Annual Review of Immunology Volume 2 1984

Serendipity in Immunology
J. H. Humphrey. Vol. 2: 1–22

The Human T-Cell Receptor
S C Meuer, O Acuto, T Hercend, S F Schlossman, and E L Reinherz. Vol. 2: 23–50

The Major Histocompatibility Complex-Restricted Antigen Receptor on T Cells
K Haskins, J Kappler, and P Marrack. Vol. 2: 51–66

The Antigenic Structure of Proteins: A Reappraisal
D C Benjamin, J A Berzofsky, I J East, F R N Gurd, C Hannum, S J Leach, E Margoliash, J G Michael, A Miller, E M Prager, M Reichlin, E E Sercarz, S J Smith-Gill, P E Todd, and A C Wilson. Vol. 2: 67–101

Tumor-Associated Carbohydrate Antigens
Sen-itiroh Hakomori. Vol. 2: 103–126

Suppressor Cells and Immunoregulation
Martin E. Dorf and Baruj Benacerraf. Vol. 2: 127–157

Regulation of IgE Synthesis
K Ishizaka. Vol. 2: 159–182

Transplantation of Pancreatic Islets
P E Lacy, and J M Davie. Vol. 2: 183–198

Collagen Autoimmune Arthritis
J M Stuart, A S Townes, and A H Kang. Vol. 2: 199–218

Natural Suppressor (NS) Cells, Neonatal Tolerance, and Total Lymphoid Irradiation: Exploring Obscure Relationships
S Strober. Vol. 2: 219–237PDF (604K)

Transfer and Expression of Immunoglobulin Genes
S L Morrison, and V T Oi. Vol. 2: 239–256

Chemoattractant Receptors on Phagocytic Cells
R Snyderman, and M C Pike. Vol. 2: 257–281

The Cell Biology of Macrophage Activation
Dolph O. Adams and Thomas A. Hamilton. Vol. 2: 283–318

Interleukin 2
K A Smith. Vol. 2: 319–333

The Role of Arachidonic Acid Oxygenation Products in Pain and Inflammation
P Davies, P J Bailey, M M Goldenberg, and A W Ford-Hutchinson. Vol. 2: 335–357

Heterogeneity of Natural Killer Cells
J R Ortaldo, and R B Herberman. Vol. 2: 359–394

Antigen-Presenting Function of the Macrophage
E R Unanue. Vol. 2: 395–428

Immunobiology of Eosinophils
G J Gleich, and D A Loegering. Vol. 2: 429–459

Complement and Bacteria: Chemistry and Biology in Host Defense
K A Joiner, E J Brown, and M M Frank. Vol. 2: 461–492

CH Isotype 'Switching' During Normal B-Lymphocyte Development
J J Cebra, J L Komisar, and P A Schweitzer. Vol. 2: 493–548

Annual Reviews

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John Humphrey

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SERENDIPITY IN IMMUNOLOGY

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PART I: TRAINING IN IMMUNOLOGY

This is an essay about the role of luck, in one guise or another, in immunology. Since the Editors intend that the introductory essays should also be in some degree personal, before considering luck (as serendipity in a philosophical and historical context) I shall write about the good fortune that has enabled me to live through and even to take part in the adolescence of immunology as it grew to its present flourishing adulthood.

In last year's *Annual Review of Immunology,* Elvin Kabat discussed getting started 50 years ago. My beginning was 5 years later. I had been reading preclinical medicine at Cambridge, in the naive but currently accepted belief that having a medical qualification would qualify anyone to do medical research. Even more naively I believed that medical research was bound to be of some good to mankind—that it could escape perversion by the war which, even in the mid-1930s, many students feared would be inevitable unless the governments of the time were prepared to realize that *Mein Kampf* was to be taken literally. It was possible to take a year to do an advanced course (so-called Part II) in Biochemistry in a lively department that included not only Gowland Hopkins, but Joseph and Dorothy Needham, N. W. Pirie, Hans Krebs, Ernest Baldwin, Marjory Stephenson, Malcolm Dixon, Frank Young, and a group of research students among whom were two future Nobel Prize winners (R. L. M. Synge and L. F. Leloir). The message from this course was that enzymes and their specificity were the most exciting objects of study. Antibodies were never mentioned, but on reading J. R. Marrack's remarkable monograph on

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Annual Reviews

The Chemistry of Antigens and Antibodies (27a) I realized that antibodies had specificity comparable to enzymes and appeared much easier to obtain at will. Marrack's book, and W. W. C. Topley's section on "Immunity" in *Principles of Bacteriology and Immunity* (45), convinced me that immunology was what I wished to study. In order to do the three clinical years required for a medical qualification I went to University College Hospital Medical School (UCHMS). The Professor of Bacteriology was Ashley Miles—a superb teacher. Miles was working at the time on the antigens of *Br. melitensis*. He subsequently became Deputy Director and Head of the Biological Standards Division at the National Institute for Medical Research (NIMR), and later Director of the Lister Institute for Preventive Medicine. The Professor of Chemical Pathology at the Medical School was Charles Harington, later the Director of the NIMR. Also at UCHMS were Sir Thomas Lewis and George Pickering, interested in histamine as well as in heart disease, and the versatile experimental pathologist Roy Cameron, under whom was working Leonard Glynn. It was a small school by modern standards, but lively and friendly; and it did something quite exceptional at the time—namely, allow students who were keen enough to take part in the work of the departments. In this atmosphere it was possible to learn some of the techniques of immunology for which at the time there were no textbooks [the first edition of Kabat & Mayer's *Experimental Immunochemistry* (22a) appeared in 1948, and Landsteiner's revised classic *The Specificity of Serological Reactions* (26a) in 1945].

