrated that although the incidence of SLE tripled over four decades between 1950 and 1990, the survival of patients with SLE has improved. They suggested that recognition of mild disease and better approaches to therapy may contribute to the improved survival, but this is not supported by the literature.

Bjomadai et al. (62) also demonstrated reduced mortality hazard ratios in Sweden for hospitalized SLE patients identified in the three decades ending in 1994. They identified a decline only in causes related to SLE, suggesting that the disease process was better treated. Unfortunately, there was no information on the actual disease manifestations or treatment provided these patients.

Although it is not clear exactly what has been associated with improved survival in SLE, it is possible that the tendency to follow patients with SLE in specialized clinics has contributed to this improvement. Ward recently documented the role of the experience of an individual physician in looking after SLE patients in the observed reduced mortality (63). After adjustment for demographic characteristics, severity of illness and hospital characteristics, the mortality risk was reduced by 20% and 42% for physicians with 1 to 3 or greater than 3 SLE admissions per year compared to less than 1. This was most noticeable for patients with lupus nephritis, for whom the adjusted odds of mortality were 60% lower among those in the highest category of physician volume.

Urowitz et al. (64) have also noted that specialty care may be advantageous for patients with lupus, even when their disease is under control.

Mortality Rates

Several published reports allow trends in overall mortality rates among patients with SLE to be interpreted. Data from the studies by Cobb (65) and Siegel et al. (66) demonstrate surprisingly low death rates. Between 1968 and 1972 (67) and between 1972 and 1977 (68), improvements in mortality rates were seen in all subsets; both of these studies suggest that mortality in African Americans is accentuated in early adulthood and then declines and that mortality in whites consistently increases with age. Death rates increased earlier in females than in males. These figures cannot be interpreted to imply a greater incidence of SLE in African Americans than in whites, however, because socioeconomic differences between the groups are still large and consequently, influence mortality.

Kaslow (69) also studied mortality patterns in 12 U.S. states that include 88% of all Americans of Asian ancestry. Between 1968 and 1976, the mortality rate for Asian Americans was 6.8 per million person-years, compared with 8.05 for African Americans and 2.8 for whites. Serdula and Rhodes (70) noted the mortality rate in Hawaii (1970–1975) to be 1.89 per million person-years for whites and 14.46 for nonwhites who were almost entirely of Asian ancestry. This finding also implied greater disease severity, increased incidence of SLE, or more socioeconomic hardships among Asian Americans, but these contentions remain to be proven.

Several reports have addressed mortality rates of SLE in Europe. Helve (71) derived a 4.7 per million person-years mortality rate among patients with SLE in Finland, but this study considered only hospitalized patients. Hochberg (72) accessed the Office of Population Censuses and Surveys data from 1974 to 1983 for England and Wales. He concluded that females have a fourfold higher mortality rate than men and that the highest mortality rates were in the 65- to 74-year age group. The annual mortality rate among females fell from 4.47 to 2.99 per million person-years between 1974 and 1983. These mortality patterns are similar to those observed in the United States. In the Danish multicenter cohort (43), the overall mortality rate was 2.9% per year, with a standardized mortality ratio of 4.6. This standardized mortality ratio is identical to that reported for the Toronto cohort, as well as to a British cohort (37 ,73). A study from Mexico City also identified the mortality rate at 2.4% per year, but does not provide comparative data for the Mexican population (39). Mortality rates in Greece and Germany were found to be lower than those in other centres, with standard mortality ratios (SMRs) of 1.2 and 2 respectively (48 ,50).

Ward demonstrated that in-hospital mortality among patients with SLE admitted through the emergency room is lower at hospitals in which there is more experience caring for patients with SLE (74). Mortality for emergency admissions as a result of SLE was 1.7% for hospitals with high experience compared to 10% for hospital with less experience. Ward further demonstrated that for patients with private insurance there was no difference in mortality rates related to hospital experience (75). However, patients without private insurance had much lower mortality rates if hospitalized in experienced hospitals. Differences in mortality between the highly experienced and less experienced hospitals was likely not related to general medical care since the difference was not seen for all subgroups of patients with SLE, nor for all reasons for hospitalization. It is more likely related to better management of the SLE itself. This is further documented in the fact that death rates were lower when experienced physicians were looking after the patients (63).

A U.S. study revealed geographical variation in mortality associated with SLE (76). The study identified increased mortality in Alabama, Arkansas, Louisiana, and New Mexico. Rates were higher in all racial groups but relative risks were lower in whites than other racial groups, and were higher in women than in men. Minnesota, Vermont, Virginia, and Washington had lower than expected SLE deaths. Clusters with elevated mortality had higher poverty rates and/or greater concentrations of ethnic Hispanics than those with lower mortality.

In the LUMINA (Lupus in Minority populations: Nature versus nurture) study, which includes patients who are seen within 5 years of disease onset, there were 34 deaths (11.8%) among the first 288 patients within the first 5 years of observation. Mortality was higher among Hispanics (12.2% and African American (15%) than Caucasians (7%) (77).

Causes of Death

Despite their improved survival, patients with SLE still die at a rate that is three to four times that of the general population (61 ,62 ,73). Causes of death may be divided into those related to the SLE disease process itself, those related to therapy, and those from unrelated causes (Table 69-2). Causes related to the SLE include active disease, including nephritis, vasculitis leading to CNS disease or intestinal perforation, intractable bleeding, and end-organ failure (e.g., renal, cardiac, or pulmonary). These certainly have improved with the more appropriate use of steroid therapy, the introduction of antimalarials, and immunosuppressive therapy. Better treatment for hypertension as well as cardiac and pulmonary failure, and the advent of renal dialysis and transplantation, may have averted some of the deaths that otherwise would have resulted from intractable organ failure in these patients.

The primary cause of death may at times be difficult to determine, but early studies followed the detailed protocols that were given in Klemperer's classic paper of 1941 (78). Before 1962, the most common cause of death was progressive renal failure and its associated complications. The incidence of uremia increased from 5% of patients who were treated in the 1930s to as high as 36% in Estes and Christian's report in the 1960s (16), before declining in the 1970s. Since then, uremic demise has occurred much less frequently as a result of better care for patients with end-stage renal disease, including dialysis and the use of cytotoxic therapy for lupus nephritis. Between 1956 and 1973, it decreased from 36% to 14% for all causes of death. The second most common clinical pattern was evidence of active CNS lupus, but with high-dose steroid therapy, CNS lupus became a much less frequent cause of death after 1956 (78). In Dubois' series (79), it decreased from 26% to 8% between 1956 and 1973. The more recent multicenter study (80) revealed mortality because of CNS disease to be reduced to 7%, and in the most recent study from Toronto (37), CNS disease contributed to 5% of deaths.

Table 69-2: Primary Causes of Death and Their Contributing Factors in SLE

	Primary Cause N (%)	Contributing Factors N (%)
I. Active SLE	20 (16)	38 (30.6)
II. Infections	40 (32)	12 (9.7)
III. Other morbidity related:	38 (31)	2 (1.6)
Acute vascular events	19 (15.4)	
Myocardial infarction	13 (10.5)	
CVA	5 (4)	
Rupture of abdominal aneurysm	1 (0.8)	
Sudden death	10 (8)	
CHF	2 (1.6)	
Pulmonary embolism	2 (1.6)	1 (0.8)
Renal failure	2 (1.6)	3 (2.4)
Pulmonary fibrosis	2 (1.6)	
Others	1 (0.8)	
IV. Unrelated to SLE	13 (10.5)	5 (4)
Malignancy	8 (6.5)	
Suicide/accident	3 (2.4)	
Chronic obstructive lung disease	2 (1.6)	
Aplastic anemia		1 (0.8)
V. Unknown	13 (10.5)	

From Abu-Shakra M, Urowitz MB, Gladman DD, et al. Mortality studies in systemic lupus erythematosus. Results from a single centre. I. Causes of death. *J Rheumatol* 1995;22:1259–1264, with permission.

Most patients in Klemperer's series (78) died from infections because of low resistance and the unavailability of antibiotics. Common bacterial pathogens and tuberculosis accounted for most of these infections (16 ,81 ,82 ,83); opportunistic organisms were rare in the presteroid era. By 1975, despite the benefits that may follow steroid or cytotoxic therapy, the primary cause of death remained progressive renal damage. The second most common cause was infection, particularly bronchopneumonia caused by opportunistic pathogens. Ropes (84) emphasized that the major causes of death before 1949 were infection, active lupus, and uremia, but that by 1964, they were uremia, infection, and CNS disease.

In 1976, Urowitz et al. (17) demonstrated a bimodal mortality pattern in SLE. Of 81 patients who were studied, 11 died, and six of these died within a year of diagnosis, usually from complications of active lupus or sepsis. All were on high-dose steroids. The remaining five patients died a mean of 8.6 years after diagnosis. None had active nephritis or sepsis. The mean dose of steroids was minimal, and four patients had myocardial infarctions. Their follow-up studies (37 ,85), as well as the work of others cited in this section, confirmed these findings.

Urman and Rothfield (19) also demonstrated a change in the causes of death as patients with SLE age. Among 209 patients who were studied in New York City from 1957 to 1968, the main cause of 49 deaths was active lupus (excluding uremia; 39%), lupus nephritis (27%), and infection (22%). In 19 deaths among their 156 Connecticut patients who were followed from 1968 to 1976, the causes were lupus nephritis (42%), active lupus (excluding uremia; 21%), and infection (16%).

The multicenter study of 1,103 patients by Rosner et al. (80) reported 222 deaths between 1965 and 1976. As in Urman and Rothfield's report (18), no patient died from a malignancy. The major causes were infection (18%), renal disease (18%), CNS disease (7%), and cardiovascular disease (6%).

Karsh et al. (86) reviewed 94 deaths in 428 patients with lupus nephritis who were seen at the National Institutes of Health (NIH) between 1954 and 1977. The bimodal pattern first reported by Urowitz et al. (17) was observed. The causes of death were renal complications (40%), vascular disease (25%), and infection (16%). Only one death occurred from cancer, although most of the patients were treated with immunosuppressive drugs.

In a similar study of 138 patients with lupus nephritis who were followed at Guy's Hospital in London between 1964 and 1982 (87), 42 died. Of these, 12 died, usually from active lupus or infection, within a month of starting dialysis. The bimodal curve with late vascular deaths was borne out.

Wallace et al. (20 ,88) reported their experience with 128 deaths among 609 private patients who were seen between 1950 and 1980. Only 38 of those in the group had kidney disease, but they accounted for 67% of the deaths. The most common causes of death without nephritis were cardiovascular disease (primarily atherosclerotic; 30%), CNS disease (mostly vasculitis; 24%), and sepsis (17%). These authors confirmed Urowitz's finding that most early deaths result from active SLE, and that the preponderance of later deaths result from cardiovascular complications (17).

Information was available on 55 of 67 patients with SLE who died at the University Hospital in Jamaica between 1972 and 1985 (89). Of these, 23 were early demises, and 32 were late. Deaths were caused by infection (37%), renal disease (24%), hemorrhage (17%), and CNS disease (17%). Of 88 deaths observed in a SLE cohort by Studenski et al. (32) at Duke University, 71 resulted from the disease. Reveille et al. (31) reviewed the charts of 389 patients with lupus at the University of Alabama who were seen between 1975 and 1984. Of the 89 patients who died, 74 had a determinable cause of death. The principal causes of death were infection (39%), active SLE (11%), and cardiovascular disease (9%). Pistiner et al. (35) completed a 10-year update of 570 patients with lupus who were seen between 1980 and 1989. None of the patients with discoid or drug-induced lupus died. The most common causes of deaths among patients with SLE were active disease (35%), sepsis (19%), stroke (15%), and cardiovascular disease (15%).

Cardiovascular disease emerged as a major cause of death in a population study from Sweden (62). Mortality risk because of heart disease was highest in the 20- to 30-year age group. Other important cause of death was malignancy. This study was based on an administrative database and based on hospital discharges. Nonetheless, it supports the concept that active lupus is decreasingly recognized as a cause of death in SLE.

Sepsis, advanced renal failure, left-ventricular failure, and pulmonary embolism were the leading causes of death in the study reported from India (52). Causes of death among 48 Chilean patients with SLE (56) included active SLE in 31 patients, with renal and CNS disease being most prominent. Seventeen patients died with inactive lupus. Four of these 17 deaths resulted from infection, with the others generally resulting from complications of the disease or its therapy. Infection contributed to death in 27 patients. A survey of African blacks who were hospitalized with SLE between 1984 and 1990 in Durban, South Africa, revealed a high mortality rate (90). Causes of death among the 23 patients who died in the hospital included a combination of infection (47%), renal failure (37%), cardiac failure (21%), and neurologic disease (16%). Other causes included pulmonary embolism, diabetic coma, and cardiovascular collapse. Infections and active lupus were the most common causes of death among SLE patients in Singapore (53). These were also the most common causes of death in the European Working Party study (44), in the Danish study (43), as well as in the LUMINA study, which reported deaths within the first 5 years of study onset (77). Infection was the most common cause of death in the cohort from London reported by Tucker et al. (38). These authors also reported on four of 16 deaths related to complications of malignancy. A more recent British study also highlights malignancy as an important cause of death among their patients with SLE (73).

Abu-Shakra et al. (37) reviewed the causes of death in 124 patients with SLE who died during follow-up at the University of Toronto Lupus Clinic between 1970 and 1994. Active SLE and infection were both primary and contributing factors in a large proportion of the patients (Table 69-2). Patients who died within the first 5 years of diagnosis were more likely to die of active disease, whereas patients who died late in the course of their disease tended to die of atherosclerotic complications, thus demonstrating the bimodal mortality once again. Of the 124 patients who died from 1970 to 1993 in that clinic, 40 have had postmortem examinations (37). Of these, 21 (40%) had evidence of moderate to severe atherosclerosis at the time of death either as a coexistent finding or as a primary cause of

death. This same group of investigators demonstrated that although mortality risks from SLE have decreased over the past 25 years, there was a decrease in mortality related to infection there no decreased mortality because of active SLE. Ståhl-Hallengegre et al. (45) also demonstrated that late deaths among patients with SLE were related to atherosclerosis.

Hernandez-Cruz et al. (91) identified 76 SLE in-patients who died and had autopsies in their institution between 1960 and 1994 and matched them with SLE in-patients on the basis of age, decade of SLE onset, and disease duration. Major causes of death included infection, lung hemorrhage, CNS hemorrhage, and active SLE. Myocardial infarction as a cause of death was noted only after 10 years of disease.

Accelerated atherosclerosis has been identified as a major cause of mortality and morbidity in SLE. Studies at the University of Toronto cohort revealed that at any point in time 10% of the patients will have features of clinical atherosclerosis either manifesting as angina, myocardial cardiac infarction, or peripheral vascular disease alone or in combination. This is comparable to the prevalence of myocardial infarction and angina noted in other established lupus cohorts including Pittsburgh (6.7%) and Baltimore (8.3%) (92 ,93). Ward (94) also has identified atherosclerotic morbidity and mortality among patients with SLE. Bruce et al. (95) further demonstrated that persistent hypercholesterolemia in the first 3 years of SLE was a risk factor for mortality in their inception cohort.

Factors Associated with Mortality and Morbidity in SLE Demographic Factors

Several specific factors have been implicated as predisposing factors for mortality in patients with SLE (Table 69-3). These include demographic features, which are unrelated to the disease process itself, such as race, gender, age at onset, and socioeconomic status, including the type of healthcare delivery system that is available.

Race

Race distribution and its influence on SLE diagnosis is discussed in Chapter 4 , The Epidemiology of Systemic Lupus Erythematosus, and in the previous sections of this chapter. Siegel et al. (96 ,97) analyzed the relationship between survival and race among a black, Puerto Rican, and white population in a series of 292 cases in the 1960s, and they observed no differences. The 5-year survival rates by group ranged from 62.7% to 65.1%.

Table 69-3: Factors Associated with Mortality

Non-SLE-related factors	SLE related factors
Demographic	Year at diagnosis
Race	Time of Onset to diagnosis
Gender	Disease manifestations
Age at onset	Disease activity
Socioeconomic status	at presentation
Health care delivery system	Treatment
Environmental	
Geographic	

In general, whites have a better outcome than nonwhites, with mortality rates being three times higher in nonwhites than in whites (55 ,57). Although race did not appear as an important prognostic factor in a logistic regression analysis of the multicenter study published by Ginzler et al. (22), it was found to be a factor adversely affecting survival of patients with SLE when Cox multivariate analysis was applied to a group of 389 patients by Reveille et al. (31), and was also an important factor in the LUMINA study (77). It has been difficult to separate the effects of race and socioeconomic status, particularly regarding the differences between white and African-American patients in the United States. In Reveille et al.'s study (31), white patients with private insurance fared better than African-American patients with private insurance; however, there was no difference in outcome when African-American patients with and without private insurance were compared. Studenski et al. (32) found that nonwhite race and socioeconomic status contributed independently to mortality. Also, although the overall survival was better for whites than for African Americans in a recent report from Duke University (33), this was related to the socioeconomic status, which was poorer among the African Americans. Abu-Shakra et al. (98) analyzed the factors associated with mortality in patients with SLE who were followed at a single centre. Race was not found to be associated with mortality. Levy et al. (99) identified high unfavorable outcomes among their black and North African patients. This was based on a descriptive analysis, not on a formal statistical evaluation. They suggest that although this may be related to poor socioeconomic status, noncompliance with medical treatment is another factor. Thus, although there may be racial differences in the expression of this disease and its outcome, the effect of race has been confounded by other factors. Wang et al. (57) demonstrated a reduced survival among Asian Indian patients compared to Chinese and Malay patients in Malaysia. Ward found that hospital mortality was mostly affected by the experience of the physician regardless of ethnicity (75). However, in a subsequent study Ward found that among whites, higher education levels were associated with lower mortality becuase of SLE. The same associations were not noted among ethnic minorities and it was suggested that underascertainment may represent underreporting of SLE on death certificates or underdiagnosis of SLE in ethnic minorities with low education levels (100).

Just as with survival studies it has been difficult to isolate the role of race in morbidity in SLE. A recent study from Toronto shows that among patients followed in the same centre with the same health care availability there were some differences in morbidity, although not in mortality (101). Similarly among SLE patients in Manitoba, although Caucasians had slightly higher survival rates, they were not substantially different from Manitoba Indians (41).

Gender

The relationship between gender and prognosis has been controversial. Although Kaslow suggested higher mortality in females than in males with SLE (69), Wallace et al. (20) and Kaufman et al. (102) demonstrated the opposite: a better prognosis for women than for men. Kellum and Haserick (8) also reported that male SLE was more severe: 34.5% of men were alive at 8 to 10 years, compared with 56.3% of women. Swaak et al. (30), a Soviet group (103), as well as a study by Ward et al. (33), have confirmed these findings. On the other hand, gender did not appear to be a significant predictor in the statistical analysis performed by Ginzler et al. (22). Chang et al. (104) found no statistically significant differences in survival between male and female patients in Taiwan. Abu-Shakra et al. (98) found no effect of gender on prognosis. Miller et al. (105), Ward and Studenski (106), and Wang et al. (57) concluded that the spectrum and severity of SLE tended to be the same in males and females. In a cohort of patients from Puerto Rico men had more sever disease as manifested by a higher prevalence of serositis, proteinuria and renal insufficiency, higher accumulation of damage, and more deaths (107). Although a similar increase in disease manifestations among Mexican SLE male patients compared to female patients was noted, there was no increased mortality among these men (108). Thus, the issue of the effect of gender on prognosis in SLE remains unanswered.

Age at Onset

Age at onset of SLE was found to be a significant predictor of survival at both 1 and 5 years in the U.S. multicenter study (22), with better survival rates in older patients. Onset of SLE in the pediatric-age group has been associated with a worse prognosis, probably because these patients still may die earlier than their counterparts with older-onset disease (109). Studenski et al. (32) found no significant effect of age on survival (cutoff at 55 years). On the other hand, Reveille et al. (31) found that increasing age of onset adversely affected survival. A comparison with the estimated survival of the age-matched segment of the U.S. population showed that patients with SLE fared worse at all age groups. Abu-Shakra et al. (98) found that older patients (≥50 years) at diagnosis or presentation to the Lupus Clinic were at a slightly higher risk for death than patients who were diagnosed before age 50. Older age also was associated with decreased survival in the study reported by Ward et al. (33). Hagelberg et al. (110) reported the survival of a pediatric cohort with lupus nephritis to be 94% at 11 years. However in an Indian pediatric cohort the survival was not as good, with only 68% of the patients surviving at 3.2 years (111). It is of interest that when experimental lupus was induced in young and old BALB/c mice by immunization with 16/6 idiotype in complete Freund adjuvant, old mice produced fewer antibodies and demonstrated a milder renal lesion than young mice, suggesting that aging modifies development and expression of the autoimmune disease (112). Rood et al. (113) recently observed a significantly higher mortality in patients who developed SLE during reproductive years than in patients who developed lupus in the nonreproductive years. Whether this is an effect of age or hormonal status is unclear. Age at enrollment was not a significant predictor of mortality in the LUMINA study (77).

SLE in Children

Childhood SLE is characterized by more organ-threatening disease than adult-onset SLE (38). In the past childhood SLE was thought to be associated with a poorer prognosis (1). Lupus in children is managed the same way as in adults (114), with particular attention being given to their specific psychosocial needs and special problems (see Chapter 6 , Serum and Plasma Protein Abnormalities and Other Clinical Laboratory Determinations in Systemic Lupus Erythematosus). All studies from before 1977 were associated with a less than 50% 10-year survival rate (115 ,116 ,117 ,118 ,119 ,120). Because of the more widespread use of cytotoxic agents, improved antihypertensive agents, dialysis, renal transplantation, cyclosporine, and other diagnostic advances, the 10-year survival now has improved to an average of 85% for children who are treated in optimal settings (110 ,121 ,122 ,123 ,124 ,125 ,126 ,127 ,128 ,129). In China, the survival rates are lower, with most recent data revealing a 5-year survival rate of only 76.3% (128), and in India the survival at 3 years was only 68% (111). A practice containing largely indigent African-American and Hispanic patients in Brooklyn reported a 25% 5-year mortality rate and a 25% 5-year rate of renal failure requiring dialysis (129). Lehman's group in Los Angeles was unable to find any prognostic differences among patients with onset at an age of less than 10 years versus 10 to 20 years (109). Wallace et al. (20) could not find any differences in survival between 55 patients who survived to adulthood and who were diagnosed before 20 years of age versus 409 patients who were diagnosed at a later age. Tucker et al. (38) identified identical 5-year survival rates of 94% among the childhood-onset and adult onset SLE patients.

In summary, the bleak prognosis for childhood SLE that was reported in the 1960s and 1970s has improved substantially. Children now have only a slightly worse outcome than adults.

Older Adults

Idiopathic SLE developing in individuals after the age of 50 years as compared with adults with onset before the age of 50 is characterized by a milder serologic picture, infrequent renal disease, and more serositis and arthritis (130). These patients with older-onset SLE had 92% and 83% 5- and 10-year survival rates, respectively, in Wallace et al.'s 1980 survey (20) and in a study by Baker et al. (131). Fewer elderly patients required corticosteroids, and and when they did, lower doses were needed, and for shorter durations (132). Nephritis did not appear to alter overall survival (20). However, Reveille et al. (31) noted that older age of onset was associated with a poorer outcome, and Abu-Shakra et al. (98) noted that patients who were diagnosed or presented at 50 years of age or older had a slightly higher risk for mortality.

A comparison of 47 patients with late onset SLE (greater than age 50 at diagnosis) and 114 patients with age of onset younger than 50 revealed that there were more men among patients with late onset SLE. The disease appeared milder with less evidence of renal involvement and arthritis, less serological abnormalities. Survival was better in patients with early onset SLE 95% at 5 and 10 years and 92% at 15 years, compared with 85%, 71% and 59% for 5, 10, and 15 years respectively in the older age group (132). The authors performed a pooled data analysis and found that serositis, pulmonary involvement, rheumatoid factor positivity were more common among late onset than early onset SLE patients.

Socioeconomic Factors

Dubois et al. (14) concluded that patients who were treated in his private practice did better than those who were treated in publicly funded clinics where he worked. Studenski et al. (32) found that patients with lower socioeconomic background had a worse prognosis; they used Medicaid insurance as a marker of low socioeconomic status, which is not necessarily the case in other practices. The issue of noncompliance with medical therapy was proposed by Levy et al. (99) to be as a factor that must be separated from socioeconomic status. Bruce et al. (133) recently demonstrated that poor compliance was associated with poor outcome with respect to renal disease, but suggested that cultural differences may contribute to noncompliance with medications. Petri et al. (134) demonstrated that both noncompliance and type of medical insurance were important factors in the morbidity of SLE. Karlson et al. (135) studied the relationship between lupus morbidity (defined as disease activity measured by the Systemic lupus activity measure [SLAM]) and socioeconomic predictors in five centers; they found that higher education, private insurance/Medicare, and higher income were associated with less disease activity at diagnosis. The GLADEL cohort documented that mortality was associated with lower education and poor medical coverage among Hispanics (51).

Methods of Healthcare Delivery

Patients who are treated in different healthcare settings (e.g., private practice, university medical center, prepaid health plan, local clinic, or within a government-controlled system such as the Veterans Administration) probably are different. Therefore, the healthcare setting from which a series of patients with SLE is obtained can influence prognosis. The availability of services and specialists varies widely. Sicker patients often are funneled into tertiary university centers, thus lowering their reported survival curves for SLE (136). As noted earlier, patients from India, Chile, and the island of Curaçao fared worse in terms of survival than patients from North American centers. Whether this is related to a lack of specialists or to poorer standards of medical care in general is not clear.

In the United States, healthcare is funded by private insurance (e.g., fee for service, managed care, or prepaid health maintenance plan), Medicare, Medicaid (i.e., an extension of the welfare system), cash, or local governments that provide subsidized care to indigent patients. Fessel (137), working with middle-class patients who had at least one family member employed and therefore enrolled in the Kaiser-Permanente prepaid health plan, reported good survivals in patients with SLE. Ginzler et al. (22) studied 1,103 patients with SLE at nine centers and found that privately funded patients had better survival rates than those receiving public funding. Reveille et al. (31) documented that African Americans with private insurance have improved survival compared with those without it.

Reports of patient outcome also probably are influenced by the specialty of the physician who is analyzing the data. For example, Wasner and Fries (138) found that rheumatologists and nephrologists usually agree with each other on general treatment approaches for SLE, but that nephrologists place more emphasis on renal biopsy and use immunosuppressive drugs more frequently. Stewart and Petri (139) recently suggested that patients followed in a health maintenance organization had higher creatinine levels, and less were treated with immunosuppressive agents compared to patients followed by an academic rheumatologist. Ward demonstrated that in-hospital mortality among patients with SLE is lower in hospitals that have more experience in looking after patients with SLE (74).

Wallace's group (20 ,34) has observed higher mortality rates in their clinic patients compared with their private-practice patients. Esdaile et al. (140), on the other hand, found that socioeconomic status had no correlation with health outcomes among Canadian patients with SLE, but all of them had health insurance. Ward also found that hospitalized SLE patients who had private insurance fared better than those who did not (63 ,74).

Environment

Environmental considerations, such as climate, occupation, exposure to chemicals, diet, lifestyle, exercise, and drug-induced SLE, are described in Chapter 3 , Environmental Aspects of Lupus, Chapter 4 , The Epidemiology of Systemic Lupus Erythematosus, and Chapter 59 , Antimalarial Therapies. Whether they influence prognosis is uncertain.

Geography

Geography may be a factor in survival patterns. Chapter 4 discusses the incidence and prevalence of SLE in various parts of the world. Generally, Canadian, Japanese, and European patients with SLE have outcomes similar to those of patients in the United States. SLE might present differently in certain locales, which may alter prognosis. For example, black patients with SLE in Zimbabwe have an unusually high incidence of renal disease and a low incidence of photosensitivity (141). In India (27), Egypt (142), and Thailand (143), mortality rates are high. However, as noted

earlier, the differences in survival that have been noted among patients from India, Chile, or the Caribbean may be related to general medical care and not to certain features related to SLE itself.

Systemic Lupus Erythematosus-Related Factors

SLE factors that may affect prognosis include time between the onset of symptoms and the diagnosis of SLE, change in disease expression over time, presence of specific disease manifestations, overall disease activity, and use of therapeutic modalities.

Year of Diagnosis

The year of disease onset is a critical factor in the prognostic equation. As noted earlier, the overall survival of patients with SLE has improved dramatically over the last 50 years. Treatment practice has changed and varied depending on the year and location of treatment and the source of healthcare delivery. For example, corticosteroids were used at higher doses and for longer periods in the 1950s; similarly, certain immunosuppressive agents were used more extensively in the late 1960s and early 1970s than they are at present. The advent of steroids, dialysis, newer antihypertensives and antibiotics, and parenteral cyclophosphamide has had an impact on patient survival as well (144).

Time from Onset to Diagnosis

The time difference between onset of symptoms and the diagnosis of SLE has not been formally used as a predictor of survival in SLE. It has, however, been considered as a factor in the calculation of survival rates, because there would be a prolonged survival in those instances where the date of onset of symptoms has been used as the entry point of a survival study. Seleznick and Fries (36) actually demonstrated the point by providing survival rates calculated from first symptom (99%, 96%, and 89% for 1, 5, and 10 years, respectively) and from first visit (96%, 88%, and 64% for 1, 5, and 10 years, respectively). Similar observations were made by Drenkard et al. (39) who showed 96% and 92% 5- and 10-year survival rates calculated from the onset of symptoms as opposed to only 91% 5-year survival calculated from the first visit. Wallace et al. (20) pointed out that the time lag between onset of symptoms and diagnosis in their population did not vary significantly in the three decades of their study and, therefore, might not have influenced the improved survival that was noted over that time period. Their 1990 analysis of 464 patients suggested that the time from onset of symptoms to diagnosis in those older than 60 years is 3.2 years, the longest of any age group (35). Urowitz et al. (61) showed that disease duration to first visit did not vary significantly in the three epochs of their study (i.e., early 1970s, late 1970s to mid 1980s, late 1980s to early 1990s). Most investigators calculate survival rates from the time of diagnosis particularly because not all patients who present with symptoms of SLE go on to develop clear-cut lupus (7). Only one third of the patients with latent lupus described by Ganczarczyk et al. (145) went on to develop clear-cut SLE. Similarly some 20% of 122 Dutch patients (146) and 57% of 28 Swedish patients (147) with “incomplete lupus” went on the fulfill criteria for SLE. If the remaining patients with “incomplete” or “incipient” lupus are included in an analysis of survival, they might yield higher survival rates for the sample population. Of interest in this regard is a paper describing SLE in Iceland (148), which describes a nationwide survey allowing the investigators to include milder cases; the mortality rates in this study are similar to those in other reports. Hernandez-Cruz et al. (91) also found that their patients who died had a longer delay between symptoms and diagnosis compared to those who survived.

Change in Disease Expression over Time

Another important issue is whether the nature of SLE has changed with time. Hashimoto et al. (149) conducted a comparison by decades of 229 Japanese patients who were studied since 1955. They concluded that the incidence of Raynaud phenomenon, alopecia, oral ulcers, and nephritis has increased in the last decade. In contrast, Wallace's (35) group compared 464 U.S. patients with SLE (diagnosed between 1980 and 1989) with the 520 patients who were seen by Dubois (diagnosed between 1950 and 1963) in the same office. The percentage of patients with organ-threatening disease decreased from 65% to 52%, and acute CNS vasculitis practically disappeared. This might be attributed to earlier recognition and treatment of the symptoms and signs of SLE, an evolutionary change in the disease process, changes in referral patterns, and/or the availability of antinuclear antibody (ANA) testing and other serologic procedures that can help to identify milder cases. Urowitz et al. (95) found that the disease expression did not change in an SLE cohort over a 24-year period; thus, survival could not be attributed to milder disease. Moreover, there was a similar delay from diagnosis to presentation to clinic in patients who were seen in the early 1970s compared with late 1980s.

Effect of Disease Manifestations

The presence of certain disease manifestations may predispose patients to mortality. Between 1973 and 1985 at the University of Mississippi, 50 deaths occurred in patients with SLE (150). Serositis, nephritis, CNS disease, and leukopenia were associated with a fatal outcome, and the mean interval from diagnosis to death was 4.1 years. Reveille et al. (31) found that thrombocytopenia, nephritis, CNS disease, anemia, and hypertension were associated with a poorer outcome. In the Toronto study (98), renal damage, thrombocytopenia, cardiac complications, hypertension, and lung involvement were associated with mortality by univariate analysis, but the best-fitting multivariate model included renal damage, thrombocytopenia, SLEDAI of 20 or higher at presentation, lung involvement, and age older than 50 years at presentation. In the Mexican autopsy study lung involvement, severe thrombocytopenia

and heart involvement were features that were statistically different between cases and controls (91).

Although CNS disease commonly was found in patients who died with active lupus (37) and has been found to be associated with decreased survival (22), most studies have not confirmed the role of CNS disease as a predictor for mortality. On the other hand, the presence of nephritis carried a poor prognosis for patients with lupus who were followed in hospital centers (20 ,22 ,31 ,137) as well as in outpatient facilities (31). The presence of proliferative and chronic lesions on kidney biopsy specimens was associated with a higher risk of all-cause mortality, particularly in patients with normal serum creatinine (151). Similar results were obtained by Esdaile et al. (152) using the conventional Cox model, although when a time-dependent model was developed, only subendothelial deposits were contributory. Massardo et al. (56) also found that survival curves for World Health Organization (WHO) types II and III were better than for type IV on univariate analysis. It is of interest that the survival rates calculated for the subgroup of Chilean patients who underwent kidney biopsy was somewhat lower than that of the total population, with rates of 89%, 72%, and 58% for 1, 5, and 10 years, respectively. However, renal disease did not remain significantly associated with prognosis on multivariate analysis once disease activity was in the model. Additionally, the Lupus Nephritis Collaborative Group did not find that renal activity or chronicity indices predicted either death or renal failure (153).

Overall Disease Activity as a Predictor of Mortality

The assessment of disease activity in SLE has become easier with the development and validation of a number of instruments over the past several years. The most commonly used instruments include: the SLEDAI (154), the SLAM (155), the British isles lupus assessment group (BILAG) (156), the Lupus activity index (LAI) (157), and the European consensus lupus activity measurement (ECLAM) (158). These indices have been shown to be comparable (159 ,160). Thus, overall disease activity now can be evaluated as a prognostic factor in SLE.

Table 69-4: Risk Factors for Mortality in Published Series

Author (ref)	Year	Time	Age	Race	Sex	SES	Renal	CNS	BP	Plat	DA
Estes & Christian (16)	1955	A	-	-	-	?	+	+	?	?	?
Wallace et al. (20)	1981	D	+	-	-	?	+	-	-	-	?
Ginzler et al. (22)	1982	E	+	+	-	+	+	-	?	?	?
Studenski et al. (32)	1987	D	-	+	-	+	?	?	?	?	?
Swaak et al. (30)	1989	D	?	?	+	?	+	+	?	?	?
Reveille et al. (31)	1990	D	+	+	-	-	+	?	+	+	?
Pistiner et al. (35)	1991	D	-	-	+	-	+	?	?	+	?
Seleznick & Fries (36)	1991	E	-	-	-	-	+	?	+	?	?
Massardo et al. (56)	1994	A	-	-	-	-	+	-	-	+	+*
Drenkard et al. (39)	1994	A	-	-	-	?	+	-	-	+	+
Abu-Shakra et al. (37)	1995	A	+	-	-	-	+	-	-	+	+*
Ward et al. (33)	1995	A	+	-	-	+	?	?	?	?	?

A, anytime prior to death; BP, hypertension; CNS, central nervous system disease; D, at diagnosis; E *, at study entry; Plat, thrombocytopenia; SES, socioeconomic factors; +, risk factor present; -, risk factor absent; ?, risk factor not assessed or not reported.

Indeed, overall disease activity at the time of renal biopsy has been shown to be a prognostic factor for mortality in two cohorts of patients (151 ,161). It also was demonstrated to be the most important predictor of mortality in other cohorts of patients with SLE (162). Disease activity (as measured by the SLEDAI) at the time of presentation to the Lupus Clinic was a predictor for mortality in a recent study (98). Although high SLEDAI (>10) was not a predictor among black Caribbean patients, a high-weighted SLEDAI score, depicting disease activity over the course of disease, was associated with decreased survival in both univariate and multivariate analyses (55). The disease activity index had the strongest association with outcome in Chilean patients with SLE (56). Disease activity, although evaluated by an unvalidated measure, was also associated with mortality in patients from Mexico (39).

Cook et al. (163) found that in a univariate analysis total SLEDAI score was highly prognostic for mortality within 6 months of the last clinic visit. Increasing relative risks were obtained for increasing SLEDAI scores (1.28 for SLEDAI 1–5, 2.34 for SLEDAI 6–10, 4.74 for SLEDAI 10–19 and 14.11 for SLEDAI ≥20 compared to SLEDAI = 0). A time-dependent Cox regression analysis identified the individual components of SLEDAI as risk factors for death, including organic brain syndrome, retinal changes, cranial nerve involvement, proteinuria, pyuria, pleurisy, fever, thrombocytopenia, and leucopenia.

Ibañez et al. (164) developed a method to describe disease activity over time in patients with SLE. The adjusted mean SLEDAI (AMS) was found to be a strong predictor for mortality.

The effect of the various factors on survival in SLE is summarized in Table 69-4 .

Effect of Treatment on Mortality

Treatment may be an important factor affecting mortality. Improved therapeutic approaches for SLE may provide better disease control through suppression of the inflammatory process and thus reduce mortality that is related to active disease. There have not been many prospective, randomized, controlled drug trials in SLE. The Canadian Hydroxychloro-quine Study Group published its findings of a randomized trial of withdrawal of hydroxychloroquine in patients with stable SLE (165). They found that hydroxychloroquine is effective in controlling disease exacerbations in SLE; patients who continued to take the drug were less likely to have clinical flares than those who were taken off the drug. However, they did not study its effect on mortality.

That steroids have made a difference in the control of SLE is widely accepted, such that it is considered to be unethical to perform a placebo-controlled trial of steroid therapy in SLE. On the other hand, the contribution of steroid therapy to the changing pattern of mortality and morbidity in SLE has been noted (166). Sturfelt et al. (167) noted that prolonged corticosteroid therapy was associated with valvular abnormalities and myocardial infarction in their prospectively studied patient population. Because atherosclerotic complications are a major cause of death, particularly late in the disease, this is an important observation. Petri et al. (168) further demonstrated that an increase in prednisone dose of 10 mg was associated with an increase in cholesterol levels of 7.5, thus predisposing patients to coronary artery disease, whereas hydroxychloroquine therapy was associated with a lower serum cholesterol level, possibly protecting patients from coronary-artery disease. Use of medications did not appear as a predictive factor in the analysis performed by Abu-Shakra et al. (98).

The role of treatment as a potential confounder of the relationship between major predictors and mortality was investigated by McLaughlin et al. (151). They found that when the treatment variable was added to the baseline model, the relative risk estimates did not change significantly, suggesting that these estimates were not confounded by the treatment variables that were considered, which included steroid and immunosuppressive drugs.

Spontaneous Remissions

During periods of remission, either no symptoms or minor complaints, such as slight morning stiffness or occasional pleuritic discomfort, may occur. Laboratory abnormalities such as leukopenia, elevated sedimentation rate, and positive ANA may persist or disappear.

SLE can spontaneously improve and remit. Dubois (169) reported that 35 of 520 patients had multiple spontaneous remissions of varying lengths of time not associated with treatment. Some were of 10 to 20 years' duration. Ropes (8 ,9) noted spontaneous remissions of a few months to several years in 70 of 72 patients. Tumulty (170) in 1954 observed spontaneous remission in 19 of 34 patients who were treated symptomatically. Tozman et al. (21) remarked that four of 160 patients with SLE with a history of severe organ involvement who were followed for a mean of 75 months had no treatment and no disease activity. Heller and Schur (171) noted that 13 of their 305 patients (4%) developed clinical and serologic (with ANA becoming negative) remissions between 1967 and 1981 and lasting for at least 18 months; only 8 of 13 patients, however, were off all medications. Drenkard et al. (172) defined remission as “at least 1 year during which lack of clinical disease activity permitted withdrawal of all treatment for lupus proper.” The period of remission was considered from the time the patient stopped all medications. They did not use a validated disease activity measure to describe disease activity. Of their 667 patients followed for a median of 3.7 years, 156 fulfilled their definition of remission, and 62 went into remission within the first 2 years of disease, and 81 were still in remission at the time of the analysis. The mean duration of first remission was 4.6 years (range, 1 to 21 years). Patients who achieved a remission period had increased survival, independently of the effect of other disease manifestations that were associated with increased mortality among their patients. They concluded that lupus was a milder disease than previously considered, and that remission was common. Urowitz et al. (173) found that of the 703 patients followed in their clinic between 1970 and 1997 who had not been absent from the clinic for more than 18 months 46 (6.5%) achieved complete remission for at least 1 year, whereas only 12 patients (1.7%) had prolonged complete remission of at least 5 years on no treatment. Although the frequency of disease manifestations was similar to the nonremission patients, the 5-year remission group was distinguished by lower overall disease activity as measured by the adjusted mean SLEDAI (AMS), the lower prevalence of anti-DNA antibodies, and lower use of steroids and antimalarials.

Overall, it seems that 2% to 10% of patients who fulfill ACR criteria for SLE can enter true disease remissions that can last for months to years; in other words, they have no symptoms and require no therapy for SLE. This must be remembered when considering therapy for the individual patient or when evaluating the efficacy of treatment.

Other Outcome Measures of Prognosis in Systemic Lupus Erythematosus

Cumulative Organ Damage

As patients with SLE live longer, their prognosis needs to be assessed by other outcome measures. Individual organ damage, most notably the presence of end-stage renal disease, has been used as an outcome (174). More recently, a global measure of damage namely, the SLICC/ACR damage index for SLE has been introduced as a measure of outcome in SLE (175). This measure includes descriptors of nonreversible change that occur after the onset of SLE, whether or not they are related to

the disease process or its treatment (Table 69-5). This instrument has been shown to be valid and reproducible (176).

The SLICC/ACR damage index may serve as a predictor for other outcomes, or may be an outcome of morbidity in itself (178). Early damage, measured by the SLICC/ACR Damage Index within the first year of disease was found to be a predictor of mortality in SLE in the Toronto Cohort (177). The presence of a SLICC/ACR damage score of two or more within the first 5 years of SLE was found to be highly predictive of mortality in a Swedish cohort (179). An increase in SLICC/ACR damage index score within the first 3 years of follow-up in a Chinese cohort was also associated with mortality risk (180). Stoll et al. (181) identified renal damage at 1 year to be predictive of end-stage renal disease, and pulmonary damage to be predictive of death within 10 years. This study also demonstrated that Afro-Caribbean and Asian patients accumulated more damage than Caucasian patients, suggesting a racial influence on disease expression in this disease. A study of the Montreal cohort, which included only Caucasian patients, revealed that the SLICC/ACR damage index scores predicted poor outcomes defined as either death or hospitalization (182). Nossent (183) found that over an observation period of 71 months the median Damage Index score was 2.4 in a population of

patients from Curaçao. Despite the higher SLICC/ACR Damage Index scores in this patient population, there was no demonstrable association with poorer survival in that study. The LUMINA study showed that deceased patients experienced more active disease and accrued more damage from the outset of their disease compared to survivors (77). Zonana-Nacach et al. (184) used the SLICC/ACR Damage Index to assess damage among 210 Mexican patients with SLE. They found that damage increased with disease duration so that after 10 years of disease 70% of their patients demonstrated damage recorded by the SLICC/ACR. A Danish study (185) also demonstrated that deceased patients had accrued more damage than patients who remained alive. In a recent multicenter cohort of patients with SLE there was evidence of damage within a mean of 3.8 years after onset. Although race and socioeconomic status did not influence early damage, older age at diagnosis of SLE, greater disease activity at diagnosis, and longer disease duration were associated with damage (186). Maddison et al. found that older patients were at higher risk of accumulating damage than younger patients with SLE (187).

Table 69-5: SLICC/ACR SLE Damage Index

Item	Score
Ocular (either eye, by clinical assessment)	
Any cataract ever	1
Retinal change OR optic atrophy	1
Neuropsychiatric	
Cognitive impairment (e.g. memory deficit, difficulty with calculation, poor concentration, difficulty in spoken or written language, impaired performance level)	
OR major psychosis	1
Seizures requiring therapy for 6 months	1
Cerebral vascular accident ever (Score 2 if >1)	
OR surgical resection not for malignancy	1 2
Cranial or peripheral neuropathy (excluding optic)	1
Transverse myelitis	1
Renal	
Estimated or measured GFR <50%	1
Proteinuria 3.5 g/24 hours	1
OR end-stage renal disease (regardless of dialysis or transplantation)	3
Pulmonary	
Pulmonary hypertension (right ventricular prominence, or loud P2)	1
Pulmonary fibrosis (physical and radiograph)	1
Shrinking lung (radiograph)	1
Pleural fibrosis (radiograph)	1
Pulmonary infarction (radiograph)	
OR resection not for malignancy	1
Cardiovascular	
Angina	
OR coronary artery bypass	1
Myocardial infarction ever (Score 2 if >1)	1 2
Cardiomyopathy (ventricular dysfunction)	1
Valvular disease (diastolic murmur, or a systolic murmur >3/6)	1
Pericarditis × 6 months,	
OR pericardiectomy	1
Peripheral Vascular	
Claudication × 6 months	1
Minor tissue loss (pulp space)	1
Significant tissue loss ever (e.g., loss of digit or limb) (Score 2 if >one site)	1 2
Venous thrombosis with swelling, ulceration, **OR** venous stasis	1
Gastrointestinal	
Infarction or resection of bowel below duodenum, spleen, liver or gall bladder ever, for whatever cause (Score 2 if >one site)	1 2
Mesenteric insufficiency	1
Chronic peritonitis	1
Stricture	
OR upper gastrointestinal tract surgery ever	1
Pancreatitis	1
Musculoskeletal	
Muscle atrophy or weakness	1
Deforming or erosive arthritis (including reducible deformities, excluding avascular necrosis)	1
Osteoporosis with fracture	
OR vertebral collapse (excluding avascular necrosis)	1
Osteonecrosis (Score 2 if >1)	1 2
Osteomyelitis	1
Ruptured tendon	
Skin	
Scarring chronic alopecia	1
Extensive scarring or panniculum other than scalp and pulp space	1
Skin ulceration (not because of thrombosis) for more than 6 months	1
Premature Gonadal Failure	1
Diabetes (regardless of treatment)	1
Malignancy (Exclude dysplasia) (Score 2 if >one site)	1 2

Note: Damage (nonreversible change, not related to active inflammation) occurring since onset of lupus, ascertained by clinical assessment and present for at least 6 months unless otherwise stated. Repeat episodes mean at least 6 months apart to score 2. The same lesion cannot be scored twice.

Non-Caucasian race, longer disease duration, higher disease activity, and lower level of education were associated with more organ damage in SLE in two cohorts of patients from the United Kingdom (188). A study of patients followed for at least 15 years documented increases in SLICC/ACR Damage Index over time, and identified corticosteroids as important contributors to damage (189). Similarly Stoll et al. (190) found that patients accumulated damage over a 5 year period and that disease activity was contributing to the damage. Patients followed in the LUMINA study who did not have damage at presentation were studied to determine the predictors for the occurrence of initial damage (191). Independent predictors of a shorter time to initial damage were Hispanic ethnicity from Texas (HR 2.11, 95% confidence interval [CI], 1.15–3.88), greater disease activity according to the Systemic Lupus Activity Measure (HR 1.09, 95% CI, 1.04–1.15), the occurrence of thrombotic events (HR 7.66, 95% CI, 2.13–27.51), and prednisone at a dosage of less than 10 mg/day (HR 2.53, 95% CI, 1.15–5.55). A dose of prednisone 10-30 mg/day was found to be protective (HR 0.46, 95% CI 0.22-0.96). Antiphospholipid syndrome was found to be important to the development of damage in a Spanish study (192). Ibañez et al. (193) documented that the risk of damage increases with increases in AMS, age at diagnosis, disease duration, and use of corticosteroids and immunosuppressive medications but is not affected by sex, SLEDAI at presentation or use of antimalarials (193).

Thus the assessment of damage in SLE is important both as a predictor of mortality and as an important outcome.

Specific Organ Damage in Systemic Lupus Erythematosus

Although the overall SLICC/ACR Damage Index provides a global measure of accumulated damage in SLE, it also identifies damage in individual organ systems. Individual organ damage is described elsewhere in this book in the appropriate chapters.

Malignancies in Systemic Lupus Erythematosus

Data concerning the incidence of malignancies in SLE are of great interest, because it is believed that the failure of immune surveillance is a cause of the induction and spread of tumors. In SLE, two reasons for faulty immune mechanisms are known. One involves abnormalities in immune regulation that are associated with the disease process. The second involves long-term treatment with cytotoxic agents, which increases the risk of developing cancer. In fact, cancer is an extremely infrequent cause of death in SLE. Eight large surveys (17 ,19 ,20 ,28 ,31 ,32 ,80 ,86) were composed of 3,683 patients, and of the 667 who died, 16 had malignancies, representing 2.5% of patients. Approximately 1,000 of the total number of patients had received some type of immunosuppressive treatment; however, these studies did not compare the frequency of malignancy to the general population. In a study of 205 patients with SLE who were followed over 2,340 patient-years, Petterson et al. (194) found a 2.6-fold increased risk for all cancers as compared with that of the total Finnish population. Sweeney et al. (195) did not find an increased frequency of cancers among their cohort of patients with SLE, but follow-up was short. In a more recent study of malignancy in 724 patients with SLE who were followed prospectively for 24 years at a single lupus clinic in Toronto, 24 cancers were identified in 23 patients (3.2%) during 7,233 patient-years of follow-up (196). The most frequent cancer types/sites were hematologic. None of the six patients with hematologic malignancies received cytotoxic drugs before the diagnosis of cancer. Compared with the general population in Toronto, the overall estimated risk for all cancers was not increased in the lupus cohort (SIR, 1.08; 95% CI, 0.70 to 1.62). A 4.1-fold increased risk for hematologic cancers was observed; however, when these malignancies were analyzed separately, only non-Hodgkin lymphoma was associated with an increased risk (SIR, 5.38; 95% CI, 1.11 to 15.7). Using the cancer rates in patients with rheumatoid arthritis or systemic sclerosis who were in the same geographic area, the risk for cancer was found to be significantly lower in the SLE cohort compared with that of patients with rheumatoid arthritis (SIR, 0.65; 95% CI, 0.41 to 0.96) and patients with systemic sclerosis (SIR, 0.4; 95% CI, 0.26 to 0.60). An increased frequency of non-Hodgkin lymphoma among patients with SLE also was reported from Denmark (197). Ramsay-Goldman et al. (198) found an increased risk for malignancy in their lupus cohort. Breast, lung, and gynecologic malignancies were the most common malignancies observed in the cohort and breast cancer was significantly increased in Caucasian women.

Bernatsky et al. (199) assembled a multisite (23 centers) international cohort of 9,547 patients diagnosed as having SLE and observed for a total of 76,948 patient-years. Patients at each center were linked to regional tumor registries to determine cancer occurrence. Standardized incidence ratios (SIRs) were calculated. Within the observation interval, 431 cancers occurred. For all cancers combined, the SIR estimate was 1.15 (95% CI, 1.05–1.27); for all hematologic malignancies, it was 2.75 (95% CI, 2.13–3.49), and for non-Hodgkin lymphoma, it was 3.64 (95% CI, 2.63–4.93). The data also suggested an increased risk of lung cancer (SIR 1.37; 95% CI, 1.05–1.76), and hepatobiliary cancer (SIR 2.60; 95% CI, 1.25, 4.78). These results support the notion of an association between SLE and cancer and more precisely define the risk of non-Hodgkin lymphoma in SLE. It is not yet known whether this association is mediated by genetic factors or exogenous exposures

Quality of Life in Patients with Systemic Lupus Erythematosus

In addition to disease activity and damage, quality of life and disability also are considered important outcomes in patients with SLE (200). These have been measured by the Medical Outcome Study (MOS) Short Form 20 (SF-20) as well as by the SF-36. The quality of life of patients with SLE was found to be reduced compared to healthy controls (201 ,202 ,203 ,204 ,205 ,206). Although fibromyalgia has been shown to be a contributor to the reduced quality of life in patients with SLE (202 ,204), disease activity and damage have been reported to contribute by some investigators (201 ,205), but not all (202 ,203 ,206). It seems that the investigators who used either the SLAM or the BILAG as their disease activity measures were more likely to find a relationship between quality of life and disease activity or damage. This may be related to the fact that these two instruments include items that reflect quality of life as well as damage.

Early work disability was reported in 40% of patients with SLE and was related to low education level, higher physical demands of the job, and higher disease activity at diagnosis (207). Ward et al. (208) suggest that patients who participate in their own care tend to have less morbidity. This is an important concept when considering the prognosis of patients with SLE.

Summary

The information in this chapter can be summarized as follows:

- Overall survival and duration of disease:
  - More than 90% of patients with SLE survive for at least 2 years after diagnosis, compared with 50% of such patients 50 years ago. More recent surveys reveal a 80% to 90% 10-year survival rate and 80% at 20 years. The mechanism for improved survival over the past five decades is unclear.

- SLE can become inactive for many years; 15% to 20% of patients at any time have no evidence of clinical activity and are on minimal or no medication.
- African Americans, males, children, patients who receive care in publicly funded systems, and those with thrombocytopenia have a poorer prognosis, especially if nephritis is present.

- Mortality rates and causes of death:
  - A bimodal mortality curve in SLE is prevalent. Patients who die within 5 years of disease onset usually have active SLE, high steroid requirements, and infections. Patients who die later usually have evidence of atherosclerotic cardiovascular disease; in contrast, active SLE, infection, and high steroid requirements are uncommon.
  - Most patients with SLE die from active SLE, nephritis, sepsis, and cardiovascular disease. Mortality from CNS disease or malignancies rarely occurs.
- Other outcome measures in SLE

With improved survival, morbidity and damage related to the disease process or its treatment become important outcomes, as is the quality of life and functional disability of patients with SLE.

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Chapter 70

Biologics and Stem Cell Therapies for Lupus

Daniel J Wallace

Bevra Hannahs Hahn

Despite all of the advances in therapy of SLE over the last few years, half of our patients with organ-threatening disease (which is half of the lupus population) succumb within 20 years. For the 75% with SLE who survive 20 years, quality of life is usually less than optimal. There has not been a drug approved for the treatment of SLE in the United States since 1966. The reasons for this are many. The first problem is the length of duration of studies to show prevention of end-stage renal disease (the landmark NIH nephritis study in the 1970s and 1980s showed it took 5 years for prednisone with parenteral cyclophosphamide to demonstrate a superior response to prednisone alone (1)). Pharmaceutical companies took note and decided they simply cannot afford to fund studies of that duration, particularly if the product being tested is not more effective than standard treatments. A second problem is the heterogeneity of the disease, presenting problems in studying outcomes—can the same study include SLE patients with nephritis and others with no nephritis, but hematologic disease or CNS disease? In this regard, it has taken a long time for the lupus investigator community to devise, test, and validate a set of measures to assess the responses and outcomes of new therapies studied in different organ systems. The recently published Food and Drug Administration (FDA) Guidance Document represents a major effort to bridge these failings and provide the pharmaceutical industry a roadmap that specifies what is expected in a clinical trial, which might lead to a drug's ultimate approval for a lupus indication (see Chapter 68) (2).

The search for innovative therapies that are targeted, safe, and effective is well underway. The majority of approaches involve the use of biologics. The 1990s saw the development and approval of a group of highly effective agents used to manage rheumatoid arthritis (RA) and other inflammatory disorders, but not lupus. Now that investigators have largely agreed that clinical activity assessments, response metrics, damage indices, quality of life assessments, organ specific measures, and safety issues are available valid and reliable in a clinical trial, no fewer than 10 biologics are in various stages of testing in humans with SLE as of this writing (July 2006). A major effort is underway to evaluate and screen a variety of surrogate markers and biomarkers that could accelerate the approval process by shortening the length of a study.

This chapter will address biologics and stem cell transplantation and their potential use in lupus. It will largely restrict itself to agents that have been tested in humans. Chapters 61 and 66 discuss niche therapies and miscellaneous immune suppressive drugs with uses applicable to lupus.

Early Human Trials

Credit for early testing of a biologic in patients with SLE goes to Richard Weisbart, Debra Zack, and their colleagues at UCLA who synthesized MAb3E10, a murine anti-double-stranded (ds) deoxyribonucleic acid (DNA) monoclonal antibody in the early 1990s. A phase I study in 9 patients showed that vaccination with the mAb produced an anti-idiotypic response (probably desirable to suppress the idiotype-bearing anti-DNA) and was safe (3 ,4). Biologics being tested for rheumatoid arthritis were studied in a small number of lupus patients. Lipsky's group and Klippel's group at the National Institutes of Health (NIH) treated a total of 10 patients with anti-CD5 ricin A chain immunoconjugate (Xoma) (5 ,6). Modest T cell depletion and transient $CD5^{+}$B cell depletion occurred. Over a 10-year period, a cell-depleting anti-CD4 antibody (MAX.16H5) was given to 35 German lupus patients. The synthesis of IL-6 was significantly decreased. However, in RA trials, tachyphylaxis occurred; safety concerns caused abandonment of this approach (7 ,8). Finally, Klippel's NIH unit also performed a phase 1b, randomized, double-blind, placebo-controlled trial of recombinant human Dnase I in lupus nephritis in an attempt to inhibit antibody deposition (9). Although safety was demonstrated among the 17 patients entered, clinical effects were slight, and achieving sustained therapeutic concentrations was problematic.