Harington, who had earlier synthesized thyroxine and glutathione, had become interested in immunochemistry and was testing the possibility of controlling the effects of hormones and some pharmacologicals by antisera raised against their protein conjugates. As Elvin Kabat pointed out in his chapter last year, accurate quantitative studies were possible even though it was not until 1939 that antibodies were recognized as being γ-globulins. Harington and his colleagues were able to show that rabbit antisera to thyroxine could prevent the metabolic effects of thyroxine in rats, and antisera to aspirin could prevent the antiphlogistic action of aspirin. He and Gordon Butler (later Director of the Chalk River Laboratory) were investigating antisera against stilbestrol when in 1939 the Second World War put an end to such esoteric research. It was almost 20 years later that research on antisera to hormones was taken up again by Erlanger (10a). A puzzle at the time was why some proteins, such as insulin and gelatin, were apparently not immunogenic. The concept of immunological tolerance had not been formulated, and Ehrlich's notion of "horror autotoxicus" was forgotten or not considered relevant. As an explanation for this non-immunogenicity Harington suggested that insulin lacked carbohydrate and gelatin lacked tyrosine. He assigned me the project of attaching carbobenzyloxyglucosidotyrosyl groups to these proteins and testing their immunogenicity. Without realizing what a privilege it was, I was accepted as a

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member of the team. By neglecting clinical studies and working mainly at night, I was able to show that the subject rabbits made detectable antibodies against the attached groups but not against the parent proteins (18a). Not a very surprising result, but splendid training!

When war finally broke out UCH was closed, except for a few beds in the basement. Students had to find places wherever they could until the teaching could be reorganized.

Having scraped through the final examination in 1940 I got a post as intern in the Department of Medicine at the Postgraduate Medical School at Hammersmith, in quick succession under John McMichael, Edward Sharpey Shafer, and Eric Bywaters—all able scientists as well as good clinicians. Soon, however, I came down with pulmonary TB. In 1941, when back on my feet again, I went to work with Douglas McClean on hyaluronic acid and hyaluronidase at the Lister Institute of Preventive Medicine at Elstree. Although I knew little about polysaccharide chemistry, I obtained advice from Walter Morgan, also at the Lister Institute, who was working at the time on *Shigella* antigens and beginning his classic studies on human blood group antigens. I thus gained some insight into complex polysaccharides and the enzymes that hydrolyzed them at a time when few people were studying them.

It was difficult to relate working on hyaluronidase and hyaluronic acid—interesting though they were—to what was going on in the war. Despite lacking any special training in pathology (owing to the disruption of medical studies at UCH I had rarely attended and never conducted an autopsy), I applied for a post as assistant pathologist at a large (900 bed) general hospital in North London. Ashley Miles agreed to act as a referee on the condition that if I got the job I would spend two months in the wartime Sector Bacteriology Laboratory he was running, so as to learn some real bacteriology. To my astonishment I got the job, and in the specified two months of intensive training I was taught enough practical bacteriology to cope. The morbid anatomist was Walter Pagel, an authority on tuberculosis and a noted medical historian, who had an enquiring mind and a splendid sense of Jewish humor. There was no other pathologist (the head of department being a prisoner of the Japanese), and I found myself in charge of the hematology, biochemistry, and bacteriology sections of the laboratory. Each section had a well-trained technician in charge, with whom I reached an amicable agreement: Each knew more about the job than I did; I would learn from them, but in the end I would probably know more than they; when innovation was needed, or novel problems arose, we would tackle the situation together. This arrangement worked well. Of all possible trainings for biomedical research, I can think of none better than being Lord High Everything Else in a busy general hospital during wartime! We were in close touch with an able clinical staff and had constantly to introduce new techniques and take on ad hoc research problems.

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There were no central reference laboratories to which problems or specimens could be referred. One solved problems by asking friends and acquaintances and/or looking up the literature and getting on with the job oneself. Medical students, seconded from the Middlesex Hospital, were pressed into helping and it was possible to get a surprising amount done. Since Walter Pagel was liable to asthma and severe bronchitis, we both knew that sooner or later he would become ill and I would have to do the autopsies and make the histopathology reports. He taught me to cope with the commoner problems, though I jibbed at reporting on frozen sections of brain in mid-operation, and got the neurosurgeon, who was quite competent to do so, to look at these himself. When Walter Pagel did fall ill, and the dreaded moment came in which I had to perform the autopsies, I made an arrangement with the mortuary attendant whereby I kept the staff and students busy with talk while he inconspicuously indicated on the corpse where I was to cut next. Our first such autopsy was on an elderly man who had died from type III pneumococcal pneumonia. (We typed the strains at the time as part of a study of the etiology of pneumonias, and of their response initially to sulphonamides and later to penicillin.) When I lifted the knife after opening the lung, long viscous strands of type III pneumococcal polysaccharide hung from it, and I was able to discourse on why type III was the commonest cause of severe pneumococcal pneumonia in the elderly. In the course of another 400–500 autopsies (almost all patients who died were autopsied) I realized what a lot can be learned from them. For example, there is no clearer demonstration of normally invisible lymphatics than the spread of carcinoma along them.