Recent Targeted Therapies in Human SLE Trials

See Table 70-1 and Table 70-2 .

Table 70-1: Summary of Drugs for SLE Recently Studied in Human Clinical Trials

1. Therapies that target T/B/APC cell interactions
   a. Anti-CD40L (Biogen/IDEC)
      i. One product was safe, but not effective and another was thrombogenic; new products being developed
   b. CTLA4Ig (Bristol Myers Squibb)
      i. Approved for RA (2006) lupus trials underway
2. Targeting the activation of complement
   a. Anti-C5a and SLE (Alexion)
      i. Improves paroxysmal nocturnal hemoglobinuria, modestly effective in RA, phase I trial documents safety in SLE
3. Inducing immune tolerance
   a. LJP 1082 (La Jolla Pharmaceuticals)
      i. Binds to $ß_2$-glycoprotein I; safety documented in phase I trial
   b. LJP 394 (La Jolla Pharmaceuticals)
      i. An anti-DNA B cell tolerogen; tested in nearly 1,000 patients and has received an approvable letter from the FDA
   c. Edratide (TV4710, TEVA)
      i. A peptide tolerogen that inhibits anti-DNA production probably by inducing regulatory T cells. Safety demonstrated in phase I study; phase II-III trial in progress
4. Agents that target B cells
   a. BlyS protein stimulator protein antagonists and related compounds (Human Genome Sciences <HGS>, Amgen, Lilly, Serono, Biogen)
      i. HGS product safe in phase I trial, improves lupus if seropositive in Phase III trial. Other agents in phase I trials.
   b. anti-CD20 (Rituximab—Genentech)
      i. Approved for lymphoma, RA. Phase III lupus trials in progress, many promising case reports, fully humanized versions being studied (Genmab, Trubion, Genentech)
   c. anti-CD22 (Epratuzumab—Immunomedics)
      i. Phase I trial demonstrates safety in lupus and Sjogren; phase III trial in progress for lupus, Sjogren, and lymphoma
5. Agents that remove autoantibodies from the circulation
   a. Antibody to CR1 linked to a DNA antigen (Elusys, ETI 201)
      i. Promotes clearance of anti-DNA, phase I trial demonstrated safety
6. Agents that target cytokines
   a. TNF blockers: Infliximab (Remicade-Centocor/Schering)
      i. Approved for RA, articles suggest efficacy in selected cases of SLE, but may increase autoantibody production
   b. Agents that target interleukins: anti IL-1Ra (Kineret-Amgen), anti IL-6 (Roche), and IL-10 (Schering)
      i. Approved for RA, Kineret is disappointing in lupus, NIH trial of anti-IL-6 suggests efficacy, 6 patients given anti IL-10 had a modest response
   c. Agents that target interferons—in development

NIH, National Institutes of Health; RA, rheumatoid arthritis.

Therapies That Target T/B/APC Cell Interactions

Anti-CD40L Monoclonal Antibodies

CD40 is expressed constitutively on B cells, antigen-presenting cells (APCs), endothelial, and epithelial cells and interacts with CD40L (CD154, gp39) induced on activated T and B cells, basophils, and eosinophils. CD40/CD40L interaction is necessary for normal immune functions such as T cell activation, B cell differentiation and activation, apoptosis, germinal center formation, and antibody isotype switching (10 ,11). $CD4^+$ and $CD8^+$ T and B cells from SLE mice and patients express increased quantities of CD40L compared to healthy controls. CD40 knockout mice have defective humoral immunity. Glomeruli from lupus nephritis patients show upregulated CD40 and CD40L expression. Inhibition of the interaction of CD40-CD40L should inhibit T cell activation, B cell responses, and autoantibody production. In murine lupus, anti-CD40L therapy can delay, but not prevent development of disease, if given prior to the onset of disease. Early therapy prevents antibody production, whereas later therapy prevents tissue damage (12).

A phase I clinical trial of a humanized monoclonal antibody against CD40L (IDEC 131) showed that the antibody binds to CD40L on T cells, prevents CD40 signaling in B cells, and is safe and well tolerated (13). A phase II randomized, double-blind, placebo-controlled trial of 85 patients with mild to moderately active SLE who received six infusions over 6 weeks showed improved SLEDAI scores in both treatment and placebo groups; the differences were not statistically significant. Many patients clearly improved

on the drug; the study design might have been improved regarding entry criteria, dose of mAb, and so on (14). On the other hand, 28 patients with active proliferative nephritis who received a different mAb anti-CD40L (BG9588) showed mean reduction in anti-DNA antibody, improved C3 complement, and diminished hematuria (15). The trials were halted when several patients in SLE and in transplantation trials developed thromboembolic events. CD40-CD40L interactions occur on endothelial cells and on platelets; these effects might account for the prothrombotic properties of at least one of these mAb (16). Further work regarding these interactions will be required before anti-CD40-CD40L blocking agents are tested further in humans.

Table 70-2: Additional Approaches Likely to Be Utilized in Human Trials

1. T cell vaccination
2. Agents that target chemokines
3. Blockade of the alternative complement pathway
4. Peptide tolerogens to HLA molecules, Sm, and immunoglobulins
5. Promotion of T regulatory cells
6. Approaches that block interferon-α
7. Gene therapies
8. Upregulation of TGF-ß
9. Targeting toll receptors that promote inflammation
10. Targeting Fc receptors
11. Developing gene transcriptional regulators
12. Inhibition of IL-12,15, 17, or 18
13. Induction of anergy in helper T cells

CTLA4Ig (Cytotoxic-T-Lymphocyte Antigen 4 Fused with a Portion of Immunoglobulin)

The CD80/86 (B7.1/2)-to-CD28 pathway is the major second signal that causes T cell activation, proliferation, survival, and cytokine production (17 ,18 ,19). CD28, which is constitutively expressed on T cell surfaces, binds CD80/86 on APCs and cell signaling resulting in cytokine production and proliferation ensues. Once T cells are activated, they express surface CTLA4. This molecule is structurally related to CD28, but binds B7 molecules with a much higher avidity than CD28. CTLA4/CD80/86 interactions usually generate a negative signal to T cells, downregulating proliferation and cytokine production. The biologic agent CTLA4Ig, a soluble recombinant humanized fusion protein with an IgG-Fc portion, mops up CD80/86. Therefore, CD80/86 molecules are not available to bind CD28; T cell activation to proliferation and cytokine secretion cannot occur. CTLA4Ig administration inhibits predominantly naïve and $CD4^+$ T cells with little or no effect on $CD8^+$ T cells. Thus, the undesirable, rapidly proliferating $CD4^+$ helper T cells driving B cells to make autoantibodies are inhibited, and a variety of both pro-and anti-inflammatory cytokines fall in quantity. The magnitude of effect varies depending on the APC cell with different effects on dendritic and B cells.

CTLA4Ig inhibits T cell–dependent B cell maturation in NZB/NZW F1 mice, and delays the onset of nephritis when administered early in life. Importantly, it can suppress established lupus nephritis when combined with cyclophosphamide or anti-CD40L therapy (19). In lupus, it is thought that the administration of CTLA4-Ig may additionally induce tolerance and skew the cytokine profile toward Th2.

Abatacept (a commercial preparation of CTLA4Ig; Bristol Myers Squibb) ameliorates disease activity in psoriasis and in RA in combination with methotrexate (20). It has not been compared directly to anti-TNF therapies in these diseases. In February, 2006, Abatacept (Orencia) was approved by the FDA for the treatment of rheumatoid arthritis. Clinical trials with abatacept in SLE were initiated in 2005 and another CTLA4-Ig product has entered phase I trials in SLE patients.

Targeting the Activation of Complement

Anti-C5a and SLE

Formation of the terminal components of complement has been inhibited by targeting C5 (21 ,22 ,23). Anti-C5 antibodies improved survival and delayed the progression of renal disease in murine lupus (24). Eculizumab binds specifically to C5, inhibiting its cleavage into C5a and C5b, preventing the release of C5a and formation of C5b-9. In a phase I trial, intravenous administration of eculizumab (humanized recombinant anti-C5a antibody hbG1.1, Alexian) blocked C5 activation for up to 10 days and was safe and well tolerated (25). Eculizumab treatment diminished transfusion requirements, hemolysis, and improved quality of life in 11 patients with paroxysmal nocturnal hemoglobinuria, a disorder characterized by a deficiency of surface proteins, which protect the cells against attack by the complement system (26). Disappointingly, a phase II trial of eculizumab in RA showed it to be no more effective than methotrexate and funding for future lupus initiatives have been put on hold by the manufacturer, whereas development for use in paroxysmal nocturnal hemoglobinuria continues (24).

Complement Inhibition Prevents Fetal Loss from Antiphospholipid Antibodies in Mice

Experiments in pregnant mice infused with human anticardiolipin (anti-CL) have shown that activation of C3 and C5 complement are required for antiphospholipid-induced pregnancy loss (27). Furthermore, the thrombogenic properties of antiphospholipid antibodies are reduced in C3 and C5 knock out mice (28). Holers and Salmon demonstrated that administering an anti-C5 monoclonal antibody to anti-CL–infused pregnant mice significantly reduces fetal loss (27). Similarly, administration of an antibody that prevents alternative pathway activation (Factor B interaction with complement to form the C3bBb complex), is also effective (29). Heparin, which inhibits complement activation

(probably via charge) is effective in reducing fetal loss in the model, but fondaparinux and hirudin, anticoagulants that do not inhibit complement activation, are not effective (30). Therefore, for prevention of antiphospholipid–induced fetal loss in mice, treatment that inhibits complement activation is more effective than treatment that simply anticoagulates.

Inducing Immune Tolerance

Inhibiting Antiphospholipid Syndrome by Tolerizing B Cells Making the Antibody

Another approach to inhibiting antiphospholipid syndrome is to tolerize B cells so they cannot make the antibody. LJP 1082 (La Jolla Pharmaceuticals) has a polyvalent antigenic structure aimed at cross-linking surface immunoglobulin that binds $ß_2$-glycoprotein 1 ($ß_2$GPI) (a target of most anticardiolipin antibodies). The antigen is small; therefore, B cell receptors can be linked with antigen without simultaneous binding of Fcγ receptors. Thus, the B cell receives the first activating signal but not the second; the B cell should be rendered anergic (tolerized). In a phase I trial in patients with antiphospholipid antibodies, a single intravenous dose of LPJ 1082 was safe. Further development of this promising agent depends upon the availability of funding (31 ,32).

Inhibiting Anti-DNA Production by Tolerizing B cells: LJP 394 (La Jolla Pharmaceuticals; Riquent)

LJP 394 is a synthetic compound that is designed to act as B cell tolerogen for cells with DNA-binding BCR. The active drug substance is a water-soluble conjugate composed of four oligodeoxyribonucleotides (B cell epitopes) attached though a linker to a triethylene glycol-base central branched structure or platform. LJP 394 provides multivalent epitopes capable of cross-linking surface antibody (B cell receptors that bind DNA, thus on a B cell that can make anti-DNA). The antigen is small, so that BCR can be cross-linked, but not FcγR. Therefore, there is first signal without second signal, and the B cell becomes anergic, unable to proliferate or secrete antibody. LJP394 is neither immunogenic nor antigenic; it does not affect natural killer cell activity or T cell–mediated delayed-type hypersensitivity. No anaphylaxis has been observed, and it activates complement poorly, if at all (33).

LJP 394 has been studied in 14 randomized controlled studies enrolling approximately 900 patients since 1996 (34 ,35 ,36). Administered as a 90-second intravenous push as often as every week, the agent has had no apparent safety issues or adverse reactions. The principal conclusions of the trials demonstrated: (a) significant reduction in serum levels of anti-dsDNA and increases in levels of C3 complement, (b) improvement in quality of life indices, (c) a trend toward reduction of renal flare rates and time-to-renal flare, (d) greatest efficacy in patients whose anti-DNA binds the LJP394 oligonucleotide epitope with high affinity. Riquent has received an approvable letter from the FDA pending results of an additional clinical trial.

Inhibiting Anti-DNA Production by Tolerizing T Cells: Peptide Tolerogens

There are several examples of small peptides (9 to 34-mers) from autoantigens or autoantibodies that bind to HLA/MHC Class II and/or HLA/MHC Class I molecules—complexes that then can engage T cell receptors (TCR) that recognize the MHC/peptide complex on $CD4^+$ T or $CD8^+$ T cells. Mechanisms of tolerance induced by IV or subcutaneous inoculation of such peptides include induction of: (a) anergy in $CD4^+$ helper T cells, (b) $CD4^+$ regulatory T cells that by secretion of IL-10 suppress proliferation in $CD4^+$ helper T cells, (c) $CD4^+CD25^+$ regulatory T cells that on contact inhibit proliferation in $CD4^+$ helpers and/or Ig synthesis by B cells, and (d) $CD8^+$ inhibitory cells that inhibit $CD4^+$ proliferation by secretion of TGF-ß. Hahn and Singh, working in the NZB/NZW F1 mouse model of lupus, have shown that the administration of high doses of selected peptides, including an artificial peptide, pConsensus, that contains T cell epitopes that can activate both $CD4^+$ T cells through I-Ed (MHC class II) and $CD8^+$ T cells through Kd (MHC class I) can delay or suppress anti-DNA production and nephritis (37). T cell anergy and induction of peptide-binding $CD4^+CD25^+$ regulatory T cells and $CD8^+$ TGF-ß-secreting cells occur in that system (38 ,39). Datta et al. have shown similar results in murine lupus with peptides selected from the histones in nucleosomes (40); both $CD4^+CD25^+$ and $CD8^+$ regulatory/inhibitory T cells are induced in that system; high doses and very low doses (nanomolar quantities) are effective (41). Reimekasten et al. (42) have shown similar results after administration of a peptide from the Sm-D antigen, with IL-10 secreting $CD4^+$ T cells as major regulators.

Mozes et al. have brought this idea to human trials. They constructed a peptide (Edtratide: TV4710, Teva Pharmaceuticals), which contains amino acid sequences from a human mAb Ig hypervariable region. Similar peptides from murine anti-DNA suppress autoantibodies and autoimmune disease in murine lupus (43). Administration of the peptide also prevents anti-DNA synthesis by human B cells transferred to SCID mice (severe combined immunodeficiency mice, which lack thymus, and therefore, most T cells) (44). In a phase I safety and dose-finding trial, Edratide was administered subcutaneously to patients with SLE. Safety and tolerability were good. Phase II/III trials are enrolling SLE patients (45).

Agents That Remove Autoantibodies from the Circulation

Elusys has developed a heteropolymer (ETI-201) that consists of a murine antibody to CR1 (CD35) linked to DNA antigen. CR1 receptors (also called C3b C4b receptor) on erythrocytes of primates, including humans, bind C3b produced by complement activation. As complement is activated within immune complexes, the complexes bind to CR1 via C3b and are carried by erythrocytes to liver, where they are phagocytized and cleared. The heteropolymer should produce immediate clearing of anti-DNA; its DNA portion is recognized by a patient's anti-DNA, and because

the polymer is already bound to CR1 via anti-CR1, clearing of the undesirable antibody should occur promptly. In SLE patients, phase I trials have demonstrated that ETI-201 can lower anti-dsDNA (both high avidity and total anti-DNA) and appears safe (46). From published data, it is not clear how frequently the heteropolymer has to be administered to produce long-lasting reduction of anti-DNA quantities.

Agents That Target B Cells

B Lymphocyte Stimulator (BLyS) Protein Antagonists and Related Compounds

A member of the TNF superfamily, BlyS protein (also known as BAFF, TALL-1, THANK, TNFSF20, zTNF4) is a 285 amino acid protein that upon binding to receptors on B cells promotes survival, expansion, and differentiation of those cells (47 ,48 ,49 ,50). There are three receptors that bind BlyS—BCMA, BAFF-R, and TACI. First described in 1999, BLyS expression is highly restricted to myeloid lineage cells (e.g., monocytes, macrophages, dendritic cells) and some activated T cells. Mice that develop spontaneous lupus have increased serum levels of BLyS; transgenic mice overexpressing soluble BlyS develop a lupus-like disorder (49 ,50). Many patients with SLE, RA, lymphoma, and Sjogren syndrome have elevated serum levels (51 ,52 ,56). BlyS is 35% homologous with APRIL, a 250 amino acid protein that binds to BlyS receptors BCMA and TACI but not BAFF-R. In some systems, signaling through APRIL results in downregulation of B cell expansion. Levels of APRIL inversely correlate with anti dsDNA (53).

Human monoclonal antibodies against BLyS and its related proteins have been developed. In a phase I study, 75 patients with SLE received anti-BlyS (LymphoStat B, Human Genome Sciences). The product reduced quantities of circulating B cells and had acceptable safety and tolerability (54). Anti-BLyS is modestly effective for RA (55). In a phase II SLE trial, the efficacy outcomes of reduced disease activity measured by SLEDAI, and reduced numbers of flares, compared to placebo, were not met (55). However, a subset of patients could be identified that had good responses, and there was significant reduction of anti-DNA in the treated groups. The manufacturer has announced that it intends to pursue phase III trials, with modifications that will concentrate on the subset of patients most likely to be responsive. Other agents in development include fusion proteins of soluble BAFF-R (Biogen) and soluble TACI (Serenoa, Amgen). These should mop up soluble BlyS, preventing it from binding to receptors on B cells, thus preventing full B cell expansion and maturation in marginal zones of lymphoid tissues.

Rituximab (Anti-CD20 Therapy)

CD20 protein consists of 44 amino acids exposed in the extracellular space of pre-B lymphocytes and mature B lymphocytes but not hematopoietic stem cells, pro-B lymphocytes and plasma cells. Antibodies to CD20 thus specifically eliminate B cells without preventing their regeneration. A chimeric anti-CD20 whose variable region is derived from a mouse antibody contains a human IgG1 κ constant region and a variable region from a murine antibody IDEC-C2B8, which binds with high affinity to cells expressing the CD20 antigen. It is thought to have anti-inflammatory actions in rheumatic diseases via five mechanisms. These include activation of complement with lysis of the targeted cell, antibody-dependent cell-mediated cytotoxicity, alteration of the ability of B cells to respond to antigen (with decreases in B cell derived IL-6 and IL-10), decreases in T helper cell activation via down regulation of the T cell CD40L costimulatory pathway, and promotion of apoptosis. Since its introduction in 1997, rituximab (Rituxan) has been given to over 750,000 lymphoma patients (56 ,57).

Rituximab has therapeutic effects in RA (especially in combination with methotrexate) in several large trials. As of this writing approximately 120 patients with SLE given rituximab have been published in case reports and open label trials (58 ,59 ,60). It appears to reduce the quantities of B cells acting as APC to activate helper T cells (59), without drastically lowering autoantibody titer, quantitative immunoglobulins or anti-dsDNA. Quantities of circulating B cells nadir 1 to 2 months posttreatment and correlate well with higher serum rituximab levels and the Fc-γRIIIa genotype (61 ,62). In uncontrolled, open trials in patients with SLE, disease activity indices (e.g., SLEDAI, SLAM) improved in 40% to 80% of patients and remain favorable in most for 2 to 6 months. The dosing in these reports ranges from a total of 1,000 mg/m^2 to 1,500 mg/m^2 given intravenously one to four times over 4 weeks. Concomitant therapies have included corticosteroids (which if given intravenously as methylprednisolone with rituximab decreases infusion reactions), and in some series cyclophosphamide or other immune suppressives. Nearly all manifestations of SLE improve, especially if autoimmune hemolytic anemia, thrombocytopenia or nephritis are present. The treatment produces fever, chills, asthenia, and headaches the first day or so, but these effects are short-lived. Such reactions are most common during and after the first infusion of Rituximab. The emergence of inactivating antibodies to the Rituximab mouse/human chimera probably accounts for the general observation that Rituximab's effects diminish with repeated dosing several months later (63 ,64). Several humanized versions of Rituximab are being studied in clinical trials in RA (Trubion, Amgen, Genmab, Genentech) and in SLE and a large-scale trial with rituximab in SLE has been initiated (Genentech).

Epratuzumab (hLL2; Immunomedics, UCB) Therapy

CD 22 is a B cell restricted, 130 to 140 kd transmembrane glycoprotein expressed in the cytoplasm of pro-B, pre-B, and mature B cells, but not plasma cells (65 ,66). It internalizes rapidly followed by degradation in lysosomes, without detectable recycling. The binding of CD22 to endogenous ligands triggers B cell activation and proliferation.

Epratuzumab (hLL2; Immunomedics) is a humanized anti-CD22 monoclonal antibody that binds with high affinity to the third loop of CD 22, and blocks binding of natural ligands. It inhibits B cell receptor mediated signals, elicits moderate antibody-dependent cellular cytotoxicity, promotes apoptosis, activates sialic acid bearing ligands, and decreases CD 22 cell surface expression. These actions play an important role in SLE. Epratuzumab causes a temporary, moderate B cell depletion, which is less than noted with its closely related cousin, rituximab.

Epratuzumab has been studied in lymphoma, Sjogren syndrome, and SLE (67 ,68). Intended to be given weekly for 4 weeks every 3 months for 1 year in its uncontrolled clinical trial for lupus that began in 2005, the agent improved salivary and lacrimal gland flow, fatigue, and tender joint counts in 14 Sjogren patients, and appeared safe and well tolerated among 15 lupus patients. In the latter group, it appeared to improve disease activity in those with mild to moderate inflammation as defined by BILAG scores.

Agents That Target Cytokines

TNF-a Inhibition

The widespread availability of etanercept, infliximab, and adalimumab for RA has led to its off-label use in patients with SLE who were refractory to standard therapies. There is considerable evidence that TNF-α plays a pro-inflammatory role in lupus; renal biopsies contain significant amounts of TNF-α, and serum levels are elevated in some patients. However, caution has been advised, because 10% to 45% of patients with RA, Crohn disease, or psoriasis receiving TNF-α inhibitors have developed antinuclear antibodies, anti-dsDNA and/or anticardiolipin antibodies (69 ,70 ,71). Disease flares have been reported in this setting and about 1% developed drug-induced lupus (reviewed in Chapter 44). Smolen's group has reported improvements in synovitis and proteinuria in lupus patients receiving infliximab even though anti-dsDNA and antiphospholipid titers increased in some of the patients (71 ,72). At the time of this writing (July 2006), the safety and efficacy of TNF-α inhibitors in SLE is not clear.

Agents That Target Interleukins

Anakinra (Kineret) is an anti–IL-1RA therapy that has transient but unimpressive effects in SLE (73). IL-6 plays a critical role in B cell hyperactivity in SLE (74). Preliminary work at the NIH suggests that mononuclear cells from lupus patients might be inactivated by treatment with a humanized anti IL6-R monoclonal antibody (75 ,76). The therapy has been impressively effective in children with Still disease in Japan (77). Six patients given an anti–IL-10 murine antibody intravenously for 21 days showed a reduction in SLEDAI score, cutaneous lesions, and joint symptoms, but all developed antibodies against the agent (78).

Agents That Target Chemokines

Although antagonism to monocyte chemoattractant protein 1 failed to improve human rheumatoid arthritis, since it retards nephritis and renal vasculitis in MRL/lpr mice, studies in human SLE are anticipated (79 ,80).

T Cell Vaccination

Li et al. successfully vaccinated six patients with autoreactive T cell clones, which resulted in decreases in SLEDAI scores and steroid dose reduction (81).

Stem Cell Transplantation and SLE

In the early 1990s, the idea arose of treating patients with severe SLE who were unresponsive to conventional therapies with immunoablation followed by infusion of autologous hematoprogenitor stem cells (82 ,83).The theoretical benefits would be (1) the procedure would “debulk” the immune system of its autoreactive cells, providing the opportunity for normal regulatory networks to exert control of autoimmune disease; (2) “new” immune cells would develop in the environment of upregulated display of self-antigens to which the person had reacted previously, and such cells would “learn” to be tolerant to those self-antigens. Against these potential benefits would be the fact that the cells repopulating the immune system have the same genetic content as the original cells; therefore if SLE depends as much or more on genetic predisposition as on unfortunate environmental “hits,” the results would be recurrence of SLE.

Supporting the potential benefits were observations that in several patients with cancers treated with autologous bone marrow transplantation, concurrent SLE improved (84 ,85). These reports were followed by reports of one or a few cases of SLE treated with high dose immunosuppressive with bone marrow rescue by autologous stem cells (86 ,87 ,88). With this encouraging beginning, U.S. and European centers began treating larger numbers of SLE patients and recording results in widely available databases (88 ,89 ,90 ,91 ,92 ,93 ,94 ,95 ,96). As of October 2004, 66 patients with SLE had been treated, followed for at least 36 months, and enrolled in the EBMT/EULAR autoimmune disease database. In the United States as of November 2004, Burt and Traynor have treated 33 patients with follow-up for at least 12 months.

From data published to date, the authors have reached the following conclusions:

- Aggressive immunoablation plus myeloablation is unnecessary for response and carries a high morbidity/mortality. At the time of this writing (November 2005), most phase II/III studies are designed with immunoablation alone, generally built around a dose of cyclophosphamide of 200 mg/kg given over 4 days before autologous stem cells are administered, plus high-dose corticosteroids during the first few weeks, and GM-CSF administered after stem cell transplant to

speed the reappearance of granulocytes in the circulation.

- The conditioning regimen (to expand stem cells prior to harvest) is somewhat risky and involves administration of cyclophosphamide 2 g/m^2 and G-CSF to mobilize hematopoietic stem cells, which are then isolated by CD34$^+$ selection. To carry out transplantation, the stem cells must expand in vitro to an appropriate number to maximize repopulation of the bone marrow.
- Removal of T lymphocytes from the host gives better responses and lower GVH-like adverse events. This is achieved in most protocols by administration of ATG 90 mg/kg divided over 3 days prior to the infusion of the stem cells.
- All the regimens include standard antibiotics, antifungal, and antiviral preventive measures during the period of agranulocytosis that follows immunoablation.
- Failure of the stem cells to engraft is rare, but occurs (97).
- Autoantibodies and autoimmune syndromes that are previously absent can occur after autologous stem cell transplantation; these include autoimmune thyroid disease, hemolytic anemia, thrombocytopenia, and antiphospholipid antibodies and syndrome.
- Mortality rates in the United States, Europe, and Asia among SLE patients treated with various regimens and autologous stem cell transplantation is 5% to 15%. Deaths occur from sepsis, bleeding, new lymphoproliferative disorders, active progressive SLE unresponsive to treatment (or relapsing), and new autoimmune events.
- The rate of remission (defined variously, most commonly as (a) SLEDAI less than or equal to 3, (b) prednisone requirement less than or equal 10 mg per day and (c) no new immunosuppressive drugs) is approximately 66%. An additional 10% to 15% have a response that is not complete.
- The rate of relapse among good or partial responders is approximately 33% over a period of 3 years.
- Several manifestations of SLE in patients that have been resistant to standard therapies can respond well to these regimens of autologous hematopoietic stem cell transplantation, including nephritis (if a reversible component is present), central nervous system lupus, pulmonary lupus, and antiphospholipid syndrome (98 ,99).

The most important factor in determining whether this intervention is successful is patient selection. Death rates in the European/Asian experience were highest in patients with very active SLE (SLEDAI scores >20) (96). On the other side of the coin, a treatment associated with a 5% to 15% mortality rate is not appropriate for patients whose SLE is not life-threatening, unless they choose to take this rather large risk because their quality of life is quite poor. Finally, the patients must have reversible disease to benefit; determining that is not always easy, particularly when the major disease manifestations are extrarenal and tissue is not readily available for biopsy.

In summary, with durable remission possible in approximately 50% of SLE patients with severe disease who have entered these trials, most experts feel that additional trials are warranted. At this time (November 2005), we are aware of controlled phase II trials underway at the facilities of the NIH in Bethesda, MD, and a larger multicenter trial supported by the NIH is scheduled to begin in 2006.

Many experts think that developing safe methods of allogeneic bone marrow/stem cell transfer to patients with SLE would be a better strategy than autologous stem cells, since the donor would not have a lupus-prone genetic background. The major hazard of this approach is graft-versus-host disease. However, a method that employs small and infrequently repeated doses of allogeneic human donor T cells to SLE patients is being studied. A recent open study by Burt RK et al. (*JAMA* 2006:295:559–560.) in SLE patients in the USA showed at 5 years probability of 84% survival and 50% disease free survival.

Targeted Therapies in Development (Not yet used in Humans)

Approaches that Block Interferon-a

Using global gene expression profiling, a majority of lupus patients show dysregulated expression of genes in the interferon pathway. This signature serves as a marker for more severe disease activity (100 ,101 ,102). α-Interferon promotes B cell, T cell, and dendritic cell activity and increased MHC expression. Plasmacytoid dendritic cells are a potent source of type 1 interferons (103). Therapeutic approaches that block the activation of interferon-α include those that interfere with its production, effects on cytokines, effects on other immune cells, agents that inhibit its signaling pathway or drugs that neutralize it directly. Agents that block this pathway have potential uses in managing lupus. MedImmune is studying antibodies against interferons and interferon receptors as potential therapies for SLE (104).