When the war ended, the former head of laboratory was due to return. I thought he was bound to regard me as a usurper, so I resolved to return to full-time research. Antibiotics promised to eliminate many diseases, but it was evident that cancer, old age, and rheumatic diseases would remain serious problems. There had been many admissions for rheumatic fever during my time as pathologist, and although the connection between rheumatoid arthritis and streptococcal infection was obvious, in 1946 the causal relationship was not. I wrote a proposal to investigate why and how streptococcal infection might stimulate autoantibody production against cardiac muscle and vascular endothelium. My initial hypothesis was that streptolysin S might complex with cell surfaces and that interaction between the complex and antistreptolysin S would lead to vasculitis and valvulitis. The Medical Research Council gave me an appointment on its external staff to work in the Department of Bacteriology at UCH under Wilson Smith, a virologist from the team responsible for isolating influenza virus. Rheumatic fever promptly became uncommon in the UK, and even regular visits to the isolation hospitals to which cases of scarlet fever were sent failed to reveal fresh cases. Furthermore, since I had no clinical status at UCH I was not allowed to see patients, take blood samples,

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or even swab throats. I ended up trying unsuccessfully to reproduce streptococcal tonsillitis in rats and rabbits, discovering that antistreptolysis S was not an antibody at all but a property of plasma lipoproteins.

Partly because of the difficulties of doing immunological research on patients, and partly because progress had resumed in basic immunology, I decided that application of immunology to the understanding of disease processes (as opposed to prophylactic immunization) would require an insight into immunopathology. I began to work on the Arthus reaction and on anaphylaxis.

The best-equipped and most prestigious center of biomedical research in Britain at the time was the National Institute for Medical Research at Hampstead. Its director was Harington (now Sir Charles), who had succeeded Sir Henry Dale; the head of the Division of Biological Standards was Ashley Miles. The Division studied, and prepared national and international standards for, the control of biological materials for therapeutic use. These included the newly discovered antibiotics penicillin and streptomycin, and others as they came along. Work on antibiotics had been assigned to Bruce White (best known for the Kauffmann-White classification of Enterobacteriaceae), but he died from leukemia in 1949. Although my work with antibiotics was confined to some therapeutic trials conducted when they were first released for civilian use—trials in which I had taken part while still a pathologist—I was invited to succeed Bruce White.

To become a member of the staff of the NIMR, especially under Harington and Miles, seemed a dream come true. It was understood that persons working in the Standards Division should have at least half their time free to do research on whatever they chose, and that members of other Divisions would help and advise them when needed. Furthermore, much of the work of the Division concerned antisera and prophylactic vaccines. Once the antibiotics were under control there would be a chance to return to the study of immunology in an ideal environment. I jumped at the chance and spent the next eight years in charge of antibiotic (and later some enzyme) standards. The work itself was far from dull, and it drove home the possibility of doing really accurate bioassays; the importance of linearity and parallelism of dose response curves became clear. Above all there was the opportunity to introduce immunological techniques and immunological problems to colleagues in various Divisions and to obtain their help or to cooperate with them. Projects included application of the newly available radioisotopes of iodine and carbon to studies of immunoglobulin metabolism and the synthesis of antibodies in vivo and in vitro, pharmacological studies of cutaneous anaphylaxis and of platelet involvement in allergic reactions, and demonstration of the role of granulocytes in Arthus reactions. I mention these not because they were important advances in immunology but to illustrate how widespread were the possibilities of cooperation. So many of the technical staff wanted to learn simple immu-

Annual Reviews

nological methods that for a while I ran lunch-hour seminars to which increasing numbers of the scientific staff also came. By 1957 immunology had caught on sufficiently at the NIMR for the Director to suggest that I be relieved of duties in the Biological Standards Division in order to set up a small Immunology Division composed of the persons with whom I had been working most closely—Brigitte Askonas, Walter Brockhurst, and Brigid Balfour. This was the first formal immunological post created in Britain (though of course much first-class research in immunology was done under other titles). From then on we were in the remarkably fortunate position to do what we chose, at our own pace, in a multidisciplinary Institute staffed with able and friendly scientists. At that time, the Institute was also as well equipped as any biomedical research center in Britain. When three years later Sir Peter Medawar succeeded Sir Charles Harington as Director, and brought with him his group from University College to take over under Avrion Mitchison the Division of Experimental Biology, the NIMR became a center that for many years attracted immunologists as visiting workers from all over the world.

This all happened before immunology reached adulthood around 1960—i.e. before the basic structure of immunoglobulin was proposed and lymphocytes were shown to be the immunocompetent cells. I have written in this egocentric vein to illustrate both my luck in the able people who were willing to teach and tolerate a beginner, and the devious route by which in those early days it was possible to become an immunologist. By present criteria, when specialized training and a PhD are needed before a candidate will be considered even for a temporary position, to gain such wide experience would be almost impossible.

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PART II: SERENDIPITY IN IMMUNOLOGY

Grants committees are duty bound to see that money made available for biomedical research is spent to the best advantage—i.e. to further the discovery of how biological processes work and how to control them in the interests of better health. As a member of various such committees, I have come to realize that the manner in which grant applications must be formulated leaves little scope for discovering something new. A well-presented grant application demonstrates, as it should, that the applicant knows the field in which he wishes to work, understands why it interests him, and is familiar with the relevant literature. He or she is then expected to define a precise problem in this field, set out in considerable detail how it is to be tackled experimentally, and state what sort of findings are expected. It is useful to add a few hopeful sentences about how the findings will advance knowledge or have some practical impact in medicine, veterinary science, agriculture, etc. It is better still

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if the applicant has already done enough work on the project to know that it will succeed. The committee, most of whom have no special knowledge of the field, feel that they understand the proposal and are therefore more deeply impressed when the application is clearly set out. Such a procedure, which compels the applicant to show expertise, clear thinking, good judgment, and the ability to distinguish a wood as something more than the trees that grow in it, provides, when accompanied by favorable reports from referees who know the subject well, a means of distributing grants sensibly. It enables the grant-giving body to allot funds, which are not inexhaustible, according to its idea of priorities. The committee decides whether the applicant's project may make practical contributions to research on cancer, skin disease, dentistry, virology, population control, etc, or whether—as at least such bodies as the British Medical Research Council intend—it may contribute fundamental knowledge in an area where this is lacking.

The sort of application I have in mind is illustrated in the instructions for Project Grant Applications to the British Medical Research Council.

> The purpose of the Council's scheme of project grants is to provide support . . . for single projects—pieces of work designed to seek the answer to a single question or to a small group of related questions. . . . It is the Council's policy that the duration of support under this scheme should normally not exceed three years; a single clearly defined project may be expected to have been completed (or to have failed) in this time and the need for extension should be exceptional.

The work funded according to such requirements may well be worth doing, but if it is carried out as set forth in an application like the one just described it cannot discover anything fundamentally new. It can test an hypothesis and extend it, or show that the original reasoning was incorrect, but expectation of the general outcome is implicit in the application. On the other hand, if the application were to state that "these are the lines along which I expect to begin my experiments, but I really hope an unforeseen observation will prompt an unexpected idea," it would need an unusually enlightened committee to award the grant. Yet this process of following up surprises is how a good many important (and unimportant) discoveries are made.

This brings me to the subject of serendipity. This strange word, which literally means a property of Ceylon (Sri Lanka), is used rather commonly nowadays. It was coined by an eccentric minor English writer, Horace Walpole, 4th Earl of Oxford, in a letter to Sir Horace Mann, written in 1754. Walpole mentions a fairy tale, "The Three Princes of Serendip" (probably by the Italian Bocci), in which the princes "were always making discoveries, by accidents and sagacity, of things they were not in quest of." His own example was that "Lord Shaftesbury happening to dine at Lord Chancellor Clarendon's, found out the marriage of the Duke of York and Mrs. Hyde by the respect with which her mother treated her at table." The importance of serendipitous

Annual Reviews

discoveries obviously depends upon circumstances. The word, in any event, filled a gap in the English language and rapidly became part of the accepted vocabulary.

To discuss the role of serendipity in immunology—by which I mean the unexpected observation seized upon and turned to advantage by the prepared mind—could be invidious. Only someone getting on in years, who has lived through some of the important growing stages in immunology and has worked in several of the areas involved, would have the impudence to try.

Lest attributing importance to serendipity be regarded as trivializing discovery, an important point must be made. Immunology, being a branch of biology, is concerned with mechanisms that operate in, and have operated to produce, living creatures as they have evolved on earth. There is no guarantee that similar mechanisms function anywhere else in the universe; and although they can certainly fascinate us inasmuch as they shed light upon the mystery of Life, immunological discoveries have no *cosmic* significance. In contrast to physics and chemistry, whose generalizations when valid are expected to apply throughout the universe, generalizations in biology are—so far as we know—limited to the past, present, and future behavior of particular elaborate organisms, whose rules we try to discover. Of course these organisms do not disobey the laws of physics and chemistry, though they probably transcend them; but even the discovery of something as exciting as the genetic code in DNA concerns a particular device that has permitted living organisms to survive and evolve. Especially since the subsequent discovery of introns and exons, it is difficult to conceive of another device that would perform as well as this code; but if self-replicating entities capable of independent existence and combining certain of the other properties we associate with life had arisen in quite another way, different devices would presumably have evolved.

The process of scientific discovery has occupied minds much abler than mine—including that of Peter Medawar; see, for example, his essay on *Induction and Intuition in Scientific Thought* (32). Although it may be possible for great minds in mathematics or physics to arrive at verifiably valid generalizations by purely mental processes—thereby showing that the processes of mental logic conform in some fascinating way with causality as it operates in the physical world—in biology we can only proceed by observation and experiment. Medawar stressed the importance of hypothesis (or if not so clearly formulated as to be dignified by this term, of "hunch") in the design and choice of experiments. He separated experiments into four kinds:

1. Baconian. "I wonder what would happen if . . . ." Noncritical experimental play.
2. Deductive or Kantian. Examination of the consequences of varying the axioms or presuppositions of a scheme of deductive reasoning.

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3. Critical or Galilean. Actions carried out to test "a hypothesis or preconceived opinion by examining the logical consequences of holding it."
4. Demonstrative or Aristotelian. Intended to illustrate a preconceived truth and to convince others of its validity.

"A good methodology," writes Medawar, "must, unlike inductivism, provide an adequate theory of the origin and prevalence of error . . . and it must also make room for luck."