Gene Therapies for Lupus

Since the tragic death in 1999 of a young man undergoing gene therapy in the United States, most human trials have stopped. However, in murine models of lupus, there continues to be important work in gene therapy. Most of the treatments have targeted cytokines, T/B/APC interactions, or induction of regulatory T cells. With regard to cytokines, there is substantial evidence that an imbalance between helper and regulatory cell functions is characteristic of SLE. Increasing IL-2 levels via administration of the construct in vaccinia virus or in oral attenuated Salmonella typhimurium to MRL/lpr mice reduced autoantibody production and renal damage (105 ,106), but administration of a construct as cDNA given IM enhanced autoantibody production and shortened survival (107). In a human experiment, transfection of p65 (a subunit of the NF-κB transcription factor that regulates IL-2 production) into cultures containing T cells from SLE patients increased IL-2 promoter activity in the cells (108).

Upregulation of TGFbeta has also had mixed results—improving MRL/lpr disease given in plasmid (109), or as cDNA injected intramuscularly (106) but not when delivered

orally in *Salmonella* (107). Administration of adenovirus vector containing DNA encoding human TGF-ß receptor showed expression of the protein in glomeruli, and a brief period during which mesangial proliferation and fibrosis in glomeruli and interstitium was reduced, but the benefit was not maintained (110). Administration of plasmid containing DNA encoding IL-12 to MRL/lpr mice increased levels of IL12 and was beneficial (111). Inhibition of IFN-γ by IM injections of plasmids with cDNA encoding IFN-γR/Vc, or naked DNA from such plasmids, retarded both onset and progression of SLE in MRL/lpr mice (112).

Prevention of T/B crosstalk was induced by administration of adenovirus containing CTLA4IgG gene IV, which improved lupus nephritis in MRL/lpr (113). A single injection of an adenoviral construct for transmembrane activator and CAML interactor receptor that produces high serum levels of TACI-Fc fusion protein reduced B cell numbers and blocked autoantibody production in B6.lpr/lpr mice, and reduced B cells and nephritis in MRL/lpr. However, NZB/NZW F1 mice were not responders because they made antibodies to the TACI and did not reduce B cell numbers or disease (114).

Finally, gene therapy has been used to induce regulatory/inhibitory T cells. First, nucleosome-specific regulatory T cells were induced in NZB/NZW F1 mice by multiple gene transfer than included TCR-α, TCR-ß, of a histone of peptidereactive T cell and CTLA4Ig, with resultant suppression of nephritis (115). A second approach was designed to induce anti-DNA-immunoglobulin-derived-peptide-specific $CD8^+$ cytotoxic T cells that could kill B cells making anti-DNA. A minigene encoding Ig-derived peptides that are presented by MHC class I molecules in NZB/NZW F1 mice was inoculated into young mice. The immunized mice developed $CD8^+$ T cells cytotoxic for B cells with BCR that bind DNA. Thus, the gene therapy specifically targeted production of anti-DNA. Proteinuria was delayed and survival increased (116).

In summary, gene therapy for SLE is in an early stage (117). The ideal DNA constructs, vectors, routes of administration, and dosage schedules have not been completely worked out, particularly for human use. This is an exciting future direction.

Other Strategies

Innovative strategies that can improve lupus may employ novel approaches, but have not been attempted in humans. Some of these include:

- The promotion of regulatory cells that are deficient in SLE, such as $CD4^+CD8^-CD25^+$, which may be accomplished by “educating” peripheral blood mononuclear cells with IL-2 and TGF-ß (118), or by peptide tolerization or gene vaccination discussed above (38 ,39 ,41 ,43).
- Developing gene transcriptional regulators, such as histidine deacetylases (HDAC) inhibitor Trichostatin A, coactivators, corepressors, or nuclear hormone receptors (119).
- Targeting Fc receptors that couple antigen-antibody complexes to effector cells and initiate the inflammatory cascade (120).
- Targeting toll receptors that promote inflammation as part of our innate immunity. For example, dendritic cell activation by chromatin-immunoglobulin G complexes is Toll-like receptor 9–dependent and is probably relevant to maintaining immune activation in patients with SLE (121).

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Section VII:
Appendices

Appendix I: A Patient's Guide to Lupus Erythematosus

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Purpose of This Appendix

When first told they have lupus erythematosus (LE), many patients have never before heard the term. This appendix is intended to help you understand what lupus is, how it may affect your life, and what you can do to help both yourself and your physician in the management of the illness. It will not replace your physician's advice. Because each case of LE is different, only your physician can answer specific questions about your individual situation. It is hoped that by learning facts about LE in nontechnical terms, you may increase your knowledge of the disease. In addition to explaining what lupus is, we have tried to answer other questions that you, your relatives, and your friends may have, such as what causes LE, the difference between cutaneous LE (CLE) and systemic LE (SLE), how the diagnosis is made, and how the illness is treated.

We use easy-to-understand terms throughout this appendix. We also have provided a glossary at the end to explain the more complicated words.

Because many of the most significant studies of LE are fairly recent and are constantly in various stages of exciting change and progress, much of the information that is available is already out of date. If you look up LE in an encyclopedia or medical book, you likely will be confused and maybe even frightened. You do not need to be frightened, and it may interfere with your seeking proper diagnosis and treatment.

A Brief History of LE

Lupus means *wolf* in Latin, and erythematosus means *redness*. The name was first given to the disease because it was thought that the skin damage resembled the bite of a wolf.

LE has been known to physicians since 1828, when it was first described by the French dermatologist Biett. Early studies were simply descriptions of the disease, with emphasis on the skin changes. Forty-five years later, a dermatologist named Kaposi noted that some patients with LE skin lesions showed signs that the disease affected internal organs.

In the 1890s, Sir William Osler, a famed U.S. physician, observed that systemic LE (also called SLE) could affect internal organs without the occurrence of skin changes.

In 1948, Dr. Malcolm Hargraves of the Mayo Clinic described the LE cell, which is a particular cell found in the blood of patients with SLE. His discovery enabled physicians to identify many more cases of LE by using a simple blood test. As a result, the number of SLE cases that have been diagnosed during the succeeding years has steadily risen. Since 1954, various unusual proteins (or antibodies) that act against the patient's own tissues have been found to be associated with SLE. Detection of these abnormal proteins has been used to develop more sensitive tests for SLE (i.e., antinuclear antibody [ANA] tests). The presence of these antibodies may result from factors other than SLE.

What is LE?

LE usually appears in one of two forms: (1) cutaneous lupus erythematosus (the skin form, CLE); or (2) systemic lupus erythematosus (the internal form, SLE).

Chronic cutaneous lupus (CLE, formerly known as discoid lupus) LE has a particular type of skin rash with raised, red, scaly areas, often with healing in the centers or with scars. These eruptions most commonly are seen on the face and other light-exposed areas. Usually, patients with DLE have normal internal organs. A skin biopsy of the lesion may be helpful in confirming the diagnosis.

Subacute cutaneous lupus erythematosus (SCLE) is a nonscarring subset of lupus that is characterized by distinct immunologic abnormalities and some systemic features.

SLE is classified as one of the autoimmune rheumatic diseases, in the same family as rheumatoid arthritis, and usually is considered to be a chronic, systemic, inflammatory disease of connective tissue. Chronic means that the condition lasts for a long period of time. Inflammatory describes the body's reaction to irritation with pain and swelling. LE involves changes in the immune system, so that elements of the system attack the body's own tissues. Different organs are affected in each person, and joints usually are inflamed.

Inflammation also can involve the skin, kidney, blood cells, brain, heart, lung, and blood vessels. The inflammation can be controlled by medication.

SLE can be a mild condition, but because it can affect joints, skin, kidneys, blood, heart, lungs, and other internal organs, it can appear in different forms and with different intensities at different times in the same person. A large number of people with SLE have few symptoms and can live a nearly normal life. Therefore, while reading about the symptoms, you should not become unnecessarily worried, because all of the symptoms usually do not occur in one person.

How serious lupus is varies greatly from a mild to a life-threatening condition. It depends on which parts of the body are affected. Even a mild case can become more serious if it is not properly treated. The severity of your LE should be discussed with your physician.

In addition to CLE and SLE, there are other variants of lupus. Drug-induced lupus afflicts 15,000 Americans each years and results from over 70 different drugs. Fortunately, it goes away when the medicine is discontinued. Neonatal lupus reflects the presence of a lupus rash or abnormal heart pacing system in a newborn whose mother has certain lupus autoantibodies. The rash disappears within a few weeks and the children do not have lupus. Mixed connective tissue disease or crossover or overlap syndromes imply the presence of lupus as well as another autoimmune disorder such as scleroderma (i.e., hardening and thickening of the skin), rheumatoid arthritis or polymyositis (i.e., inflammation of the muscles). Finally, undifferentiated connective-tissue disease (UCTD) patients often have features of lupus, such as a positive ANA with swollen joints, but do not fulfill the criteria for SLE. Over time, one third evolve lupus or another autoimmune disorder, one third stay as a UCTD, and the process disappears in another third. LE is not infectious or contagious. It is not a type of cancer or malignancy. LE is not related to acquired immunodeficiency syndrome (AIDS).

Frequency of LE

No one has made an accurate estimate of the number of patients with CLE because many people have mild cases and probably do not know it. There may be as many as 1 million people with SLE in the United States.

The number of new cases of SLE diagnosed by physicians is definitely increasing, for several reasons. After the LE cell test came into use, physicians were able to diagnose the illness correctly in patients who were believed to have other rheumatic diseases, or who were thought to have neurotic complaints. Tests for ANAs and other antibodies, which usually are positive in SLE, have helped physicians to discover even more patients with milder cases, but the tests might be positive in patients without SLE.

Seven of ten patients with CLE and 90% with SLE are women, most of them developing their first symptoms between 15 and 30 years of age. LE is rare in children under the age of 5. It is found throughout the world, however, and affects all ethnic groups and religions.

SLE is more common than rheumatic fever, leukemia, cystic fibrosis, muscular dystrophy, multiple sclerosis, hemophilia, and several other well-known diseases.

What Causes LE?

The cause of CLE is unknown. In most cases, the cause of SLE also is unknown, although it is believed that many factors may be involved, including genetic predisposition and environmental factors such as excessive sun exposure, certain medicines, and infections. In families of patients with SLE, it is known that there is an increase in the number of relatives with SLE and rheumatoid arthritis compared with the normal population. Many of the relatives have abnormal proteins, such as ANAs, in their blood, although they may not have any symptoms of the disease. Using sera from new recruits to the U.S. Armed Forces, it has been shown that those who develop SLE possess lupus autoantibodies years before they have symptoms or are diagnosed.

A critical “dose” of 30 thus far identified susceptibility genes causes enough immune response abnormalities to sustain the production of antibodies to self and immune complexes (antigen and antibody complexes). The genes are “turned on” by infections, increased exposure to ultraviolet light or other environmental toxins, at least 70 known prescription drugs, increased exposure to exposure to increased estrogen or other sex hormones and severe stress. Antigens are formed which promote an immune reaction, mimic microbes, or react to debris from dead cells. There are intrinsic abnormalities of B and T lymphocytes. These cells are activated by lower concentrations of antigen than normal, have sustained surface expression of activation markers, and are relatively resistant to dying cell debris, which allows them to escape regulation. There is a consequent release of cytokines by these cells which results in inflammation. In this milieu, autoantibody subsets become pathogenic and more antibody production occurs. Immune complexes (consisting of antigen and antibody) are formed, which favor capture rather than elimination and tissue is further damaged.

In perhaps 10% of patients with SLE-type symptoms, the disease may have been caused by medications. The most common of these is procainamide (Pronestyl), which often is used to treat heart irregularities. It is essential that your physician be told of all medications you are taking, including birth control pills and estrogens for menopause, as well as medications purchased over the counter or at health-food stores. Sometimes, medication can flare lupus; for example, sulfa antibiotics can make you more sun-sensitive and susceptible to developing rashes.

Diagnosis

The skin rash of CLE may be so typical that an experienced doctor can make the diagnosis by the history and appearance of the rash. If there is any question, a skin biopsy usually helps. It is essential that each patient with CLE

have a thorough physical examination, including laboratory tests, to check the possibility of SLE being present.

Diagnosing SLE is more difficult. Finding a definite answer may take months of observation, many laboratory tests, and sometimes a trial of drugs. Because of many different symptoms, some patients are thought to have another disease, rheumatoid arthritis, with swelling of a few or many joints of the hands, feet, ankles, or wrists. If typical skin lesions are present, they are helpful in making the diagnosis. Other findings, such as fever, pleurisy (i.e., painful breathing), or kidney disease, also point to the diagnosis of SLE.

In addition to a complete medical history and physical examination, routine tests are done to learn what internal organs are involved, for example, a blood count to see if there are too few red cells, white cells, or platelets (i.e., cells that are necessary for clotting). A routine analysis of the urine is always done, and a kidney function test is obtained. A chest radiograph electrocardiogram, or echocardiogram may be recommended if clinical evidence of problems in the lung or heart is found.

Diagnostic Criteria and Autoantibody Testing

In 1997, the American College of Rheumatology (ACR) established new diagnostic criteria for SLE. After excluding rheumatoid arthritis, scleroderma, and polymyositis, a diagnosis of SLE can be made if four of the following 11 criteria are met:

- Butterfly rash on cheeks
- Cutaneous (discoid) lupus
- Sensitivity to sunlight
- Mouth or nose sores
- Arthritis (i.e., swelling or inflammation of several joints)
- More than 0.5 g of protein in the urine per day, or cellular casts in a urinalysis
- Seizures or psychosis
- Pleuritis or pericarditis
- Low white blood count, low platelet count, or hemolytic anemia
- Antibody to DNA or to Sm antigen (i.e., a fairly specific antibody found in about one fourth of patients with lupus), or to antiphospholipid antibody (a false-positive syphilis test, anticardiolipin antibody, or lupus anticoagulant)
- Positive ANA test

Table APPI-1: Principal Immune Serologies and Their Value in SLE

Autoantibody	% in SLE	% in Normals	Comment
Antinuclear	98	5-10	If absent, it's probably not lupus
Anti-DNA	50	<1	Suggests more serious disease
Anti-Sm	25	<1	The most specific test for lupus
Anti-RNP	25	<1	Many also have MCTD
Antiphospholipid	33	5	1/3 have thromboembolic events
Anti-Ro/SSA	30	<1	Associated with Sjögren's, neonatal lupus, sun sensitivity
Anti-La/SSB	15	<1	Always seen with anti-Ro; may diminish pathogenicity of anti-Ro
Antineuronal	20	<1	Putative marker for CNS vasculitis
Antiribosomal P	20	<1	Seen with psychosis, hepatitis
Low serum complement	50	5	Decreased with inflammation

MCTD, mixed connective tissue disease.

To help confirm the diagnosis, special tests for SLE are performed that measure blood antibodies. These include examinations for ANA, which is the most sensitive test for the disease. Serum complement (i.e., a protein that is decreased during active phases of autoimmune illness) often is measured. Anti-DNA antibody is a specific type of ANA that often is present in the blood of patients with SLE; its presence is helpful in confirming the diagnosis of SLE. Moreover, when the disease is active, especially if the kidneys are affected in SLE, anti-DNA antibodies usually are present in high amounts in the blood. Thus, tests for anti-DNA antibody can be useful in monitoring disease activity in SLE. Again, none of these tests is specific for SLE, and different medical centers may use other diagnostic tests depending on their individual experience tests; consequently, obtaining such a result does not confirm the diagnosis of SLE. All tests must be evaluated by the physician in regard to the signs and symptoms of the patient. Table APPI-1 lists some of the autoantibodies ordered by musculoskeletal specialists concerned about diagnosing or following SLE.

Some patients with a negative ANA may still have SLE. Usually, these patients have anti-Ro/SSA antibody or a positive, nonlesional (i.e., skin that looks normal) skin biopsy using immunofluorescence (i.e., lupus band test), or have taken steroids or given chemotherapy in the past. Patients with CLE often have a negative ANA and a positive biopsy from the skin rash.

Resemblance to Other Diseases

One problem in diagnosis is that there is no single set of symptoms or pattern of disease. Also, SLE can mimic the symptoms of many other diseases, such as cancers, infections, and hormonal problems, and it can strike many different parts of the body, sometimes confusing even the most experienced physicians. The musculoskeletal pain of SLE often is difficult to differentiate from a syndrome known as fibromyalgia. Formerly termed fibrositis, fibromyalgia is associated with poor sleeping habits, fatigue, tension headaches, numbness and tingling, and irritable bowel symptoms, in addition to spasm and pain in the muscles, especially in the upper neck and back and coexists with lupus.

Symptoms and Course

The patient with SLE may have periods of severe illness (i.e., flare or exacerbation) with extreme symptoms, intermingled with periods of no illness and complete freedom from symptoms (i.e., remission). The illness comes and goes so unpredictably that no two cases are alike. Even before the discovery of corticosteroids, some patients made a full recovery with treatment by aspirin and rest alone. Although causes for disease flare-ups may be recognized and prevented by the patient, at other times their cause is unknown. Some possibly preventable causes of flare-ups are excessive sun exposure, injuries, insufficient rest, stopping medications that have been controlling the disorder, irregular living habits, and emotional crises. It cannot be emphasized too strongly that abruptly stopping medication, particularly large doses of corticosteroid derivatives such as prednisone, can lead to severe flare of the disease or even a fatal outcome.

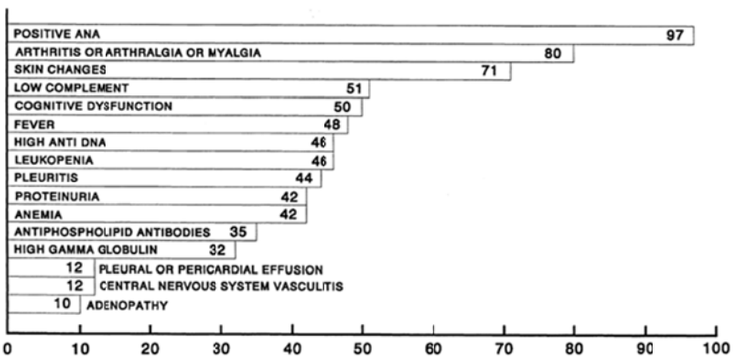


Figure APPI-1. Cumulative percentage incidence of 16 clinical and laboratory manifestations of systemic lupus erythematosus (SLE) based on major studies involving nearly 2,000 patients evaluated in seven studies since 1975.

Symptoms of the Disorder

The symptoms of SLE are varied, and no two patients have exactly the same ones. Any part of the body can be involved, so symptoms may include one or more of the following in any combination: joint and muscle pain, fever, skin rashes, chest pain, swelling of hands and feet, and hair loss (Fig. APPI.1). Joint involvement in SLE usually is less severe than that occurring in rheumatoid arthritis and usually is nondeforming. You should remember that in most patients, most of the symptoms disappear. This clearing of symptoms is called a remission. Medications usually are necessary to cause remissions, but sometimes they occur spontaneously (i.e., without treatment).

Physicians use the term remission or controlled rather than cure in speaking of the periods when patients are free of symptoms, because both doctors and patients then can be watchful for signs and symptoms, which may be a warning that a flare is beginning. Treatment then can be started before unnecessary damage occurs.

General Symptoms

Generalized aching, weakness, tiring easily, low-grade fever, and chills commonly are associated with active SLE. Although these symptoms usually are particularly noticeable during flare-ups of the disease, some patients give a lifelong history of low energy, malaise (i.e., generalized discomfort), and inability to keep up an active work schedule. A low-grade rise in temperature (99.5°F to 100.5°F), usually in the late afternoon, may be a sign of smoldering LE activity and may appear several days before the patient feels really ill. In the patient with SLE, loss of energy, development of weakness, low-grade fever, or tiring easily is each considered to be a danger sign. It may indicate that new

activity of the disease is developing. When any of these early warning signs develop, patients should consult their physician immediately, so that an examination can be made and further treatment prescribed if necessary.

The following symptoms and signs are typically found in SLE:

- Skin: A reddish rash or flush may appear involving the cheeks and nose in a so-called butterfly pattern. Other eruptions resembling CLE may occur in light-exposed areas of the body. Some patients are particularly sensitive to cold. After exposure to cold, the skin of their hands and feet may show several distinctive changes in color. Other patients may notice red, scaling changes on the back of the hands and on the fingers between the knuckles. Small areas of scarring on the scalp may produce baldness, and small red areas on the lips and the lining of the mouth may be related to SLE. Some patients have a definite sensitivity to ultraviolet rays of the sun, and even small amounts of sunlight may make these patients much worse. Easy bruising or pinpoint bleeding into the skin sometimes is related to SLE.
- Chest: Pleurisy, or irritation of the membranes lining the chest, causes painful breathing and is common in patients with SLE. Shortness of breath or rapid heartbeat sometimes is a related symptom. There may be an accumulation of fluid in the chest cavity from inflammatory changes.
- Muscular system: Tiring easily and weakness often are the first symptoms of SLE. Indeed, without these complaints, the diagnosis of systemic involvement in LE is open to doubt. Because they also are common in many other diseases and with plain nervous exhaustion, it is best to let your physician decide on their importance. Weakness occasionally can be caused by corticosteroid drugs. In these cases, a change in medication dose or type often is all that is necessary to return muscle strength.
- Bones and joints: Arthritis, joint swelling, and stiffness are common signs of SLE activity. These may involve only one joint, may move from one region to another, or, rarely, may progress to a deforming arthritis. This, however, rarely is disabling, and it is less frequent than in rheumatoid arthritis. Softening of bone (i.e., osteoporosis) can result from physical inactivity when you are ill and from corticosteroid drugs. Strategies are available to reduce these effects, including calcium, vitamin D, bisphosphonates, and hormonal replacement therapies for menopause or parathyroid disease.
- Blood: Anemia, or a low red-blood-cell count, is common in patients with LE. There may be a decrease in white blood cells, usually to around 2,500 to 4,000 per mL (normal is 4,500 to 10,000 per mL). The blood platelets, which are necessary for clotting, may become affected. Frequently, abnormalities of proteins in the blood are present as well; sometimes, a false-positive reaction for syphilis occurs when the blood is tested. Of course, this does not mean that the patient has syphilis, because this false reaction is only a manifestation of SLE.
- Heart: In some patients with SLE, swelling of the feet and ankles may occur, as well as shortness of breath or difficulty in breathing after exertion or when lying down. These symptoms may mean that the heart is affected. Fluid may collect in the pericardial sac surrounding the heart; sometimes heart muscle or valves become inflamed. The patient should remember that LE involvement does not always damage the heart permanently, because such changes disappear can completely with treatment. SLE patients are susceptible to developing accelerated atherosclerosis, even if they never took corticosteroids and should be screened for blood pressure and lipid profiles.
- Stomach and intestinal tract: Pain in the abdomen, nausea, vomiting, diarrhea, or constipation sometimes are associated with SLE. These symptoms may be so severe as to imitate acute appendicitis, a stone in the kidney, or some other condition requiring surgical treatment. If these symptoms appear, it is important for the patient to tell the surgeon that he or she has SLE as well as the type and dosage of medication being taken.
- Kidney and bladder: The kidney serves as the filtering plant of the body, filtering out waste products while preserving the many chemical parts of the blood that are essential for good health. Involvement of the kidney by SLE may cause loss into the urine of some of these essential chemical components, and there may be poor excretion of the waste products that usually are discarded in the urine. Retention and accumulation of the waste products can produce further symptoms or signs (such as foam in the urine or swelling) that may require specialized treatment. The development of SLE kidney involvement is painless and should be checked by urinalysis every few months. Kidney biopsy (i.e., removal of a bit of tissue for study under the microscope) may be helpful in confirming the diagnosis or choice of treatment.
- Lymph glands, spleen, and liver: Occasionally, the lymph glands of the neck, under the arms, and in the groin become enlarged. The spleen (an internal organ) also may become enlarged, and SLE hepatitis (i.e., an inflammation of the liver) sometimes develops.
- Nervous system (brain, spinal cord, and nerves): Temporary seizures that resemble epilepsy may be early evidence of SLE, and the diagnosis of SLE is suggested only after other symptoms appear. Mental depression, excitability, unusual worry, headache, mental confusion, forgetfulness, nerves, or even a nervous breakdown can be caused by SLE. Some patients have transient paralysis, stroke, neuritic pains, or poor bladder control related to their disease. Cognitive impairment (difficulty in thinking clearly) is common.
- Menstrual periods: Menstrual periods may become irregular, more or less frequent, or even stop completely for several months. This usually is related to the activity of SLE or to side effects of glucocorticosteroids.

When the disease is brought under control, menstrual periods may return to normal.

Thus, a wide range and variety of symptoms may announce the onset of SLE. Some patients, through the entire course of their illness, have symptoms involving only one organ. Others may have symptoms that come and go, and some may begin with one group of symptoms and acquire others as new parts of the body become involved with the SLE process. Remember that before the discovery of cortisone derivatives, 40% of patients improved with rest and aspirin alone.

Other Considerations

Early warnings that may indicate a flare-up include chills, fatigue, loss of pep, new symptoms, and fever, such as change from the normal daily temperature to a slight afternoon fever of 99.5°F to 100.5°F. If any of these changes occur, the physician should be notified.

The patient with SLE without organ-threatening disease generally can return to his or her regular occupation. Usually, after the illness is well controlled, it does not interfere with full-time work as long as the patient does not become too tired or stressed.

Childbearing

Patients with SLE usually can have successful pregnancies, provided they do not have too much kidney or heart disease. Although many women with SLE feel better during pregnancy, an occasional flare-up can occur. Physicians cannot predict the effect of pregnancy on a particular individual. Whether pregnancy is advisable in your own case should be discussed with your physician before you become pregnant.

Patients with DLE usually have no problems with pregnancy. The safety of many common medications in pregnancy, however, is not well established. Glucocorticoids (i.e., steroids) generally are safe for the fetus and can be continued throughout pregnancy and delivery if needed for disease control. Nonsteroidal anti-inflammatory drugs (NSAIDs) and high-dose aspirin can be used cautiously if necessary. Hydroxychloroquine is generally safe. Active SLE is associated with fetal loss.

A subset of patients with LE and antiphospholipid antibodies (especially those with high levels of anticardiolipin antibody) has been shown to have spontaneous recurrent fetal loss. They also may be at risk for developing blood clots. Children of mothers with SLE who have Ro/SSA antibody are at a slight risk for developing neonatal lupus or congenital heart block.

Contraception

The safest methods of contraception are the use of barrier methods such as diaphragm and jelly, foam, sponges, or condoms. Although birth control pills are safely used by many patients with SLE, the incidence of pill-related complications appears to be higher in these patients than in the normal user, especially in individuals with migraine headaches, high blood pressure, very high cholesterol, and antiphospholipid antibodies. Intrauterine devices are not advisable because of the high incidence of infections connected with their use.

Hormone Replacement Therapy

When women with SLE enter the menopause, either naturally or from the chemotherapy-type medications that sometimes are used to treat SLE, the question of taking estrogen and/or progesterone type hormones arises. Advantages include controlling hot flashes, prevention of dryness and mood swings, and reduced risk of heart attacks, osteoporosis, and fractures. Disadvantages include risking a lupus flare, risks of increased clotting and gallstones, weight gain, fluid retention, and higher blood pressure, as well as a slightly increased risk for breast cancer. Women with lupus and their doctors should weigh the pros and cons before starting this treatment. Treatment always can be stopped if problems do occur.

Treatment of LE

Several effective methods of treatment are available. Unfortunately, all of the medications that are used to treat SLE, including regular aspirin, have some potential dangers, but we must use them, optimally at low levels and for a short time. The one to be used in a particular individual depends entirely on the type of LE that is present. Patients with CLE may be treated with creams or ointments containing corticosteroid medications and sunscreens. With more extensive skin changes, antimalarial drugs often are effective.

Treatment usually is required for months to years. Stopping medication may produce a flare of skin lesions.

SLE is managed by local treatment for any skin eruptions plus various medications taken by mouth for symptoms such as arthritis, fever, rash, and kidney disease.

Aspirin and Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Aspirin and NSAIDs are not merely pain killers. When taken regularly and as often as prescribed, such as 8 to 16 five-grain tablets daily for adults, aspirin frequently controls fever, pleurisy (i.e., painful breathing), and joint discomfort. Aspirin and NSAIDs should be used with caution in patients who have had stomach ulcers; rarely, internal bleeding may result. When possible, it is advisable to stop these medications 1 week before surgery because of their tendency to slow down blood clotting. Taking the tablets with food often eliminates the stomach upsets that some patients experience. Antacids and a class of drugs known as frequently help to protect the stomach lining. NSAIDs such as indomethacin (Indocin), naproxen (Naprosyn), or ibuprofen (Motrin) frequently are effective for relieving

joint pains as well as pain at other sites of inflammation. Patients with kidney involvement should only take NSAIDs under close medical supervision, and all patients on NSAIDs or aspirin should have blood testing at 3- to 4-month intervals.

Antimalarial Drugs

This group of medications was first developed during World War II for the treatment of malaria when it became known that quinine, which then was standard treatment for malaria, was in short supply. It was discovered that many patients with LE, especially those who had skin changes of CLE, showed definite improvement after receiving the antimalarial drug quinacrine, although these chemicals also are helpful in systemic types of LE. It should be emphasized that there is no relationship between LE and malaria (which is caused by a small parasite transmitted by mosquitoes).

The exact mechanism of the antimalarial drugs in LE is not known, but by raising the pH of cells (i.e., making them more basic as opposed to acidic), inflammation is decreased. Immune responses also are reduced. In many patients with SLE, the antimalarials appear to make it possible to reduce the total daily dose of cortisone drugs. Another advantage of antimalarials is that they increase resistance to sun exposure and block the appearance of SLE rashes on exposure to ultraviolet light. Antimalarials are useful in managing skin, joint, and muscle symptoms, as well as fever, fatigue, and pleurisy. These agents often do not take effect for several months. Hydroxychloroquine (Plaquenil) is the only antimalarial approved by the U.S. Food and Drug Administration (FDA) for lupus and is the safest agent in its class.

Side effects of antimalarials in patients with LE do not often occur, but when they do, they can be important. The most common side effects usually involve the digestive system, with mild nausea, occasional vomiting, and diarrhea. Formerly, certain antimalarials, especially chloroquine (Aralen) and hydroxychloroquine (Plaquenil), were found to affect the eyes when used in doses twice as great as those now prescribed. Therefore, to be certain that no such bad effects occur, patients who are taking these medications must have eye examinations by an ophthalmologist at regular 6- to 12-month intervals. The risk of changes in the retina with Plaquenil is 3% after 10 years of continuous use. These changes are completely reversible with discontinuation of the drug and regular monitoring. Quinacrine (Atabrine) has not been reported to cause eye complications. Any changes in vision should be called to the attention of your physician.

Corticosteroids

The corticosteroid drugs (e.g., prednisone) are used primarily for treating the internal changes that are caused by lupus. However, they also help to heal the skin.

Cortisone and, later, prednisone were the first of the corticosteroid family to be used in medicine, and both they and their successors have been lifesaving in many thousands of patients with many different diseases. They are synthetic forms of hormones that normally are produced by the adrenal glands, which are the small glands above the kidneys. In addition to the beneficial effects of corticosteroids, however, these drugs have unwanted and undesirable side effects that may produce complications when they are taken for long periods.

In some cases of SLE, the physician may choose to prescribe different types of corticosteroid drugs or to prescribe them every other day instead of daily. This method reduces side effects considerably, but it may not be satisfactory for active cases.

The chief action of corticosteroid drugs is to decrease inflammation, so that these drugs control many of the symptoms and signs of SLE that are caused by inflammatory changes (e.g., arthritis and pleurisy). The drugs may be given by mouth in the form of tablets, or by injection into the muscle or joint or directly into the vein.

Another effect of the prednisone-like drugs is their shrinking effect on the adrenal glands. This occurs because the adrenal glands may stop producing the natural hormone, which is of special importance for two reasons. First, the synthetic hormone should not be stopped suddenly, because the adrenal glands may take several months to start production of natural hormone again. A sudden withdrawal of the synthetic hormone leaves the patient without this support and may cause a serious crisis. Therefore, the dosage of corticosteroids should be reduced gradually over several weeks or months, so that the patient's adrenal glands may increase their production of the natural hormone gradually over the same period. Second, any physical or mental stress, surgical procedure, dental extraction, or severe illness may increase the patient's need for large amounts of corticosteroids. When patients have taken corticosteroids for a long time, their own adrenal glands cannot satisfy this increased need, and larger, booster doses of the synthetic drug are required.

Persons who are taking corticosteroid drugs, or those who have taken them during the previous year, should carry with them an identification card or bracelet stating this fact for emergency use (much like the card carried by the diabetic person who must take insulin, or by a person who is extremely sensitive to penicillin).

Two points must be emphasized to every patient with SLE who is taking the corticosteroid groups of drugs. First, the drug should never be stopped suddenly if it has been taken for over 30 days; it should be reduced gradually over a long period (this is best done under the direct supervision of a physician). Second, when any patient is on long-term steroid therapy, he or she may need increased booster doses of the drug before, during, and after any period of general body stresses (e.g., surgery), and he or she should tell the physician or dentist of this possibility.

Because corticosteroids have an appetite-stimulating effect, an effort should be made to avoid excessive weight

gain. Damage to weight-bearing joints may occur following long-term steroid treatment and, occasionally, in some patients with untreated SLE. Additionally, steroids may induce diabetes, hypertension, cataracts, glaucoma, edema, avascular necrosis, bone demineralization (i.e., osteoporosis), poor wound healing, fragile skin that tears and bruises easily, susceptibility to infections, and ulcers. If the dose of steroids is greater than 10 mg of prednisone each day, people may have increased susceptibility to infections.

Immune Suppressives

Many powerful drugs, such as immune suppressives (e.g., antibody suppressors), are used in the treatment of severe SLE; these include azathioprine (Imuran), cyclophosphamide (Cytoxan), mycophenolate mofetil (CellCept), and methotrexate. These drugs most commonly are used in those with aggressive disease. Indications for these agents are the subject of a great deal of debate, both because they are toxic and their effectiveness has not always been demonstrated, and also because they may decrease steroid requirements. Evidence from the National Institutes of Health (NIH) suggests that intravenous, intermittent cyclophosphamide in combination with corticosteroids or mycophenolate mofetil represent the treatments of choice for severe lupus nephritis. Plasma exchange (i.e., plasmapheresis) is a very expensive, blood-filtering procedure whose results are only of uncertain benefit. Its use is reserved for those with life-threatening complications of lupus. Other agents that occasionally are used in LE are nitrogen mustard, retinoid derivatives, leflunomide, gamma globulin, danazol, chlorambucil, dapsone, and cyclosporine.

It is essential that once an effective treatment program has been started, the patient should continue the medication faithfully and not change it without the physician's advice. Severe flare-ups may occur suddenly in patients who stop their treatment abruptly.

Coping with LE: How Can You Help Yourself?

Physical Measures

- Be careful in the sun: Two thirds of lupus patients have a problem with ultraviolet A and B (UVA and UVB) radiation from the sun. If you're going to be outside for more than 5 minutes, use a sunscreen. Choose a preparation that has a sun protection factor (SPF) of at least 15 and blocks both UVA and UVB. UVB sun exposure is greatest at midday. Perform your outdoor activities earlier in the morning or later in the afternoon or in the evening. Wear protective clothing. Ultraviolet radiation is greater at higher altitudes. The exposure one gets at sea level in 1 hour is the same that one absorbs in 5 minutes a mile up, as in Denver or Mexico City or on the ski slope.
- Diet: Lupus patients should eat a nutritious, well-balanced diet. There are some suggestions that fish, or specifically eicosapentaenoic acid in fish oil, might have modest antiinflammatory properties. In double-blind controlled studies, eating the equivalent of two fish meals a week clearly helps rheumatoid arthritis pain. An amino acid, L-canavanine, is found in alfalfa sprouts and can activate the immune system and promote inflammation in lupus patients. Other members of the legume family have only a fraction of the L-canavanine that sprouts do and are safe to use. Lupus patients taking corticosteroids should watch their sugar, fat, and salt intake.
- When you hurt, apply heat: Moist heat soothes painful joints. Moist heat is superior to dry heat. Hot tubs, saunas, Jacuzzis, or hot showers are useful. We advise ice or cold applications only for acute strains or injuries for the first 36 hours.
- General conditioning exercises: Activities such as walking, swimming, low-impact aerobics, and bicycling help prevent muscle atrophy or wasting and decrease your risk for developing thin bones, or osteoporosis. On the other hand, if your joints are swollen or you have fibromyalgia, be careful before doing a lot of weigh lifting, rowing, high-impact aerobics, or engaging in tennis, bowling, or golf. If exercises tire you easily, pace yourself with frequent rest periods.
- Consult a rehabilitation specialist: Physical therapists assist patients in muscle strengthening programs, exercises, and gait training. Occupational therapists work to minimize stresses to painful areas, evaluate workstations (especially those with computers) to ensure proper body mechanics, and recommend a variety of assistive devices. Vocational rehabilitation counselors may train you for a job that involves less sun exposure or emphasis on repetitive motions involving an inflamed hand or other part of the body.
- Don't smoke: Tobacco smoke contains an aromatic amine, hydrazine, which can flare cutaneous lupus. Smoking also worsens Raynaud disease and impairs circulation to a greater extent in lupus patients that in otherwise healthy people.

Develop Preventive Coping Strategies

- Don't let the weather psych you out: Lupus patients are sensitive to changes in barometric pressure. If the weather goes from hot to cold or wet to dry, one might be a bit achier. This will pass. The best climate for lupus patients is one with the fewest changes in the barometer.
- Mastering fatigue: Fatigue in lupus is caused by inflammation, anemia, and chemicals known as cytokines, among other sources. Pace yourself. Have periods of activity alternating with periods of rest. Patients who stay in bed all day only become weaker. On the other hand, supermoms who put in a 20-hour day without a break can flare their disease.
- Have a good doctor-patient relationship: Make sure that your physician is accessible and will assist you

when it's important. Work out in advance what to do in case of an emergency. Will your physician advise you if pregnancy is contraindicated, whether or not you can take a birth control pill, know which antibiotics lupus patients need to be careful with, write a jury duty letter, or fill out a disability form if needed? In return, it's vital to keep your appointments, be honest with your physician, take medication as prescribed, and respect the physician's time.

- Genetic and prognosis counseling: Women with lupus have a 10% chance of having a daughter with lupus and a 2% of having a son with the disease, although there is a 50% chance their offspring will have a positive ANA. Twenty percent of patients with non-organ-threatening SLE will evolve organ-threatening disease, usually within the first 5 years after diagnosis. Patients with non-organ-threatening disease have a near-normal life expectancy if antiphospholipid antibodies are absent. The survival of organ-threatening lupus patients is 75% at 15 years.
- Pregnancy: 70% of lupus pregnancies are successful. Lupus patients are normally fertile but often don't conceive if they are inflamed. Kidney failure, severe hypertension, and myocarditis are relative contraindications to becoming pregnant. Patients with antiphospholipid antibodies who have miscarried may be given aspirin or heparin during a pregnancy. Mothers with anti-Ro (SSA) antibody should be advised of a 5% to 15% risk of their child being born with a transient lupus rash or a more serious heart problem that can be detected with ultrasounds at weeks 18 and 24. Find out what medicines are safe to take during a pregnancy. Most lupus activity cools down during the second trimester and mild postdelivery flares can occur.
- Address fevers or infections promptly: Call a doctor if your temperature is over 99.6°F. It could be a lupus flare or an infection. Be careful before taking sulfa-based antibiotics, which are usually prescribed for bladder and female infections. They tend to make lupus patients more sun sensitive and can lower blood counts, and up to 30% of lupus patients are allergic to sulfa drugs.
- Ask about cognitive therapy: Some lupus patients have difficulty remembering names and dates, balancing their checkbook, and processing thoughts. Termed cognitive dysfunction, cognitive impairment, or “lupus fog,” this is a reflection of vascular spasm in that insufficient amounts of oxygen are reaching the brain. These symptoms come and go. Cognitive therapists are psychologists, speech therapists, and physical therapists who can help patients cope with this by initiating biofeedback and specific strategies that improve concentration.
- Don't be afraid to ask for help: The Lupus Foundation of America (LFA) provides information about doctor referrals, lupus books, patient information brochures, and newsletters. Most local chapters have rap or discussion groups, sponsor guest speakers, and maintain a list of mental health professionals who can assist you.

Is There Hope of Conquering SLE?

There certainly is! A great deal of fast-moving research is going on throughout the world. Medical scientists are interested in SLE not only because they want to help those who suffer from it, but also because they want to find the key to other closely related rheumatic disorders, such as rheumatoid arthritis. We expect laboratory research to improve methods of treatment and, eventually, to provide a means of prevention and cure. Some of the approaches that are being studied include newer anti-inflammatory therapies with-, chemicals that block or accentuate the effects of a protein known as cytokines, hormones, vaccines with peptides, new forms of immune suppression used in transplant patients, and biologics that block specific parts of the immune system.

Glossary

ACR
American College of Rheumatology, a professional association of 5,000 U.S. rheumatologists, of whom 3,800 are board-certified; criteria, or definitions for many rheumatic diseases, are called the ACR criteria; Formerly known as the ARA (American Rheumatism Association)

Acute
Of short duration

Adrenal glands
Small organs located above the kidney that produce many hormones, including corticosteroids and epinephrine

Albumin
A protein that circulates in the blood and carries materials to cells

Albuminuria
A protein in urine

Analgesic
A drug that alleviates pain

Anemia
A condition resulting from low red blood cell counts

Antibodies
Special protein substances made by the body's white cells for the defense against bacteria and other foreign substances

Anticardiolipin antibody
An antiphospholipid antibody

Anticentromere antibody
Antibodies to a part of the cell's nucleus; associated with a form of scleroderma called CREST

Anti-DNA
Antibodies to DNA; seen in one half of people with SLE and sometimes associated with disease flares and kidney disease

Anti-ENA
Extractable nuclear antibodies that largely consist of anti-Sm and anti-RNP antibodies

Antigen
Self or foreign substance that stimulates antibody formation

Antiinflammatory
An agent that counteracts or suppresses inflammation

Antimalarials
Drugs originally used to treat malaria but that are helpful for lupus, such as hydroxychloroquine, chloroquine, and quinacrine

Antinuclear antibodies (ANAs)
Proteins in the blood that react with the nuclei of cells; seen in 96% of patients with SLE, 5% of healthy individuals, and in most patients with autoimmune diseases

Antiphospholipid antibody
Antibodies to a constituent of cell membranes; seen in one third of patients with SLE; in the presence of a cofactor, these antibodies can alter

clotting and lead to strokes, blood clots, miscarriages, and low platelet counts; also detected as the lupus anticoagulant

Anti-RNP

Antibody to ribonucleoprotein; seen in SLE and mixed connective tissue disease

Anti-Sm

Anti-Smith antibody; is found only in lupus

Anti-SSA

Antibody associated with Sjögren syndrome, sun sensitivity, neonatal lupus, and congenital heart block; also called the Ro antibody

Anti-SSB

Antibody almost always seen with anti-SSA; also called the La antibody

Apoptosis

Programmed cell death—a normal process for ridding the body of damaged cells

Artery

A blood vessel that transports blood from the heart to the tissues

Arthralgia

Pain in a joint

Arthritis

Inflammation of a joint

Aspirin

An antiinflammatory drug with analgesic properties

Autoantibody

An antibody to one's own tissues or cells

Autoimmune

Allergy to one's own tissues

Autoimmune hemolytic anemia

See hemolytic anemia .

B lymphocyte or B cell

A white blood cell that makes antibodies

Biopsy

Removal of a bit of tissue for examination under the microscope

Bursa

A sac of synovial fluid between tendons, muscles, and bones that promotes easier movement

Butterfly rash

Reddish facial eruption over the bridge of the nose and cheeks, resembling a butterfly in flight

Capillaries

Small blood vessels connecting between arteries and veins

Cartilage

Tissue material covering bone; the nose, outer ears, and trachea primarily consist of cartilage

Chronic

Persisting over a long period of time

CNS

Central nervous system

Collagen

Structural protein found in bone, cartilage, and skin

Collagen vascular disease

Antibody-mediated inflammatory process of the connective tissues, especially the joints, skin, and muscle; also called connective tissue disease

Complement

A group of proteins that are activated, promote, and are consumed during inflammation

Complete blood count (CBC)

A blood test that measures the amount of red blood cells, white blood cells, and platelets in the body

Connective tissue

The glue that holds muscles, skin, and joints together

Corticosteroid

Any natural anti-inflammatory hormone made by the adrenal cortex; also can be made synthetically

Cortisone

A synthetic corticosteroid

Creatinine

A blood test that measures kidney function

Creatinine clearance

A 24-hour urine collection that measures kidney function

CREST syndrome

A form of limited scleroderma characterized by C (calcium deposits under the skin), R (Raynaud phenomenon), E (esophageal dysfunction), S (sclerodactyly or tight skin), and T (a rash called telangiectasia)

Crossover syndrome

An autoimmune process that has features of more than one rheumatic disease (e.g., lupus and scleroderma)

Cutaneous

Relating to the skin

Cytokine

A group of chemicals that signal cells to perform certain actions

Dermatologist

A physician specializing in skin diseases

Dermatomyositis

An autoimmune process directed against muscles associated with skin rashes

Discoid lupus

A thick, plaque-like rash seen in 20% of patients with SLE; if patients have the rash but not SLE, they are said to have cutaneous (discoid) lupus erythematosus

DNA

Deoxyribonucleic acid; the body's building blocks; a molecule responsible for the production of all the body's proteins

Enzyme

A protein that accelerates chemical reactions

Erythematous

Reddish hue

Estrogen

Female hormone produced by the ovaries

Exacerbations

Symptoms reappear; a flare

False-positive serologic test for syphilis

A blood test that reveals an antibody that may be found in syphilis and is falsely positive in 15% of patients with SLE; associated with the lupus anticoagulant and antiphospholipid antibodies

FANA

Another term for ANA

Fibrositis or fibromyalgia

A pain amplification syndrome characterized by fatigue, a sleep disorder, and tender points in the soft tissues; can be caused by steroids and mistaken for lupus, although 20% of patients with lupus have fibrositis

Flare

Symptoms reappear; another word for exacerbation

Gene

Consisting of DNA, it is the basic unit of inherited information in our cells

Glomerulonephritis

Inflammation of the glomerulus of the kidney; seen in one third of patients with lupus

Hematocrit

A measurement of red blood cell levels; low levels produce anemia

Hemoglobin

Oxygen-carrying protein of red blood cells; low levels produce anemia

Hemolytic anemia

Anemia caused by premature destruction of red blood cells because of antibodies to the red blood cell surface; also called autoimmune hemolytic anemia

Hepatitis

Inflammation of the liver

Hormones

Chemical messengers made by the body that include thyroid, steroids, insulin, estrogen, progesterone, and testosterone

Human leukocyte antigen (HLA)

Molecules inside the macrophage that binds to an antigenic peptide; controlled by genes on the sixth chromosome; they can amplify or perpetuate certain immune and inflammatory responses

Immune complex

An antibody and antigen together

Immunity

The body's defense against foreign substances

Immunofluorescence

A means of detecting immune processes with a fluorescent stain and a special microscope

Immunosuppressive

A medication, such as cyclophosphamide or azathioprine, that treats lupus by suppressing the immune system

Inflammation

Swelling, heat, and/or redness resulting from the infiltration of white blood cells into tissues

Kidney biopsy

Removal of a bit of kidney tissue for microscopic analysis

La antibody

A Sjögren antibody; also called anti-SSB

LE cell
Specific cell found in blood specimens of most patients with lupus
Ligament
A tether attaching bone to bone and giving them stability
Lupus anticoagulant
A means of detecting antiphospholipid antibodies from prolonged clotting times
Lupus vulgaris
Tuberculosis of the skin; not related to systemic or discoid lupus
Lymphocyte
Type of white blood cell that fights infection and mediates the immune response
Macrophage
A cell that kills foreign material and presents information to lymphocytes
Major histocompatibility complex (MHC)
In humans, it is the same as HLA
Mixed connective-tissue disease
When a patient who carries the anti-RNP antibody has features of more than one autoimmune disease
Natural killer cell
A white blood cell that kills other cells
Nephritis
Inflammation of the kidney
Neutrophil
A granulated white blood cell involved in bacterial killing and acute inflammation
NSAIDs
Nonsteroidal anti-inflammatory drugs, agents that fight inflammation by blocking the actions of prostaglandin; examples include ibuprofen and naproxen
Nucleus
The center of a cell that contains DNA
Orthopedic surgeon
A doctor who operates on musculoskeletal structures
Pathogenic
Causing pathology, or abnormal reactions
Pathology
Abnormal cellular or anatomic features
Pericardial effusion
Fluid around the sac of the heart
Pericarditis
Inflammation of the pericardium
Pericardium
A sac lining the heart
Photosensitivity
Sensitivity to ultraviolet light
Plasma
The fluid portion of blood
Plasmapheresis
Filtration of blood plasma through a machine to remove proteins that may aggravate lupus
Platelet
A component of blood responsible for clotting
Pleura
A sac lining the lung
Pleural effusion
Fluid in the sac lining the lung
Pleuritis
Irritation or inflammation of the lining of the lung
Polyarteritis
A disease closely related to lupus that features inflammation of small- and medium-sized blood vessels
Polymyalgia rheumatica
An autoimmune disease of the joints and muscles seen in older patients with high sedimentation rates who have severe aching in their shoulders, upper arms, hips, and upper legs
Polymyositis
An autoimmune disease that targets muscles
Prednisone; prednisolone
synthetic steroids
Protein
A collection of amino acids; antibodies are proteins
Proteinuria
Excess protein levels in the urine; also called albuminuria
Pulse steroids
Giving very high doses of corticosteroids intravenously over 1 to 3 days to critically ill patients
Raynaud's disease
Isolated Raynaud phenomenon not part of any other disease
Raynaud's phenomenon
Discoloration of the hands or feet (they turn blue, white, or red, especially with cold) as a feature of an autoimmune disease
RBC
Red blood cell count
Remission
Quiet period, free from symptoms, but not necessarily a cure
Rheumatic disease
Any of 150 disorders affecting the immune or musculoskeletal system; approximately 30 of these also are autoimmune
Rheumatoid arthritis
Chronic disease of the joints marked by inflammatory changes in the joint-lining membranes, which may have positive rheumatoid factor and ANA tests
Rheumatoid factor
Autoantibodies that react with immunoglobulin G (IgG) that are seen in most patients with rheumatoid arthritis and 30% of patients with SLE
Rheumatologist
An internal medicine specialist who has completed at least a 2-year fellowship studying rheumatic diseases
Ro antibody
See anti-SSA
Scleroderma
An autoimmune disease featuring rheumatoid-like inflammation, tight skin, and vascular problems (e.g., Raynaud)
Sedimentation rate
Test that measures the precipitation of red cells in a column of blood; high rates usually indicate increased disease activity
Serum
Clear liquid portion of the blood after removal of clotting factors
Sjögren syndrome
Dry eyes, dry mouth, and arthritis observed with most autoimmune disorders or by itself (i.e., primary Sjögren)
Steroids
Usually a shortened term for corticosteroids, which are anti-inflammatory hormones produced by the adrenal cortex or synthetically
STS
False-positive serologic test for syphilis
Synovial fluid
Joint fluid
Synovitis
Inflammation of the tissues lining a joint
Synovium
Tissue that lines the joint
Systemic
Pertaining to or affecting the body as a whole
T cell
A lymphocyte responsible for immunologic memory
Temporal arteritis
Inflammation of the temporal artery associated with high sedimentation rates, systemic symptoms, and occasionally loss of vision
Tendon
Structures that attach muscle to bone
Thrombocytopenia
Low platelet counts
Thymus
A gland in the neck area responsible for immunologic maturity
Titer
Amount of a substance, such as ANA
Tolerance
The failure to make antibodies to an antigen
Uremia
Marked kidney insufficiency frequently necessitating dialysis to stay alive
Urinalysis
Analysis of urine
Urine, 24-hour collection
All urine passed in a 24-hour period is collected and examined for protein and creatinine to determine how well the kidneys are functioning
UV light
Ultraviolet light; its spectrum includes UVA (320–400 nm), UVB (290–320 nm), and UVC (200–290 nm) wavelengths
Vasculitis
Inflammation of blood vessels
WBC
White blood cell count

Appendix II: Lupus Resource Materials

Compiled by Jenny Thorn Allan on Behalf of the Lupus Foundation of America

What Organizations Provide Patient Support in the United States?

(Many such organizations exist; only those with a budget of over $1 million are listed.)

Lupus Foundation of America, Inc. (LFA), 2000 L. St. NW, Suite 710, Washington, DC 20036, 202–349–1155 or toll-free 800–558–0121, Spanish line, 800–558–0231. With a nationwide network of 267 chapters, branches and support groups, the LFA is the nation's leading nonprofit voluntary health organization dedicated to finding the causes and cure for lupus. The LFA's mission is to improve the diagnosis and treatment of lupus; support individuals and families affected by the disease; increase awareness of lupus among health professionals and the public; and find the causes and cure. The LFA publishes patient education brochures, fact sheets, articles, and a national magazine, *Lupus Now*®. E-mail: lupusinfo@lupus.org for health education information; info@lupus.org for general information; lupusnow@lupus.org for magazine information. Web site: http://www.lupus.org/.

Arthritis Foundation, P.O. Box 7669, Atlanta, GA 30357, toll-free 800–568-4045. The Arthritis Foundation is the only national not-for-profit organization that supports the more than 100 types of arthritis and related conditions with advocacy, programs, services and research. Chapters and support groups across the country provide research monies, publish literature, and offer patient support for arthritis and related conditions such as lupus. Web site: http://www.arthritis.org/.

American Autoimmune Related Diseases Association (AARDA), 22100 Gratiot Ave. E. Detroit, MI 48021, 586–776–3900 (national office); 750 17th Street, NW, Suite 1100, Washington, DC 20006, 202–466–8511 (Washington office). The AARDA is dedicated to the eradication of autoimmune diseases and the alleviation of suffering and the socioeconomic impact of autoimmunity through fostering and facilitating collaboration in the areas of education, research, and patient services in an effective, ethical and efficient manner. Literature request line: 800-598-4668; E-mail: aarda@aol.com; Web site: http://www.aarda.org/.

SLE Lupus Foundation, 330 Seventh Ave., Suite 1701, New York, NY 10001, 212–685–4118 or toll-free 800–74LUPUS (5–8787); Headquartered in New York City and Los Angeles, the SLE Lupus Foundation provides patient services, education, public awareness, and funding for lupus research. E-mail: lupus@lupusny.org; Web site: http://www.lupusny.org/.

In Addition to the above, Where Else Can Reliable Information about Lupus Be Obtained?

American College of Rheumatology (ACR) and Association of Rheumatology Health Professionals (ARHP), 1800 Century Place, Suite 250, Atlanta, GA 30345, 404–633–3777. The ACR is the professional organization to which nearly all U.S. and many international rheumatologists belong. The ARHP is a division of the ACR. Web site for both: http://www.rheumatology.org/.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), 31 Center Drive, Room 4C05, MSC 2350, Rockville Pike, Bethesda, MD 20892. Part of the National Institutes of Health, NIAMS funds $90 million dollars in lupus research each year at the Bethesda campus and elsewhere in the country. The Institute also sponsors conferences and publishes patient education pamphlets and booklets, including specific materials on lupus. Some materials are available in Spanish. E-mail: NIAMSWeb-L@mail.nih.gov; Web site: http://www.niams.nih.gov/.

The NIAMS Information Clearinghouse offers a wide variety of information on rheumatic diseases, including lupus (also information in Spanish). NIAMS Information Clearinghouse, National Institutes of Health, 1 AMS Circle, Bethesda, MD 20892, 301–495–4484 or toll-free 877–22-NIAMS. TTY 301–565–2966. E-mail: niamsinfo@mail.nih.gov. (Please include your mailing address and, if possible, a telephone number in your e-mail message.)

In Addition to the above, What Other Organizations Fund Lupus Research?

(Many such organizations exist; this list is restricted to those that give more than $1 million a year to lupus-related research at more than one institution.)

Alliance for Lupus Research, 28 West 44th Street, Suite 1217, New York, NY 10036 212–218–2840 or toll-free

800-867-1743, was founded in 1999 with the mission to support research into the cause, cure, treatment and prevention of systemic lupus erythematosus. E-mail: info@lupusresearch.org; Web site: http://www.lupusresearch.org/.

Lupus Research Institute, 149 Madison Ave., Suite 205, New York, NY 10016, 212-685-4118, was founded in 1998 to conquer lupus through identifying its cause, creating effective treatments and, ultimately, developing a cure. E-mail: lupus@lupusresearchinstitute.org; Web site: http://www.lupusresearchinstitute.org/.

Research and Education Foundation of the American College of Rheumatology. The research funding arm of the ACR funds rheumatology training and research programs that are vital to the care of patients suffering from rheumatic diseases. To find out more about the REF's programs, go to http://www.rheumatology.org/ref/, send an e-mail to ref@rheumatology.org or call 404-633-3777.

Rheuminations, Inc., 221 East 48th Street. New York, NY 10017, 212-593-5180, is a private, nonprofit foundation that has provided funding for lupus-related research and education to various institutions, including the Hospital for Special Surgery's LANtern (Lupus Asian Network) Program and Charla de Lupus Teen Chat Program, and its Barbara Volcker Center for Women and Rheumatic Disease. Web site: http://www.dxlupus.org/.