Few people, I suppose, literally follow Francis Bacon's advice about how to probe the secrets of Nature. The Baconian approach implies that one might try rubbing two sticks vigorously together, not to test whether they would generate enough heat to ignite them but simply to see what would happen. This is certainly one way of discovering something entirely original; and when a subject is in its infancy, the Baconian approach may be the only one possible. In general, however, we know too much nowadays to proceed without some sort of hypothesis. We are also aware that in science an hypothesis is only useful if its consequences can be tested. Since any hypothesis stands a good chance of being overthrown or modified in the light of later knowledge, the hypothesis must be capable of refutation rather than of verification.

Medawar's second kind of experiment involves a motivation that I suspect is not uncommon—namely, such irritation at the certainty with which some hypothesis is promulgated that one designs experiments or formulates an alternative or contradictory hypothesis out of a sheer sense of devilment. It also includes a formula once put forward for gaining a Nobel Prize: Open a standard textbook at random, choose the most dogmatic statement on any page, and test rigorously its validity. I doubt, however, whether this advice is generally applicable!

Nevertheless, when able people turn a currently acepted hypothesis upside down the result can be enlightening. The trouble is that the mental effort involved is likely to be worthwhile and the arguments are likely to be accepted seriously by others only if there are at least some hints that currently accepted hypotheses are deficient or susceptible to modification. As an example I give Niels Jerne's early suggestion (21) that the body normally contains a population of cells already able to produce specific antibodies against almost every immunogen, and that the function of an immunogen is to take the antibody back to the cell that made it—wrong, but not hopelessly wrong! Jerne would probably not have produced the hypothesis had he not been studying antibodies against viruses, especially bacteriophage. Because the methods used in these studies were extremely sensitive he could find antibodies against the different viruses in all normal sera (20).

I could list other examples where received wisdom was challenged and superseded, noting that in each case the challenge followed clear indications

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that then-current hypotheses were incapable of accounting for well-attested observations. These examples would include the clonal section hypothesis of Talmage & Burnet (5, 44), elaborated in 1959 by Burnet (6) and Lederberg (27); the demonstration (43) that a single monoclonal antibody may have combining sites capable of binding more than one distinct epitope (an observation whose relevance to the question of the size of the antibody repertoire is not always appreciated); the multigene control of the synthesis of single polypeptide chains in Ig and other proteins; and the discovery of idiotypes and anti-idiotypes by Oudin (41). This is not to state that immunologists never produce hypotheses based on the purely logical consequences of varying the axioms in a scheme of deductive reasoning, in the absence of a strong hint that such rethinking was needed. I regard Niels Jerne's (22) formulation of the network theory of the immune system as an example of this mental feat, which requires an unusually bold and clear mind.

Experiments in Medawar's fourth category are the stuff of a good many PhD and MD theses, which fill the libraries of universities and the pages of journals without adding greatly to scientific knowledge.

Most research workers do experiments that belong in Medawar's third category. They formulate an hypothesis—however limited—and carry out experiments to test whether or not it correctly predicts their results. The hypothesis provides the justification for designing the experiments. Cynics claim that the *sole* justification of any hypothesis is to make people do experiments. They above others must be aware of the importance of luck!

Luck, or in this context serendipity, has contributed to more fundamental observations in immunology—and of course in other branches of biomedical science (e.g. physiology)—than the published accounts of the observations might indicate. It seems appropriate here to mention examples from my own experience—not because my experiments were of great importance but because I know what really happened and can relate the facts without shame or risk of giving offense. It will become obvious that discoveries of mine that involved a concurrence of chance observations with what may be termed "a prepared mind" would sooner or later have been made by others.

I begin with two examples, minor and unpublished, from my years as a pathologist.

Pseudomucinous Cysts of the Ovary as a Source of Blood Group Substances

I thought that these cysts might be derived from ovarian granulosa cells and might contain hyaluronic acid. Since the viscous cyst fluid was unaffected by hyaluronidase, this was obviously wrong. However I had seen volunteers in Walter Morgan's laboratory chewing rubber bungs and thinking of lemons in order to produce saliva as a source of blood group substance, and cyst fluid

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was not unlike saliva. When the next cyst came along I checked the blood group of its owner (group A) and took the fluid to Walter Morgan. It was almost pure A substance! The role of luck is shown by the fact that the next ten cyst fluids were unidentifiable as blood group substances (they were H or Le, which had not yet been characterized). The eleventh was B substance. If the first had not been identifiable Morgan would not have kept the rest, and would have continued to rely on saliva.

Procaine Penicillin

When it first became available I was responsible for issuing penicillin to patients. At that time penicillin was impure (400 units/mg), and injections were often painful. If any batch, tested on myself, proved severely painful, I mixed it with procaine. After an hour or two a fine amorphous precipitate appeared, which could be easily resuspended and painlessly injected. I showed in rabbits that it would protect against streptococcal infection, and that most of the penicillin appeared in the urine within 24 hours. Bottles of procaine penicillin mixture were issued to the wards with instructions to resuspend before injection. A penicillin manufacturer's representative to whom I recommended the procedure was uninterested at the time, but when I met him again four years later he told me that procaine penicillin had been patented and that $1 million in royalties were owed to the patent holder! By then penicillin was almost pure, and on mixing with procaine it rapidly crystallized as large needles that would not pass through a syringe unless pretreated in a micronizing mill. It would have been impossible to issue such crystals in suspension to the wards. Although I had no intention of preparing long-acting penicillin, the fact that it had been used sufficed to prevent the patent's being enforced and saved the British penicillin manufacturers $1 million!