How Can I Find Out about Lupus Support Outside of the United States?

The Lupus Foundation of America is associated with approximately 75 international lupus groups. Call the LFA for information, or go to the LFA Web site for an up-to-date list. Some important groups are:

Canada

Lupus Canada (the national organization), 590 Alden Road, Suite 211, Markham, Ontario L3R 8N2 Canada. Phone: 905-513-0004; toll-free in Canada 1-800-661-1468; E-mail: lupuscanada@bellnet.ca; Web site: http://www.lupuscanada.org/ (in French and English).

Europe

European Lupus Erythematosus Federation. All efforts in Europe are coordinated through a central office of the ELEF. Affiliate groups are located in Belgium, Finland, France, Germany, Great Britain, Iceland, Ireland, Israel, Italy, Netherlands, Norway, Portugal, Spain, Sweden, and Switzerland. E-mail: elef@rheumanet.org; Web site: http://www.elef.rheumanet.org/.

Mexico

Fundación Mexicana de Lupus, c/o Dr. J. Humberto Orozco-Medina, Club de Lupus Centro Medico de Occidente, Pedro Buezeta 870-B, Tavascos 3469-306, 44660 Guadalajara, Jalisco, Mexico. Phone: 5233-1201-9790. E-mail: info@lupusmexico.org; Web site: http://www.lupusmexico.org/.

Latin America

Panamerican League of Associations For Rheumatology, University of Arkansas for Medical Sciences, 4301 West Markham, Division of Rheumatology, Slot 509, Little Rock, AR 72205. E-mail: Donato-Debra@Cooperhealth.edu. Web site: http://www.panlar.org/.

United Kingdom

Lupus UK, St James House, Eastern Road, Romford, Essex RM1 3NH England, 44-1708-731-251; E-mail: headoffice@lupusuk.org.uk; Web site: http://www.lupusuk.org.uk/.

How Can I Find Out about Organizations That Serve Patients With Lupus-Related Disorders?

Fibromyalgia Network, P.O. Box 31750, Tucson, AZ 85751 supports research through the American Fibromyalgia Syndrome Association. Call toll-free 800-853-2929; Web site: http://www.fmnetnews.com/.

Scleroderma Foundation, 300 Rosewood Drive, Suite 105, Danvers, MA 01923, Phone: 978-463-5843, toll-free 800-722-HOPE (4673); E-mail: sfinfo@scleroderma.org; Web site: http://www.scleroderma.org/.

Sjogren's Syndrome Foundation, 8120 Woodmont Ave., Suite 530, Bethesda MD 20814. Phone: 301-718-0300, toll-free 800-475-6473. Web site: http://www.sjogrens.com/.

What Are the Best Books on Lupus Written by Nonphysicians?

Phillips, RH. *Coping with Lupus*. Revised and Updated Ed. (Avery/Penguin Putnam, 2001). Written by an eminent psychologist and popular speaker, it is the best book on the subject. Available from the LFA and in bookstores.

Phillips RH. *Successful Living with Lupus: An Action Workbook.* Revised and Updated Ed. (Balance Enterprises, 2005). Learn to improve your emotional and social well-being, by emphasizing your positive potential. Also available as a CD-ROM. Available from the LFA.

Lupus Foundation of America, Inc. *Loopy Lupus Helps Tell Scott's Story About a Disease Called Lupus.* Written by a third-grade class after a classmate was diagnosed with lupus, this book is brightly colored and charmingly illustrated by the boy's sister. Includes information for adults on the left-hand pages with Scott's story in his own words on the right-hand pages. Available from the LFA.

Hospital for Special Surgery (HSS) Department of Patient Care and Quality Management. *For Inquiring Teens with Lupus: Our Thoughts, Issues & Concerns* (HSS 2003). This colorful teen-speak booklet is available free of charge by calling the Charla de Lupus (Lupus Chat) Program toll-free at 866-812-4494 or 212-606-1958 within New York City.

Hospital for Special Surgery (HSS) Department of Patient Care and Quality Management. *What Chinese-Americans*

and Their Families Should Know about Lupus (HSS 2003). This bilingual booklet is available free of charge by calling the Lupus Asian Network (LANtern) toll-free at 866–505–2253.

Are There Other Books About Lupus and Related Diseases Written by Physicians?

Lahita RG, Phillips RH. *Lupus Q&A: Everything You Need to Know* (Avery Press, 2004). This is the revised and updated version of the popular 1998 book, *Lupus: Everything You Need to Know.*

Lahita RG, Yalof I. *Woman and Autoimmunity: Your Body Betrayed* (Regan Press, 2004). Written in the style of a novel, this book details the lives of 15 women and their adventures with autoimmune disease, with lupus being one of the diseases highlighted. Additionally, the author explains the body's immune system and how autoimmune diseases happen.

Wallace DJ, Wallace JB. *Making Sense of Fibromyalgia* (Oxford University Press, 1999). This complex autoimmune disease is found in 20 percent of people with lupus, making this book a popular companion text to books about lupus.

Blau SP, Schultz D. *Living with Lupus: The Complete Guide*. 2nd Ed. Revised and Updated (Da Capo Press/Perseus Books Group, 2004). The authors discuss medications and therapies, current research directions, and potential disease complications. The book also offers tips for day-to-day coping, plus information on dealing with decisions on pregnancy and hormone replacement. Lupus in children and teens, possible causes of the disease, and the doctor-patient relationship also are covered.

Lehman TJA. *It's Not Just Growing Pains: A Guide to Childhood Muscle, Bone, and Joint Pains, Rheumatic Diseases, and the Latest Treatments* (Oxford University Press, 2004). In this comprehensive resource guide for parents and professionals, the author offers easy-to-understand information on the causes, symptoms, tests and treatments for a variety of rheumatic diseases and childhood pain.

Wallace DJ, Wallace JB, *Fibromyalgia: An Essential Guide For Patients and Their Families* (NY/London: Oxford University Press, 2003). In clear and accessible language the authors provide a concise explanation of the syndrome and its symptoms, and also outline the recent advances in treatments. This guide offers expert advice to sufferers and gives them the education they need to get the help they require.

Wallace DJ, Editor, *The New Sjogren's Syndrome Handbook* (3rd Ed.) (NY/London: Oxford University Press, 2005). This comprehensive and authoritative guide has been revised and expanded to provide readers with medical and practical information that brings together the current thinking about the disease. The book illuminates the major clinical aspects of the syndrome in an easily readable and understandable manner and is loaded with practical tips and advice to assist those seeking information.

Wallace DJ, Clauw DJ, *Fibromyalgia and Other Central Pain Syndromes* (Philadelphia: Lippincott Williams & Wilkins, 2005). This volume is the first comprehensive text devoted to fibromyalgia and other centrally mediated chronic pain syndromes. Chapters discuss the definition, epidemiology, and pathophysiology of chronic pain and fibromyalgia, the clinical presentations of fibromyalgia syndrome, and central sensitization syndromes associated with chronic neuromuscular pain. The contributors thoroughly examine various approaches to evaluation and management of patients with fibromyalgia and chronic pain. Other chapters focus on disability issues, prognosis, and future research directions. A critically reviewed listing of Web sites and other resources is included.

Wallace DJ, *The Lupus Book: A Guide for Patients and Their Families* (3rd Ed.) (NY/London: Oxford University Press, 2005). This revised and expanded edition is suitable for the motivated patient wanting a concise, practical overview of their disease. It contains new sections relating to disability, economic impact of the disease, biologics and other new drug treatments, clinical measures of disease activity, clinical trial methods, and proactive treatment strategies, while sections relating to inflammation and the causes of lupus have been significantly updated.

What about Rheumatology or Lupus Textbooks?

Wallace DJ, Hahn BH. *Dubois' Lupus Erythematosus*. 7th Ed. (Lippincott, Williams & Wilkins, 2007). The definitive text on the topic, this book can be ordered from the publisher's Web site: http://www.lww.com/.

Lahita RG. *Systemic Lupus Erythematosus.* 4th Ed. (Academic Press, 2004). Another excellent lupus textbook.

National Institutes of Health/NIAMS. *Lupus: A Patient Care Guide for Nurses and Other Health Professionals.* (National Institutes of Health/NIAMS, 2001, Bethesda, MD) A replacement for Terry Nass's Lupus Erythematosus: A Handbook for Nurses (1985). Books are available at no charge from the NIAMS Information Clearinghouse; see listing above.

The best general rheumatology textbooks are:

Hochberg MC, Silman AJ, Smolen JS, et al, eds. *Rheumatology.* Two-volume set, 3rd Ed. (Mosby 2003; previous edition, 1997, by J.H. Klippel).

Kelley WN, et al. *Textbook of Rheumatology.* Two-volume set, 7th Ed. E. Harris, ed. (WB Saunders, 2005; previous edition, 1997).

Klippel JH, ed. *Primer on the Rheumatic Diseases.* 12th Ed. (National Book Network, 2001).

Koopman WJ, Moreland LW. *Arthritis and Allied Conditions: A Textbook of Rheumatology.* Two-volume set. 15th Ed. (Lippincott, Williams & Wilkins, 2004).

Koopman WJ, Boulware DW, Heudebert GR, eds. *Clinical Primer of Rheumatology* (Lippincott, Williams & Wilkins, 2003).

Maddison PJ, Isenberg DA, Woo P, et al, eds. *Oxford Textbook of Rheumatology.* 3rd Ed. (Oxford Medical Publications, Oxford Press, 2004).

Appendix III: The Medical Outcome Survey Short-Form General Health Survey

Jennifer Grossman

Caroline Gordon

- In general, would you say your health is:

  1 = Excellent

  2 = Very good

  3 = Good

  4 = Fair

  5 = Poor

- *Compared to one year ago*, how would you rate your health in general now?

  1 = Much better now than one year ago

  2 = Somewhat better now than one year ago

  3 = About the same as one year ago

  4 = Somewhat worse now than one year ago

  5 = Much worse now than one year ago

- The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

  1 = Yes, limited a lot

  2 = Yes, limited a little

  3 = No, not limited at all

  - *Vigorous activities,* such as running, lifting heavy objects, participating in strenuous sports
  - *Moderate activities,* such as moving a table, pushing a vacuum cleaner, bowling, or playing golf
  - Lifting or carrying groceries
  - Climbing *several* flights of stairs
  - Climbing *one* flight of stairs
  - Bending, kneeling, or stooping
  - Walking *more than one mile*
  - Walking *several blocks*
  - Walking *one block*
  - Bathing or dressing yourself

- During the *past 4 weeks,* have you had any of the following problems with your work or other regular daily activities *as a result of your physical health*? 1 = Yes, 2 = No
  - Cut down on the *amount of time* you spent on work or other activities
  - *Accomplished less* than you would like
  - Were limited in the *kind* of work or other activities
  - Had difficulty performing the work or other activities (for example, it took extra effort)
- During the *past 4 weeks,* have you had any of the following problems with your work or other regular daily activities *as a result of any emotional problems* (such as feeling depressed or anxious) 1 = Yes, 2 = No
  - Cut down on the *amount of time* you spent of work or other activities
  - *Accomplished less* than you would like
  - Didn't do work or other activities as *carefully* as usual
- During the *past 4 weeks*, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

  1 = Not at all
  2 = Slightly
  3 = Moderately
  4 = Quite a bit
  5 = Extremely

- How much bodily pain have you had during the past 4 weeks?

  1 = None
  2 = Very mild
  3 = Mild
  4 = Moderate
  5 = Severe
  6 = Very severe

- During the *past 4 weeks* how much did pain interfere with your normal work (including both work outside the home and housework)?

  1 = Not at all
  2 = A little bit
  3 = Moderately
  4 = Quite a bit
  5 = Extremely

- These questions are about how you feel and how things have been with you *during the past 4 weeks*. For each question, please give one answer that comes closest to the way you have been feeling.

  1 = All of the time
  2 = Most of the time
  3 = A good bit of the time
  4 = Some of the time
  5 = A little of the time
  6 = None of the time

  How much of the time during the *past 4 weeks* ….

  - Did you feel full of pep?
  - Have you been a very nervous person?
  - Have you felt so down in the dumps that nothing could cheer you up?
  - Have you felt calm and peaceful?
  - Did you have a lot of energy?
  - Have you felt downhearted and blue?
  - Did you feel worn out?
  - Have you been a happy person?
  - Did you feel tired?

- During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting your friends, relatives, etc.)?

  1 = All of the time
  2 = Most of the time
  3 = Some of the time
  4 = A little of the time
  5 = None of the time

- How **TRUE** or **FALSE** is each of the following statements for you?

  1 = Definitely true
  2 = Mostly true
  3 = Don't know
  4 = Mostly false
  5 = Definitely false

  - I seem to get sick a little easier than other people.
  - I am as healthy as anybody I know.
  - I expect my health to get worse.
  - My health is excellent.

From
Stewart AL, Hays RD, Ware JE Jr. The MOS short-form general health survey. Reliability and validity in a patient population. *Med Care* 1988;26(7):724–735, with permission.

Color Plates

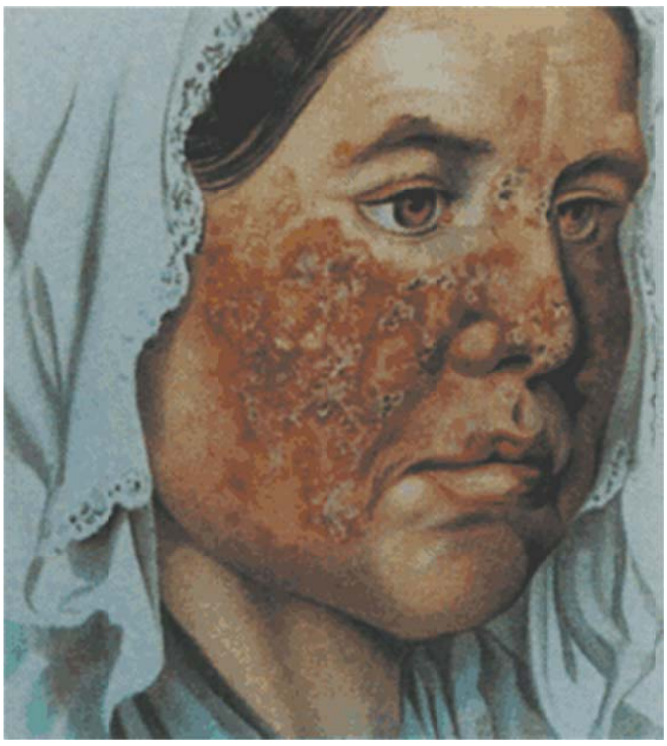
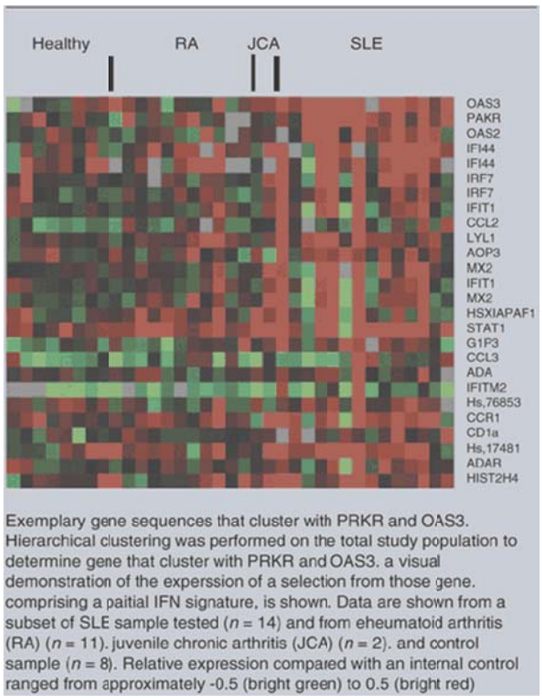


FIGURE 1.1. (See black and white image)

FIGURE 1.2. (See black and white image)

FIGURE 1.3. (See black and white image)



Exemplary gene sequences that cluster with PRKR and OAS3. Hierarchical clustering was performed on the total study population to determine gene that cluster with PRKR and OAS3. a visual demonstration of the experssion of a selection from those gene. comprising a paitial IFN signature, is shown. Data are shown from a subset of SLE sample tested ($n = 14$) and from eheumatoid arthritis (RA) ($n = 11$). juvenile chronic arthritis (JCA) ($n = 2$). and control sample ($n = 8$). Relative expression compared with an internal control ranged from approximately -0.5 (bright green) to 0.5 (bright red)

FIGURE 10.1. (See black and white image)

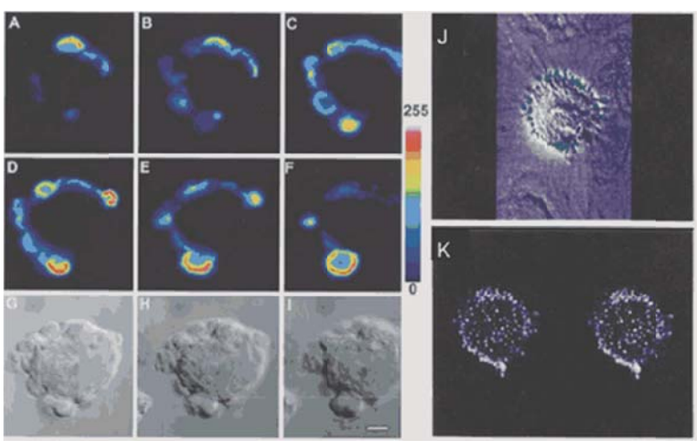


FIGURE 13.3. (See black and white image)

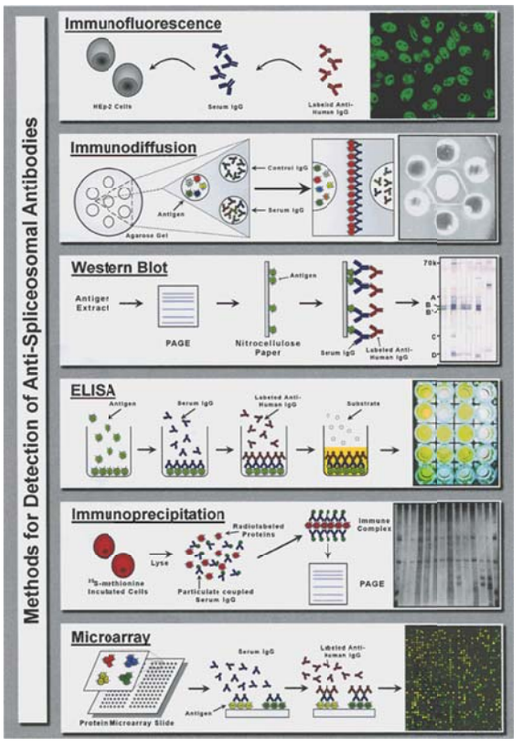


FIGURE 26.2. (See black and white image)

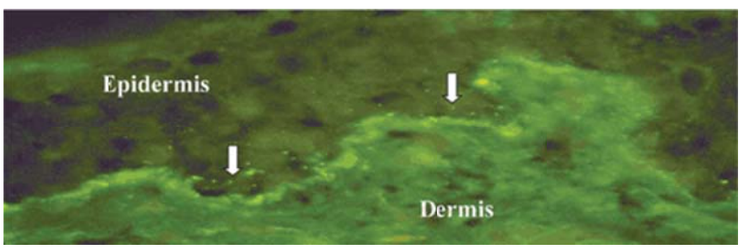


FIGURE 29.4. (See black and white image)

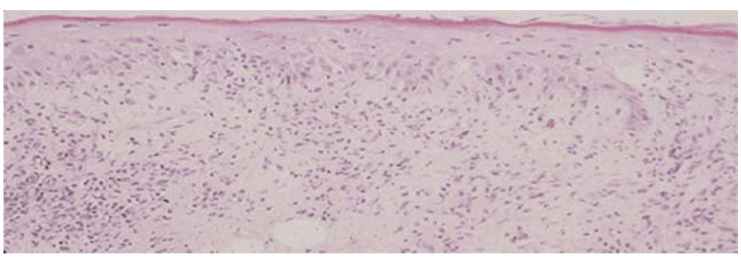


FIGURE 29.5. (See black and white image)

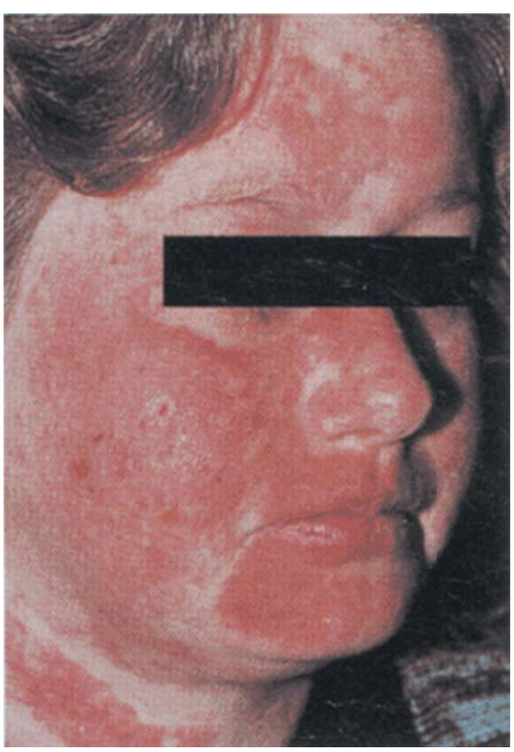


FIGURE 30.1. (See black and white image)

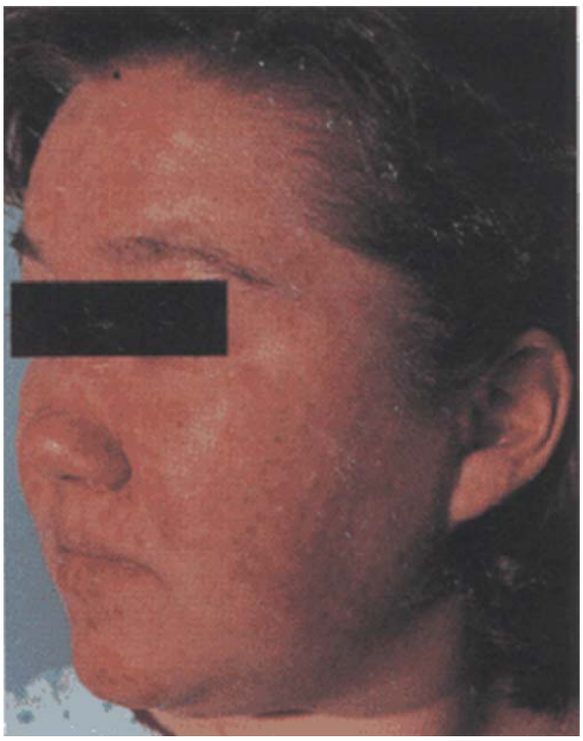


FIGURE 30.2. (See black and white image)

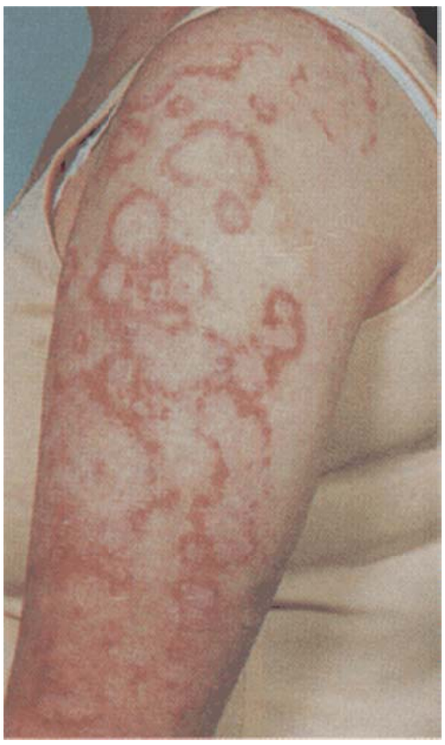


FIGURE 30.7. (See black and white image)

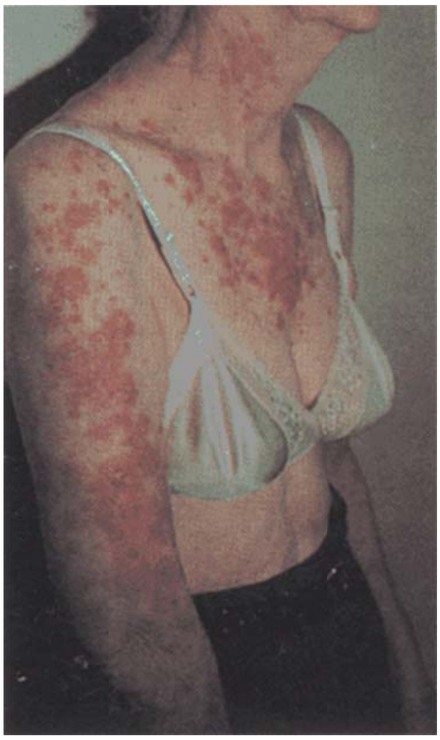


FIGURE 30.8. (See black and white image)

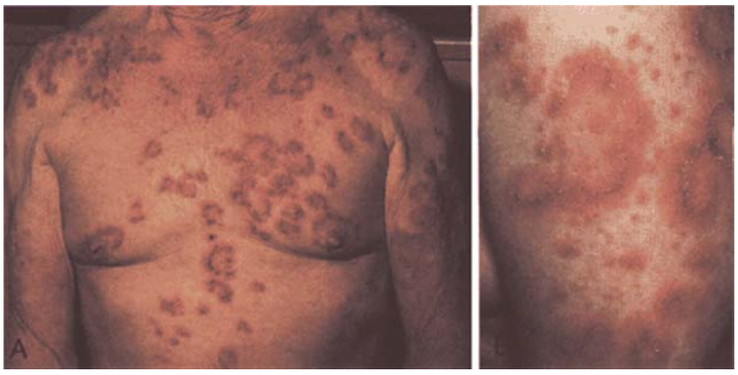


FIGURE 30.12. (See black and white image)

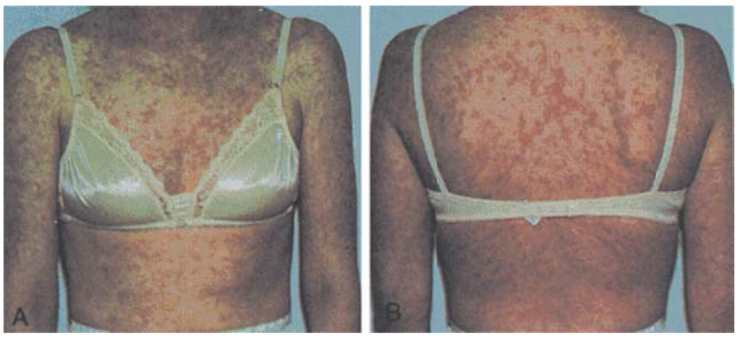


FIGURE 30.13. (See black and white image)

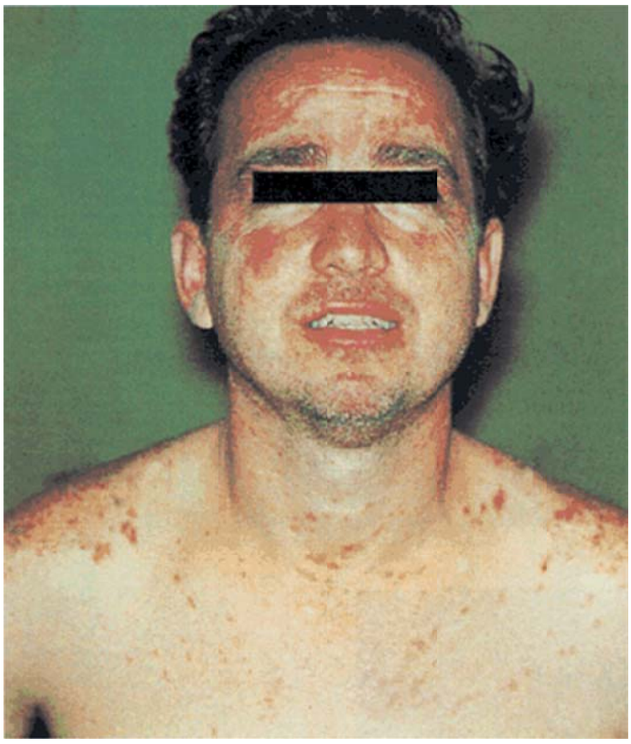


FIGURE 30.15. (See black and white image)

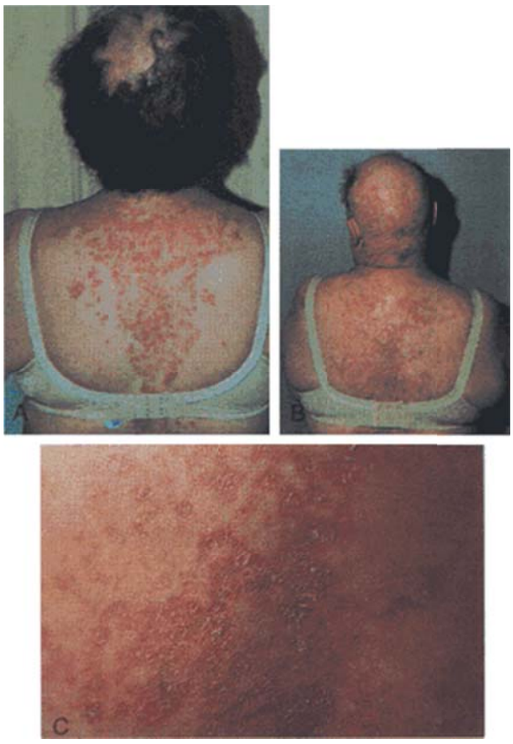


FIGURE 30.16. (See black and white image)

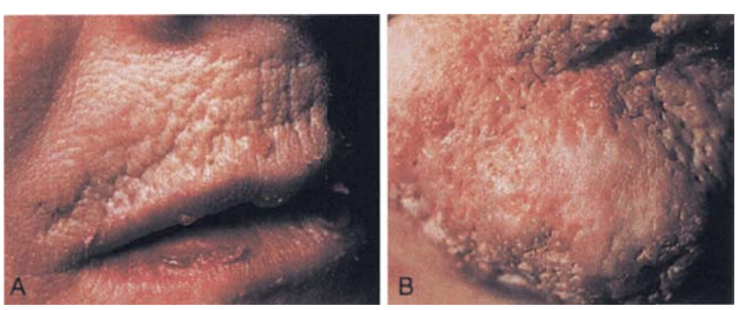


FIGURE 30.23. (See black and white image)

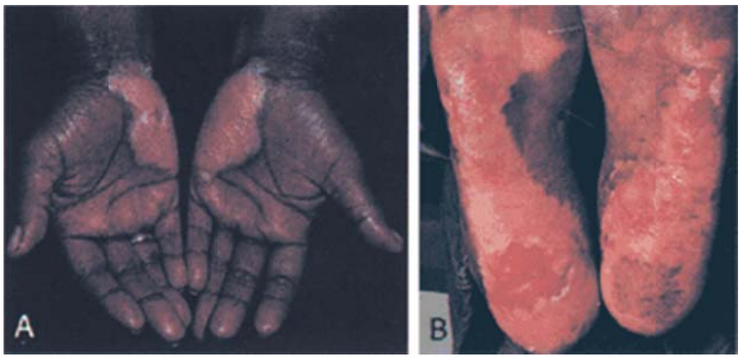


FIGURE 30.28. (See black and white image)

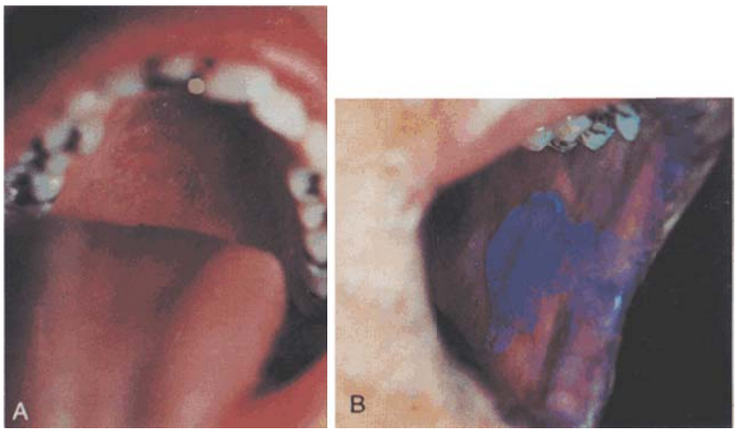


FIGURE 30.34. (See black and white image)

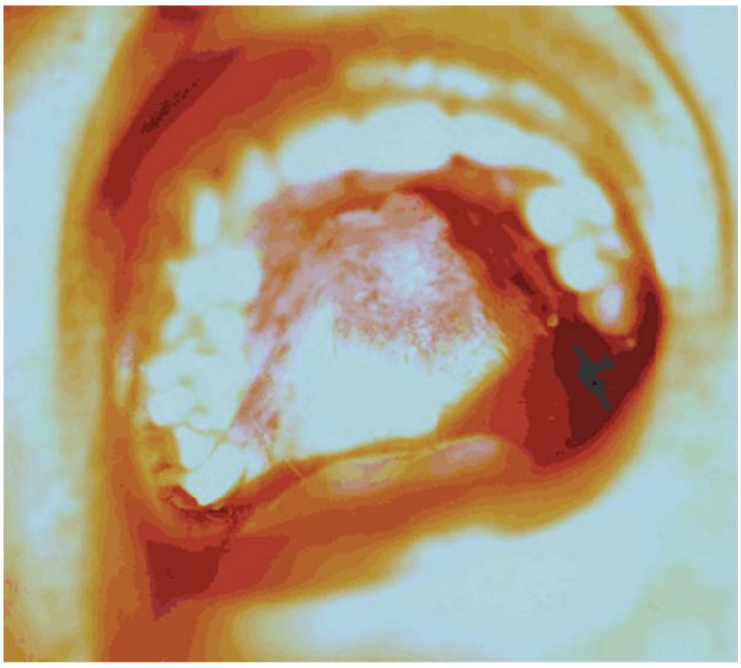


FIGURE 31.1. (See black and white image)

FIGURE 31.2. (See black and white image)

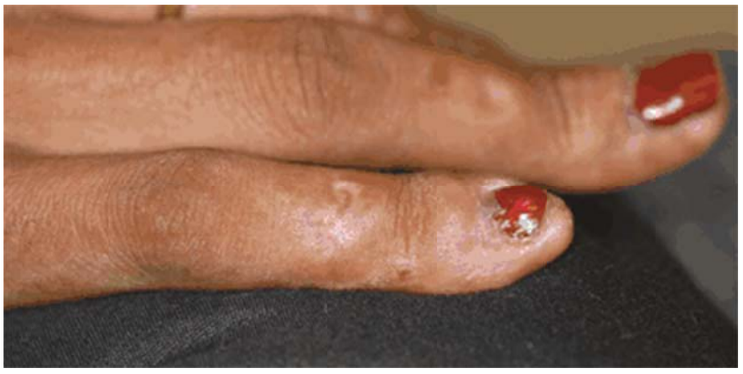


FIGURE 31.3. (See black and white image)

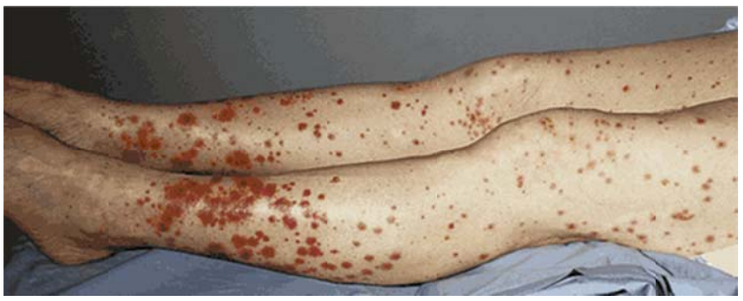


FIGURE 31.4. (See black and white image)

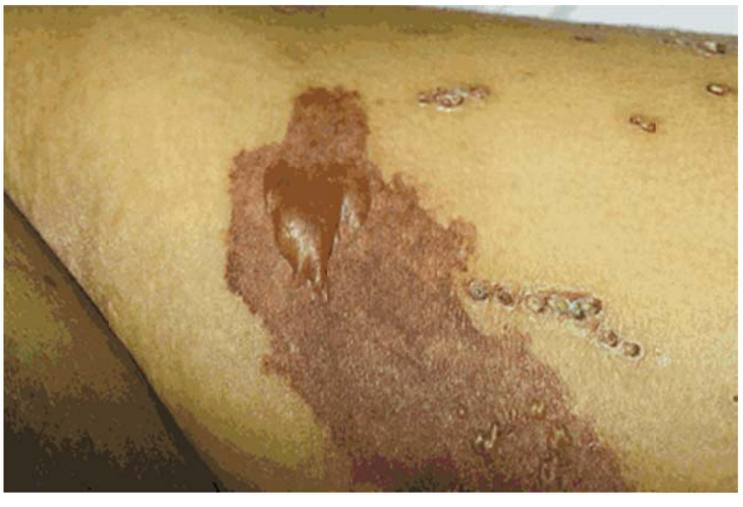
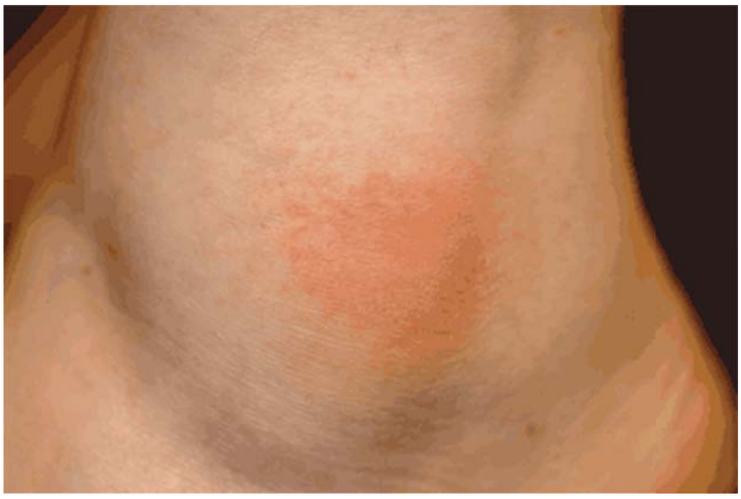


FIGURE 31.5. (See black and white image)



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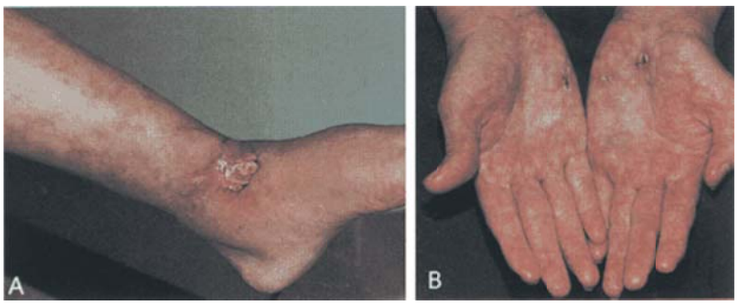

FIGURE 31.7. (See black and white image)

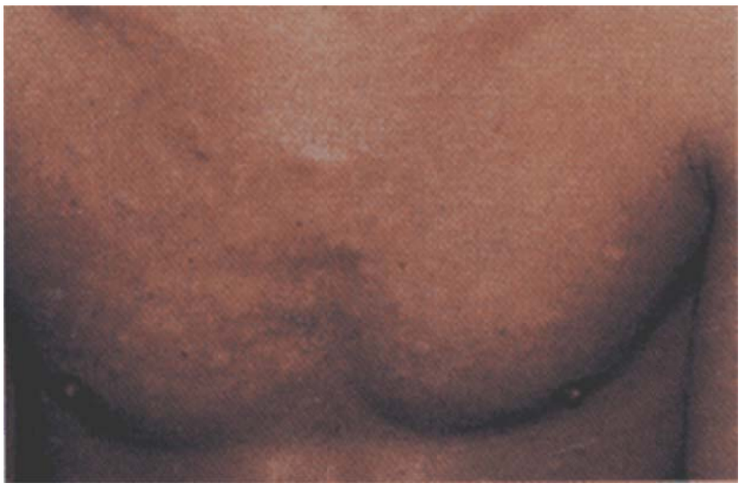

FIGURE 31.8. (See black and white image)

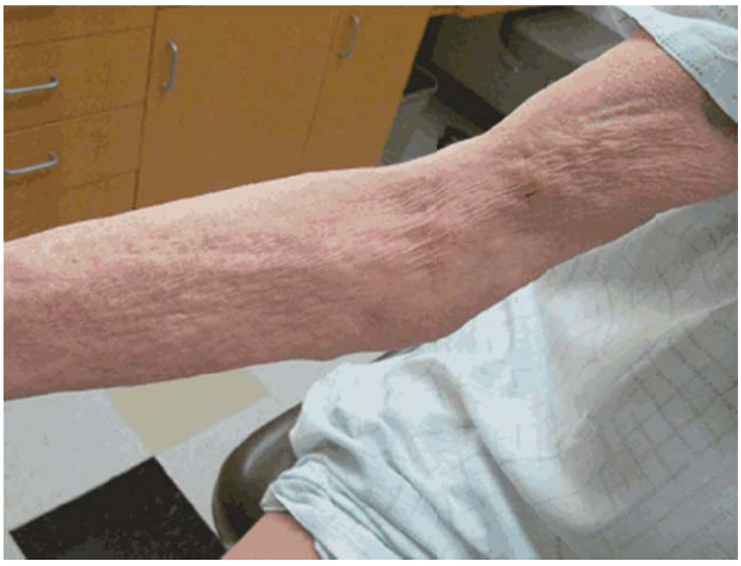

FIGURE 31.9. (See black and white image)

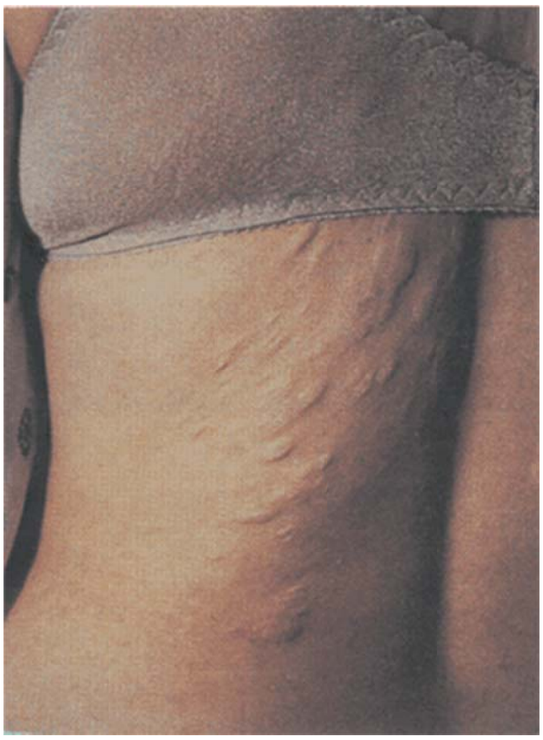

FIGURE 31.11. (See black and white image)

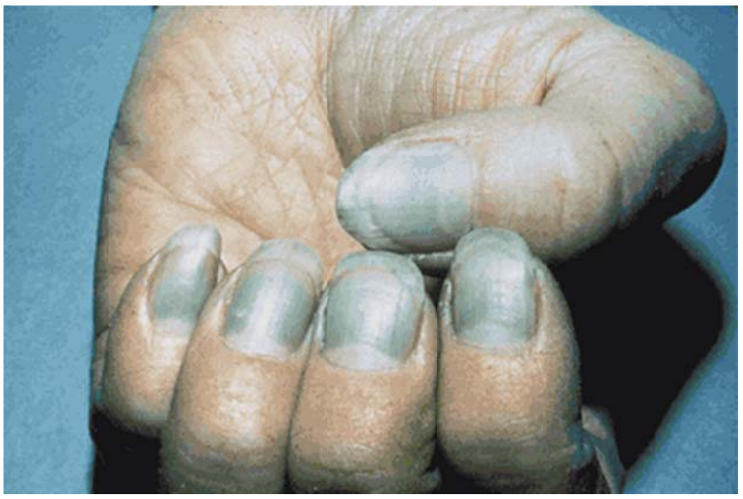

FIGURE 31.12. (See black and white image)

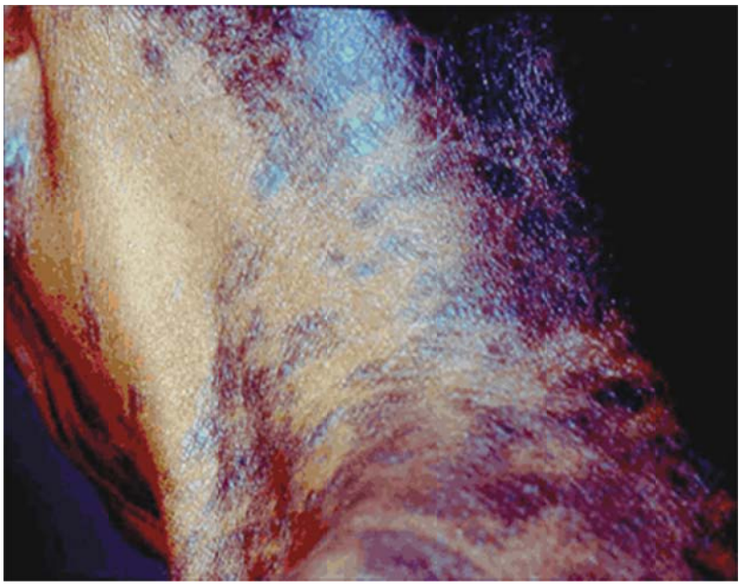

FIGURE 31.13. (See black and white image)

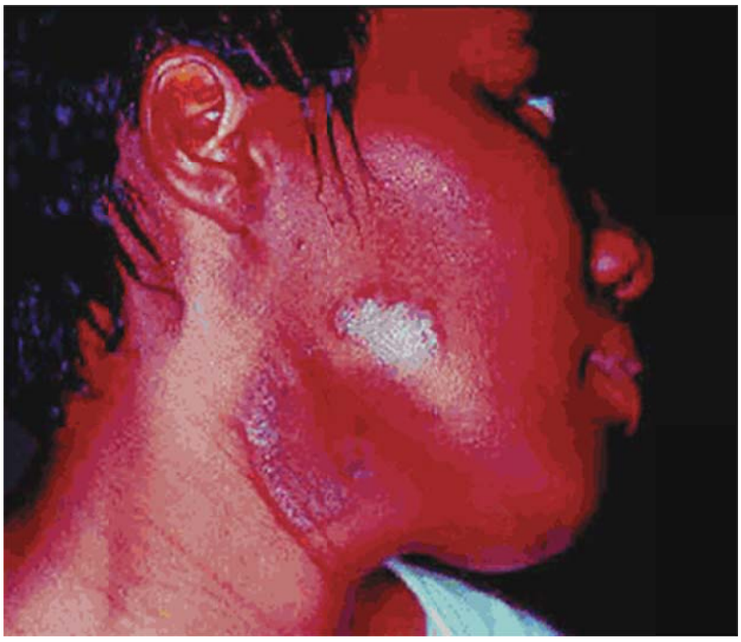

FIGURE 31.14. (See black and white image)

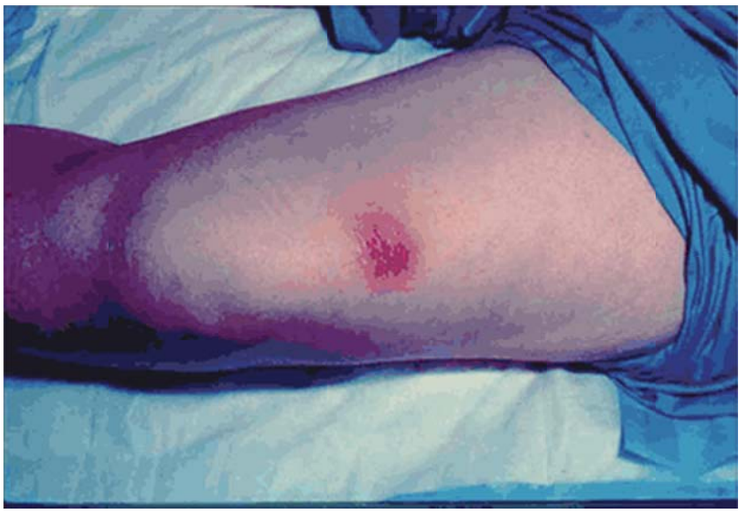

FIGURE 31.15. (See black and white image)

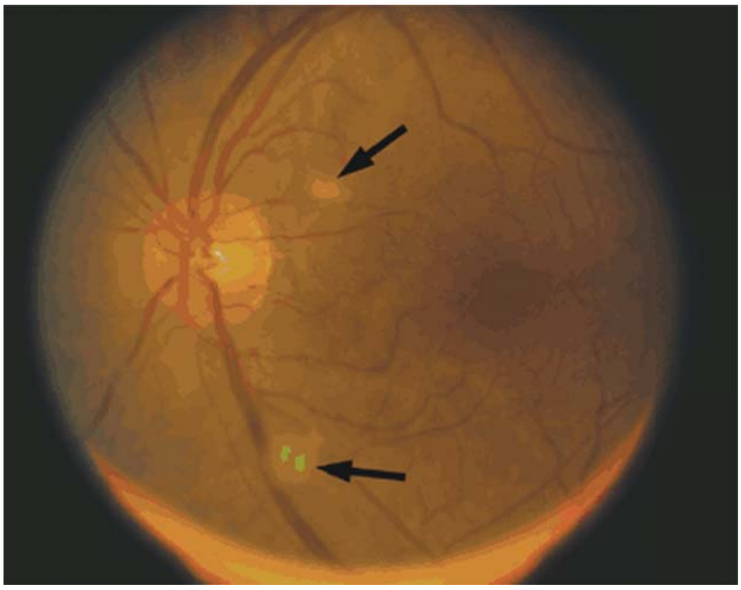

FIGURE 40.1. (See black and white image)

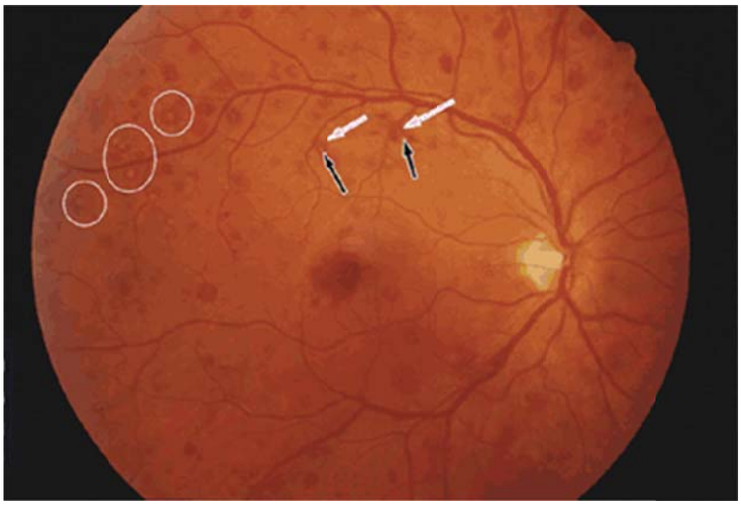

FIGURE 40.2. (See black and white image)

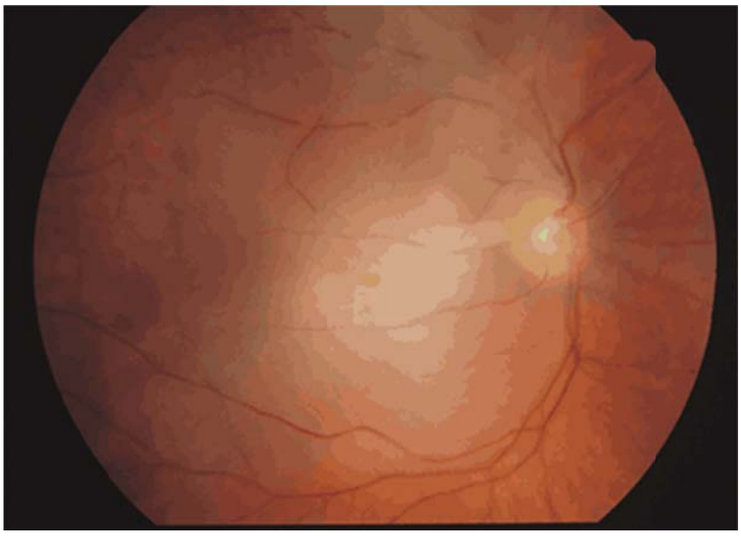

FIGURE 40.3. (See black and white image)

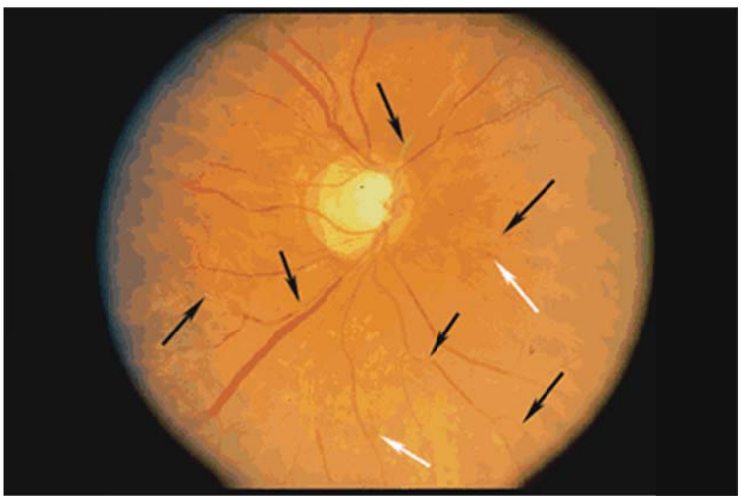

FIGURE 40.4. (See black and white image)

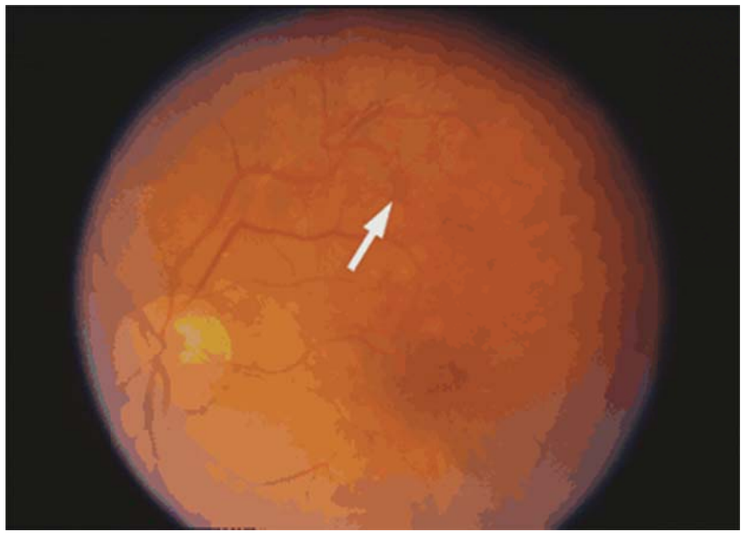

FIGURE 40.5. (See black and white image)

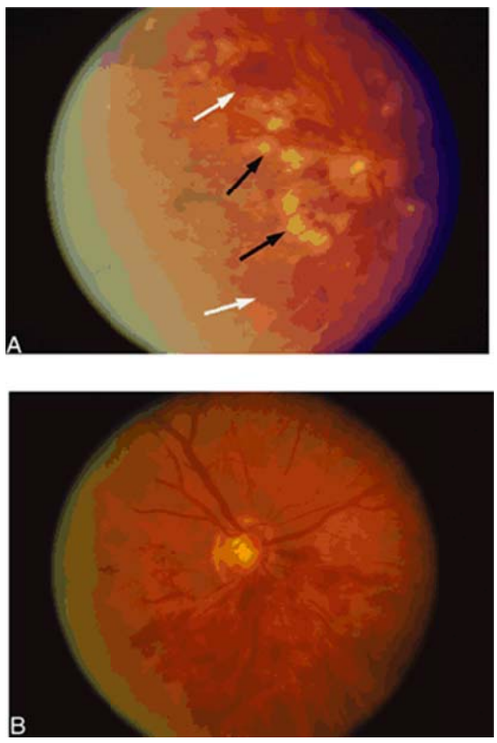

FIGURE 40.6. (See black and white image)

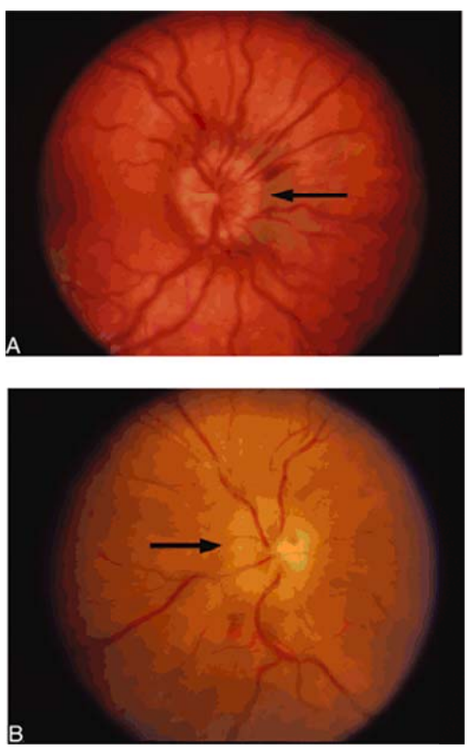

FIGURE 40.7. (See black and white image)