Platelets and Granulocytes in Arthus Reactions

During the period when I thought I was doing experiments relevant to rheumatic fever, I used to elicit reversed passive Arthus reactions (i.e. inject known amounts of antibody intracutaneously, followed later by antigen intravenously) that were reproducible, convenient, and measurable quantitatively. The intensity of such reactions was reduced by cortisone, but the only obvious histological difference between treated and control animals was that the granulocyte infiltration was diminished when cortisone had been administered. Since at that time (1952) little was known about mediators of inflammation other than histamine, I supposed that histamine must somehow be involved and that perhaps it came from granulocytes. It was not difficult to show that rabbit blood contained quite a lot of histamine (and serotonin) but that it was virtually all in platelets and not in granulocytes. The obvious experiment was to see whether interaction of antigen and antibody in plasma would cause release of

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histamine and serotonin from platelets—which it did (17), though the relevance of this finding to any phenomenon but anaphylaxis in the rabbit is questionable. More interesting was the observation that selective removal of neutrophil granulocytes in vivo, by nitrogen mustard in rabbits or specific antiserum in guinea pigs, prevented the inflammatory response so long as granulocytes were almost absent from the blood (13a). These experiments were valid, but they were based on a chance observation stimulated by a quite erroneous assumption about the role of histamine.

Antilymphocyte Antibodies

Erroneous assumptions about histamine also led to the first demonstration that antilymphocyte antibodies would prevent delayed-type hypersensitivity reactions. Theo Inderbitzin and my colleague Walter Brocklehurst had observed that when cutaneous delayed-type reaction was induced in guinea pigs, skin histamine level rose markedly at the test site. Our technique of histological fixation did not preserve guinea-pig mast cells—a fact of which we were unaware; otherwise we might have stumbled upon a role for mast cells revealed much later by Philip Askenaze (2)—so we thought perhaps infiltrating lymphocytes were the conveyors of the histamine. I prepared specific rabbit antisera against guinea-pig platelets, granulocytes, and lymphocytes and tested their effect on delayed-type response in vivo. Much to our surprise, antilymphocyte antibodies abrogated the response. This occurred whether or not lymphocytes were eliminated from the circulation. We were too unsure of what this meant to publish the findings, though they were published by Inderbitzin (19). When Medawar and Levy used antilymphocyte sera later, for much better reasons, the immunization schedule that worked was supplied from my notes.

Complement 'Holes'

Bob Dourmashkin (then with the Imperial Cancer Research Fund) had found by electron microscopy, using negative staining, that saponin-treated erythrocyte membranes apparently contained a beautiful pattern of hexagonal channels. When it was pointed out by Alec Bangham that these were simply due to arrangement of cholesterol molecules in the surface lipids around the solvated saponin, this was a disappointment. I had purified various hemolysins (streptolysins S and O, staphylolysin, *Cl. welchii* α-toxin), and I gave them to Dourmashkin to see whether he could demonstrate more interesting lesions with these. As a last-minute thought I added a complement hemolytic system. The toxins produced characteristic lesions (10), but the most regular and interesting were those produced by complement. Tibor Borsos was enlisted to prove that the lesions actually corresponded to those predicted theoretically, and to convince us that complement could be studied even by novices (4). A wholly unforeseen line of work was initiated (15).

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Radioactive Suicide

Many years ago, before the clonal selection hypothesis, it seemed relevant to ask whether any antigen molecules were present in a cell stimulated to make antibody. With Hugh McDevitt we had shown that the number of antigen molecules in an antibody-secreting cell would not be more than 15 (30). Nossal had gone further and shown that the number was less than three. However, we had examined (T,G)-A-L partially labeled with ^{125}I as the antigen, in cells making anti-(T,G)-A-L, and theoretically the radiolabeled molecules might not have been those relevant. The only way to meet this criticism was to iodinate totally the tyrosines in (T,G)-A-L, converting it to TIGAL, and to examine cells making antibody against the iodinated form. In this case the radiolabel would be part of the immunogenic determinant. Hans Uli Keller and I set out to reexamine the question using ^{125}I TIGAL with specific activity about 2000 μCi/μg. This was a somewhat academic exercise, since there was by now general agreement that antigens did no more than trigger predetermined B cells, but we had done all the spadework and decided to go ahead.

To our surprise, although mice responded by making antibody against TIGAL perfectly well, they failed to make any against highly radioactive TIGAL. Yet the same mice responded to H-pertussis antigen injected, as an adjuvant, at the same time. Only when we sought an explanation did it occur to us that the B cells with receptors for TIGAL must have been selectively killed by weak β-emission from the ^{125}I (18). By a rather better reasoning process Ada & Byrt (1) had reached similar conclusions in respect of mouse spleen cells treated with highly radioactive flagellin. These experiments provided at the time the best evidence for the validity of clonal selection.

Inhibition of Antihapten Responses by Hapten-Conjugated Polysaccharides

Intrigued by the problem of why thymus-independent (T1) immunogens appeared to be incapable of receiving T-cell help, I thought that if a suitable hapten were attached (e.g. DNP onto pneumococcus type III capsular polysaccharide, S3) and this were administered to mice sensitized by application of DNCB to the skin, the DNP-reactive T cells would enable DNP-S3 to behave as a thymus-dependent immunogen. In fact it turned out that quite small amounts of DNP-S3 not only failed to increase the response to S3 but almost completely prevented mice primed against DNP-conjugated proteins from making anti-DNP on rechallenge with the same conjugate (38). This observation, the opposite of what was expected, led to a series of experiments with my colleagues Gerry Klaus and Abul Abbas to determine the mechanism by which B cells could be switched off specifically by antigens (23).

It also led indirectly to the observation that T1-1 and T1-2 immunogens are retained in different macrophage populations in distinct compartments in

Annual Reviews

lymphoid tissues. Because any possible therapeutic applications of selective suppression of antihapten responses were unlikely to involve using S3, I tried out conjugates of a variety of polysaccharides readily available commercially. In order to study their metabolism at the same time, I also conjugated small amounts of tyramine so as to permit trace labeling with radio-iodine. Again quite unexpectedly I found out that whereas conjugates of some polysaccharides were potent suppressors of secondary antihapten responses, others were poor suppressors but potent stimulators (14). All the polysaccharides had prolonged half-lives in the body but the T1-1 and T1-2 conjugates became located quite differently in different tissues. Autoradiography revealed that T1-2 conjugates were confined to a subset of macrophages (16), whose functions are still being studied.

Follicular Dendritic Cells and B Memory Cell Generation

It had been proposed by Dukor and his colleagues that activation of C3 could be a necessary and sufficient second signal to stimulate B cells with receptors for an antigen to secrete specific antibody (9). For various reasons this seemed unlikely. We thought it could be tested by seeing whether thymus-deprived mice could make an antibody response to Cobra venom factor (CVF), a naturally occurring form of activated C3, which had already been shown to be a potent immunogen. It turned out that the response to CVF was completely thymus dependent, which was inconsistent with Dukor's hypothesis (42). But this observation also made it possible to keep thymus-deprived mice with undetectable C3 levels for weeks on end.

Evidence had been produced that in mice treated with CVF, aggregated Ig—and by inference antigen-antibody complexes—failed to become localized on follicular dendritic cells (f.d.c) in germinal centers. It was also known that B memory cells could be generated in thymus-deprived mice. Knowing that such mice could be chronically depleted of C3 with CVF, we could test whether deposition of antigen-antibody complexes on f.d.c. were important or even essential for the generation of B memory cells. This proved to be the case (24) and led to a series of interesting experiments by Gerry Klaus that have emphasized the importance of antigen presentation in special microenvironments in determining the outcome in immune response (25).

Not every experiment I undertook was based on an hypothesis that proved false, but most of those that led to anything novel or interesting arose because of some unexpected or chance observation that I was fortunate in being able to follow up.

Having revealed my own dependence on serendipity, I may now describe briefly the origin of some more significant discoveries in immunology made by others, for which the importance of unexpected or chance observations has

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been revealed by the discoverers themselves or by colleagues, or of which I know the actual sequence of events at first hand.

The H-2 System in Mice

In his early studies on the genetics of mice, to be followed by studies of genetic factors in resistance to transplanted tumors, Peter Gorer observed that sera from rabbits immunized with blood from 3 strains of mice, maintained for 25 or more generations by brother-sister mating, could distinguish two heritable markers on the mouse erythrocytes (11). He later (12) cross-immunized the mouse strains with blood or leukemic cells—which proved more potent immunogens—and tested for iso-antibodies by specially sensitive tests involving agglutination of erythrocytes. These showed that blood and tumor cells evoked antibodies with similar specificities, and that one of the specificities corresponded with that of antibody II previously obtained in rabbits (hence the name H-2). At this stage he could distinguish only three separate specificities, but their genetic association was such as to enable him to propose that "normal and neoplastic tissues contain iso-antigenic factors which are genetically determined. Iso-antigenic factors present in grafted tissue and absent in the host are capable of eliciting a response which results in destruction of the graft. Antigenic differences between normal and neoplastic tissues are not normally capable of stimulating a defensive reaction."

These observations do not so much illustrate serendipity (for he was seeking what he found) as luck. It happens that mouse erythrocytes, unlike those of humans and many other species, express small amounts of what are now termed class I major histocompatibility antigens, and do not express different conventional blood group antigens. But for this, his erythrocyte agglutination tests, which made multiple analyses possible in those days, would not have revealed the H-2 system.

The Role of the Thymus in Immunity

While Robert Good and his colleagues were moving on clinical and evolutionary grounds toward the idea (which they could not prove) that the thymus was crucially involved in some kinds of immune response, Jacques Miller was studying leukemia in mice at the Institute of Cancer Research. Leukemia in AKR mice commonly arises in thymus, and Miller was examining the effect of thymectomy on the development of leukemia. He tried removing the thymus at various ages, including from newborn mice. The neonatally thymectomized mice developed an unexpected disease syndrome—wasting, hunched backs, loss of hair, and eventually death—and were prone to infection. Their blood contained fewer lymphocytes than that of sham-thymectomized controls. Miller accordingly tested the capacity of neonatally thymectomized mice to reject

Annual Reviews

allogeneic skin grafts and to respond to *Salmonella typhi* H antigen. He found that many mice retained the grafts for long periods and that the antibody response was minimal or absent (33, 34). The fact that allograft rejection could be restored by syngeneic thymocytes constituted the sought-for proof that the thymus was essential for the development of the capacity to reject allografts and to make antibodies against certain common antigens. The observation has since been brilliantly exploited by Miller and by many other workers. A minor piece of luck was the use of *Salmonella* H-antigen to reveal immune deficiency in thymectomized mice. Had he used certain other antigens (e.g. *Salmonella* O antigen or pneumococcal capsular polysaccharide) that are now known to be thymus-independent, the evidence for the role of the thymus would not have been so clear.