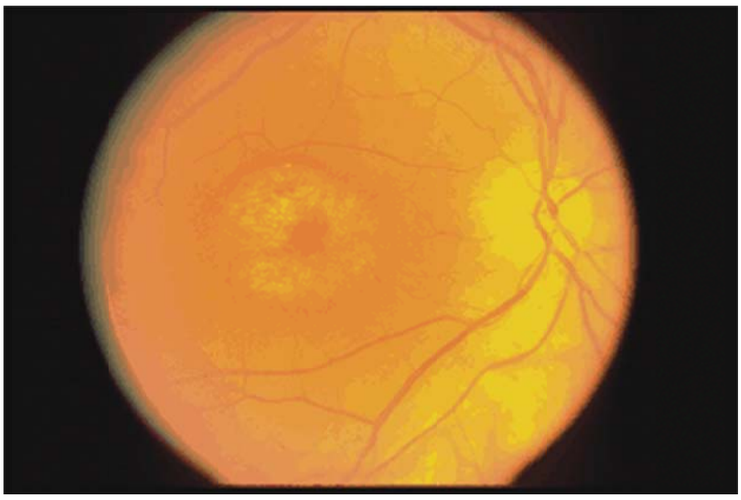

FIGURE 40.8. (See black and white image)

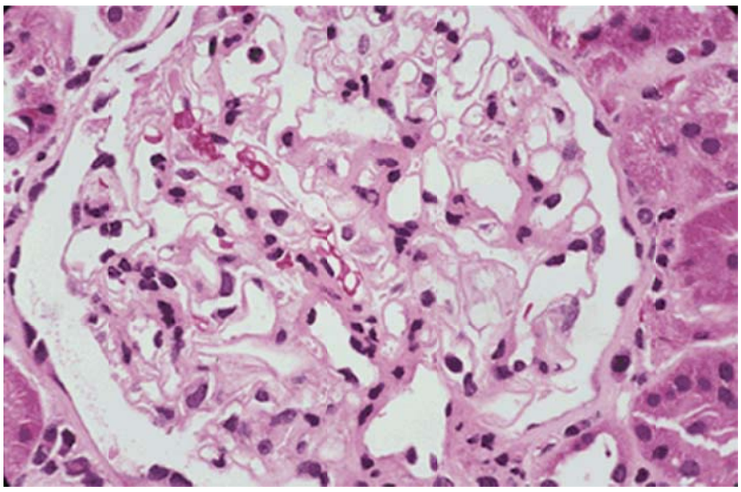

FIGURE 55.1. (See black and white image)

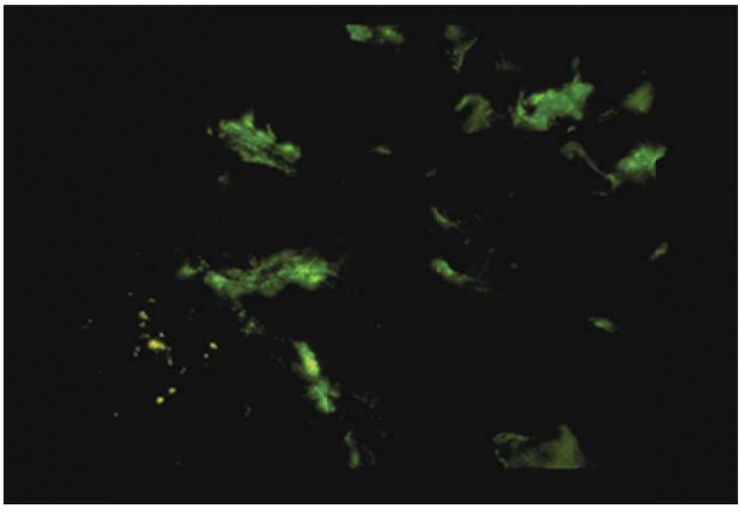

FIGURE 55.2. (See black and white image)

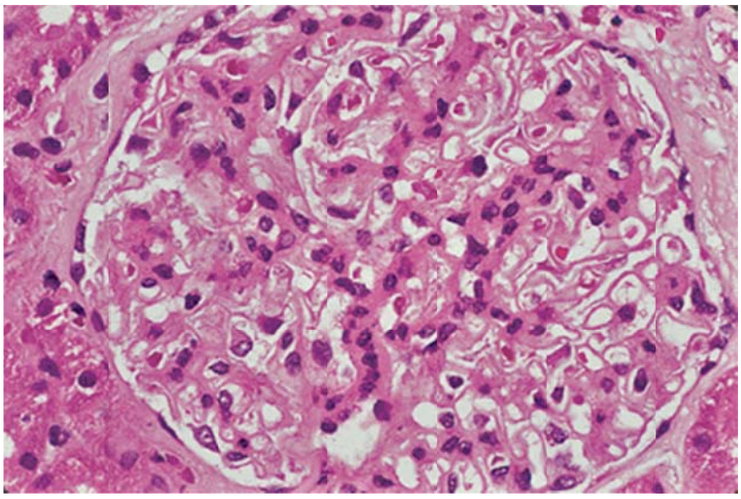

FIGURE 55.3. (See black and white image)

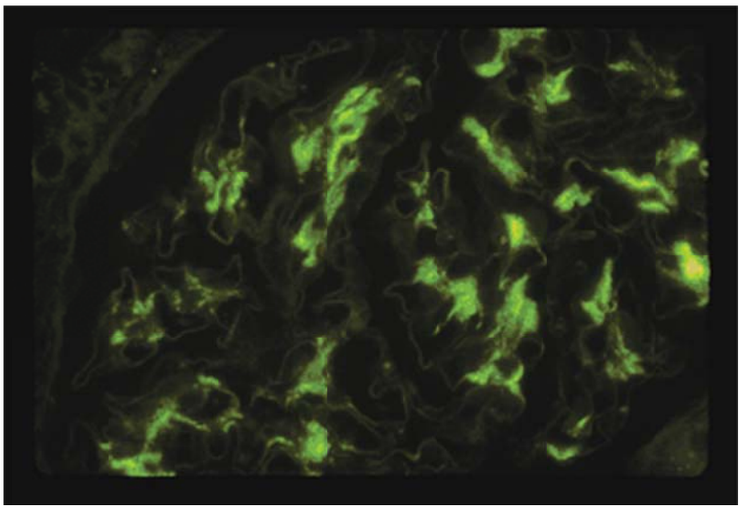

FIGURE 55.4. (See black and white image)

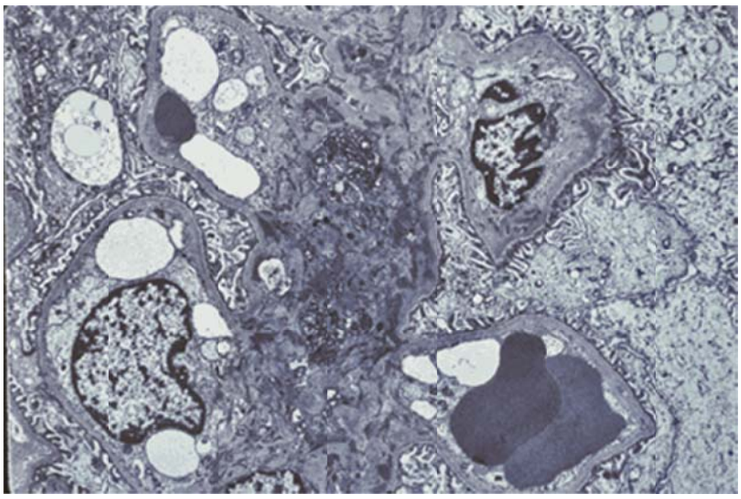

FIGURE 55.5. (See black and white image)

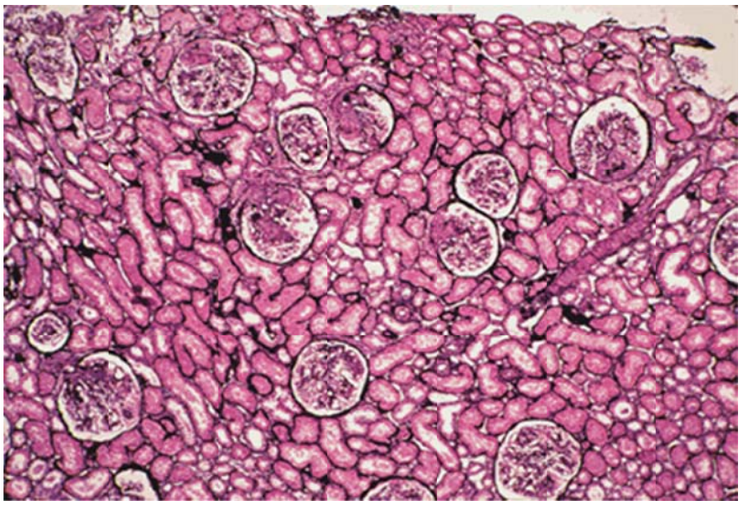

FIGURE 55.6. (See black and white image)

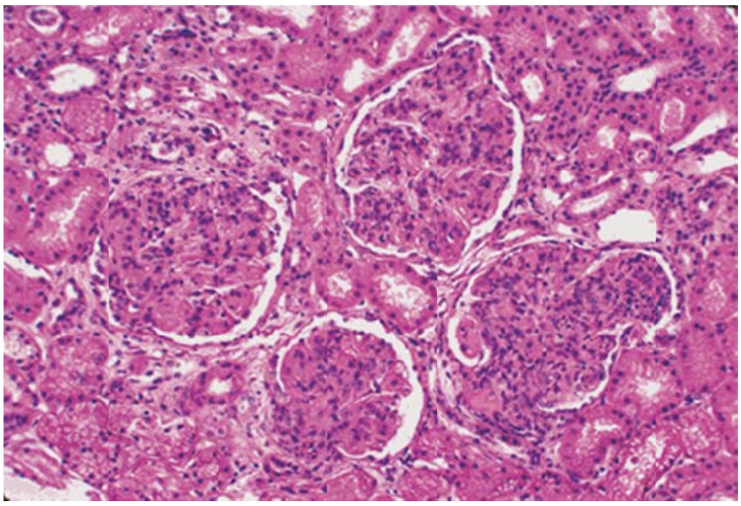

FIGURE 55.7. (See black and white image)

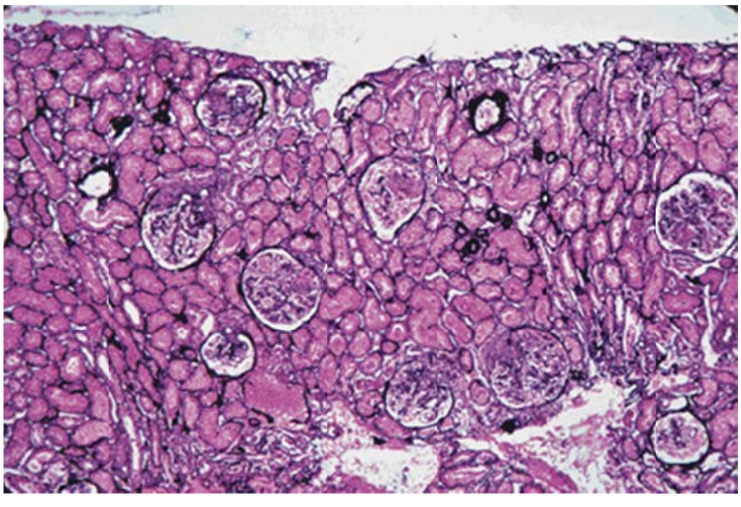

FIGURE 55.8. (See black and white image)

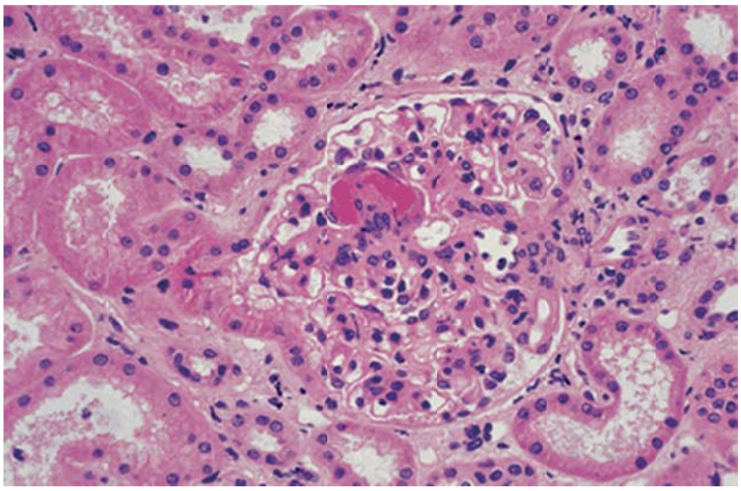

FIGURE 55.9. (See black and white image)

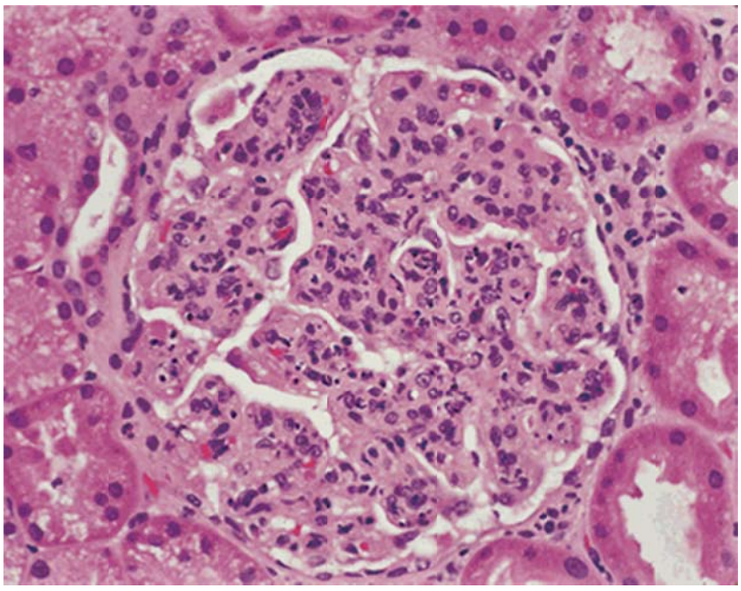

FIGURE 55.10. (See black and white image)

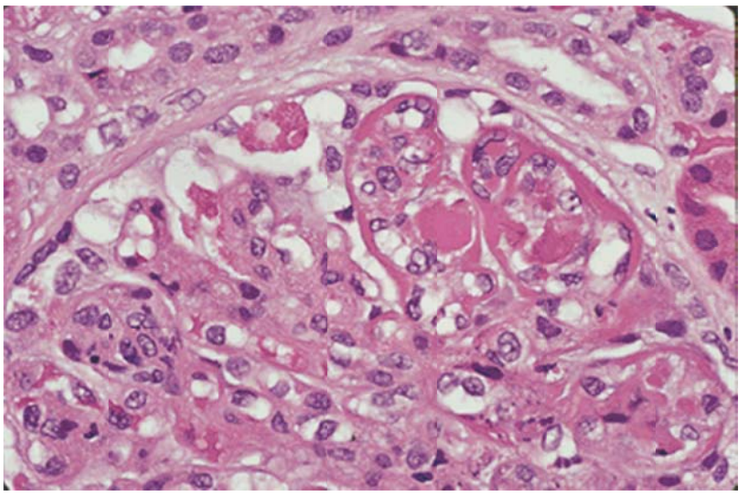

FIGURE 55.11. (See black and white image)

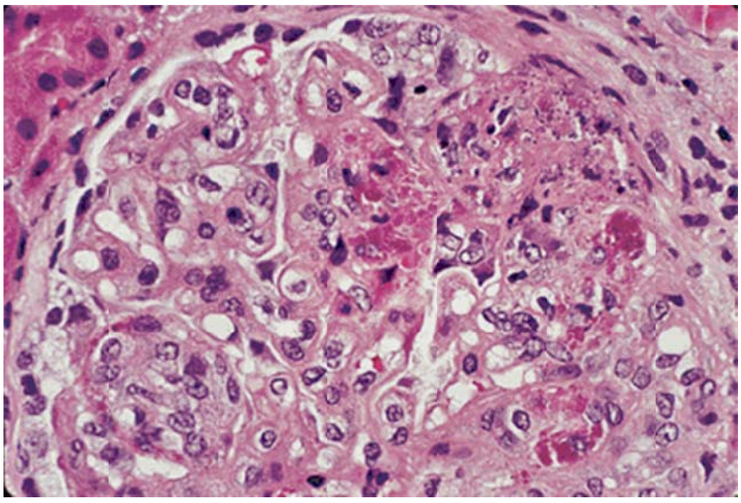

FIGURE 55.12. (See black and white image)

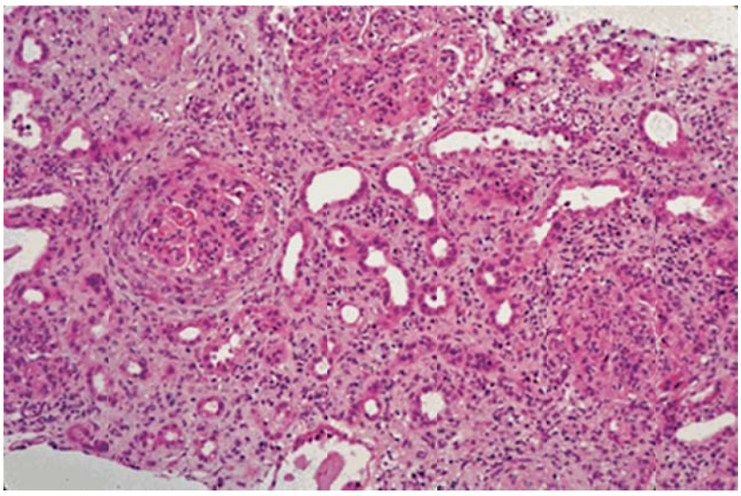

FIGURE 55.13. (See black and white image)

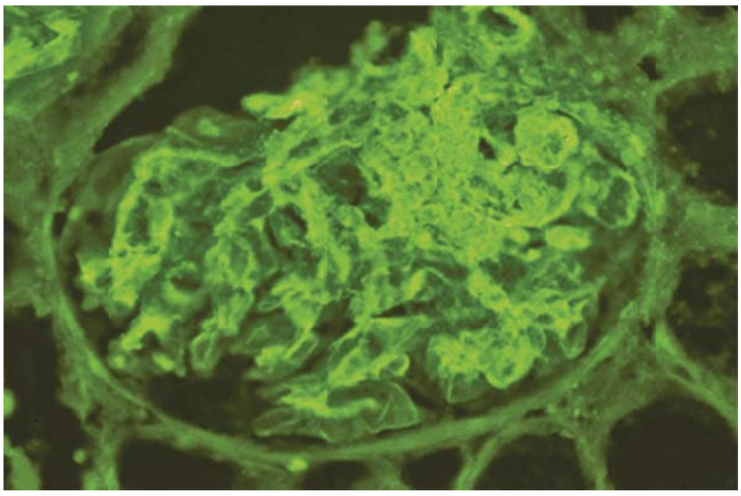

FIGURE 55.14. (See black and white image)

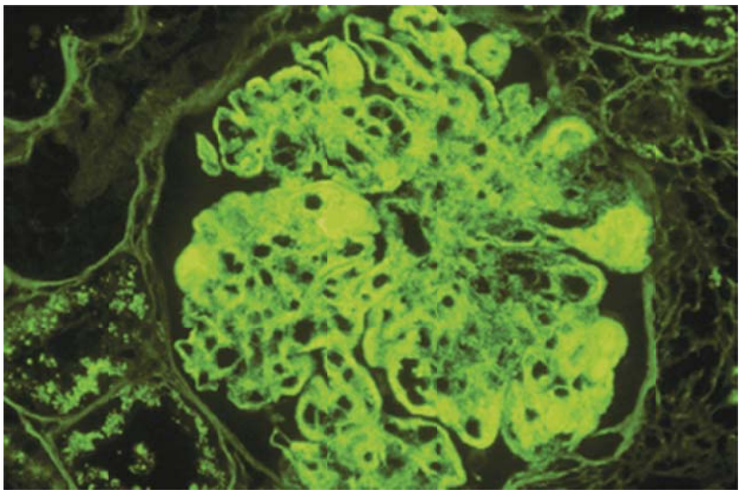

FIGURE 55.15. (See black and white image)

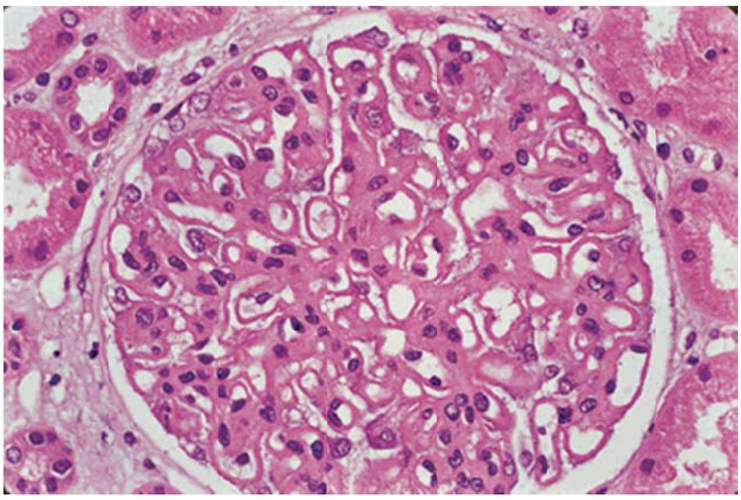

FIGURE 55.17. (See black and white image)

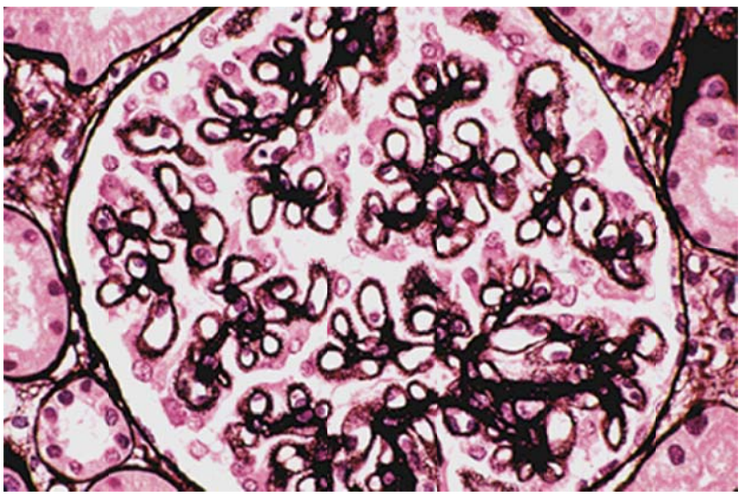

FIGURE 55.18. (See black and white image)

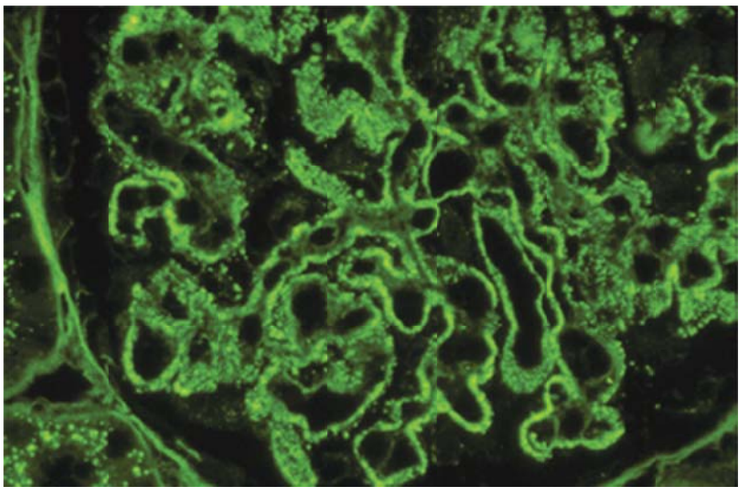

FIGURE 55.19. (See black and white image)

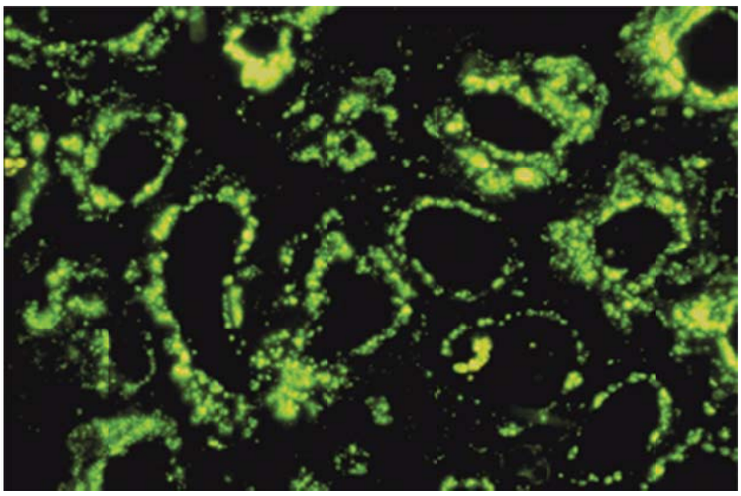

FIGURE 55.21. (See black and white image)

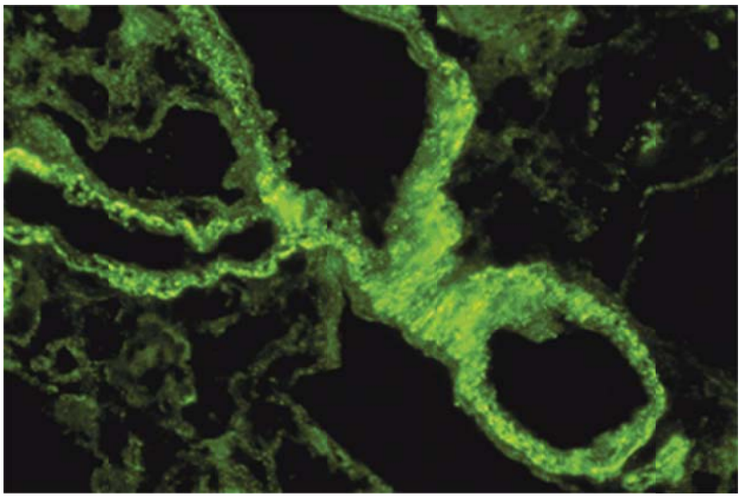

FIGURE 55.22. (See black and white image)

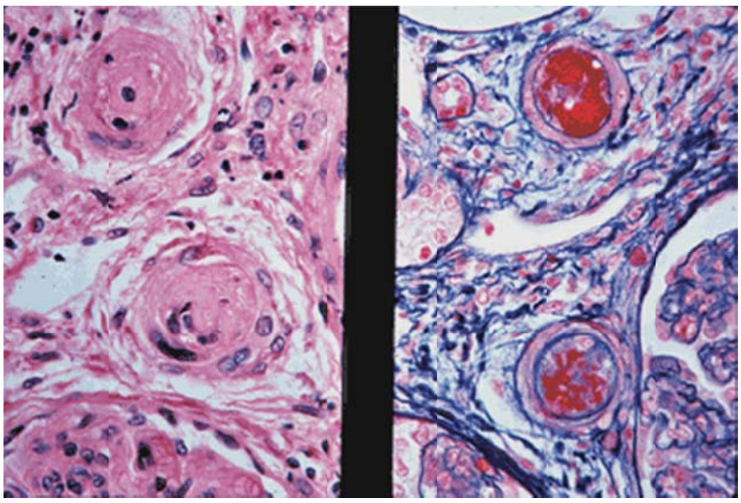

FIGURE 55.24. (See black and white image)

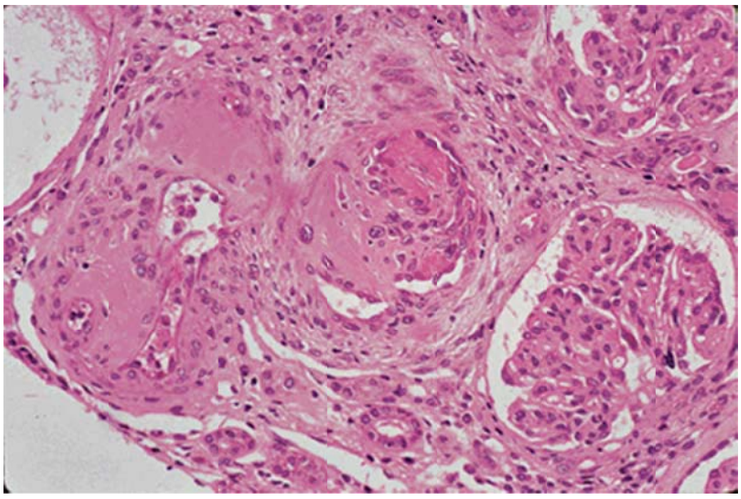

FIGURE 55.25. (See black and white image)

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