T-B Cell Cooperation

The first demonstration that antibody responses to sheep erythrocytes required cooperation between bone marrow– and thymus-derived cells came from experiments by Claman et al (7). These investigators were testing the capacity of thymocytes to give rise to antibody-producing cells by a technique involving intravenous injection of thymus-cell suspensions and sheep erythrocytes into lethally irradiated mice, and later enumeration of foci of cells making hemolysin in the spleens. Thymocytes were known not to restore erythro- and granulopoiesis after lethal irradiation, so Claman and his colleagues added bone marrow cells in some mice in the hope that these mice would survive better. In the event, mixtures of bone marrow and thymus cells resulted in many more hemolytic foci than either cell suspension on its own. Claman et al rightly concluded that thymocytes must somehow cooperate with bone marrow–derived cells to enable the latter to secrete antibody. This unexpected finding was subsequently exploited and analyzed more fully by Miller & Mitchell (35, 37, 38).

Genetic Control of Immune Responses

At a time when it was not unreasonable to consider instructive hypotheses of antibody stimulation (i.e. that antigen molecules directly influenced uncommitted potential Ig-producing cells to make specific antibody), it seemed important to determine whether there were any molecules of antigen in a cell making antibody. Michael Sela and I discussed how this could be done and concluded that if the polypeptide (T,G)-A—L were synthesized from radioactive amino acids, themselves synthesized using tritium, it might be possible to detect a single molecule. Israel Schechter undertook and accomplished the synthesis, but the end product proved to be insoluble and we eventually used the new ^{125}I label. Meanwhile I set out to make anti-(T,G)-A—L in rabbits so as to detect antibody-containing cells by the sandwich immunofluorescence

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technique. The sandylop rabbits at Mill Hill, immunized according to a schedule that was regularly successful in Israel, made no detectable antibody. Eventually we tried immunizing other breeds of rabbit and found in contrast that Dutch or Himalayan rabbits responded perfectly well. When Hugh McDevitt joined us to work on the project the first thing he did was to test all available strains of mice for responsiveness to (T,G)-A—L. Some made antibody regularly and others did not, and we then did the experiments (mentioned earlier) in F1 hybrids between two responsive strains (30).

McDevitt had realized that the strain differences were potentially important. By studying responses in F1 and F2 generations between responsive and unresponsive strains he had concluded that a single major genetic factor was responsible. He consulted Michael Sela about using another synthetic polypeptide to test whether the phenomenon was generally applicable, and they chose a similar molecule in which tyrosine was replaced by histidine. When this was tested they again found responsive and unresponsive strains, but the strains were different (28, 29). Examination of the H-2 specificities of a large number of strains and recombinants (recently worked out by Donald Shreffler) to three different synthetic polypeptides revealed that responsiveness was controlled by a gene or genes termed *Ir-1* lying between H-2K and H-2D (31). Having brought the researchers so far, the signposts to further progress were clear and the rest has followed!

Luck (converted to serendipity by McDevitt and Sela) was involved at four points: the use of (T,G)-A—L, which had very homogeneous epitopes so that the response was largely confined to these; the initial screening, for other reasons, of mouse strains; the choice of (H,G)-A—L, which behaved differently as a second immunogen; and the fact that the H-2 specificities had already been worked out, so that scrutiny of these could immediately suggest the association between responsiveness and H-2.

Lectins as Mitogens

When P. C. Nowell was culturing leukemic cells in vitro to study their chromosomes, using a technique described by Osgood (40) he employed phytohemagglutinin (PHA) to agglutinate and remove the erythrocytes. PHA had been chosen as a nontoxic lectin, as opposed to some others such as ricin. Unexpectedly, not only did leukemic cells proliferate but mononuclear cells from normal blood regularly underwent mitosis after a few days of culture (39). From this observation originated the exploitation of lectins as polyclonal mitogens, which has greatly advanced cellular immunology.

Australia Antigen (Hepatitis B)

In his Nobel Prize address, Baruch Blumberg (3) has described how he and A. C. Allison, interested in genetic polymorphism, decided to test the hypoth-

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esis that patients who received a large number of blood transfusions might develop antibodies against putative polymorphic proteins that they had not inherited, but that the blood donors had. They tested sera against one another for precipitin formation by the agar gel diffusion technique and found such a polymorphism in low-density lipoproteins. They also found a different antibody in a hemophilic patient that reacted with an antigen present in the serum of an Australian aborigine but in that of very few normal Caucasians in the United States. It was relatively common in sera from individuals in some tropical countries and in sera of patients treated for leukemia with blood transfusions. Blumberg considered the hypothesis that the presence of Australia (Au) antigen was somehow correlated with susceptibility to leukemia and investigated its presence in sera of patients with Down's syndrome, who have a much increased tendency to develop leukemia. In Blumberg's study about 30% of the sera from children with Down's syndrome contained Au antigen. The serum of one child, originally negative, later became positive; this coincided with the development of chronic anicteric hepatitis. Further study of hepatitis patients showed that many had Au antigen in their blood early in the disease but that the antigen usually disappeared within a few days or weeks. This was the clue to recognizing that the Au antigen was part of the elusive hepatitis virus. Blumberg readily acknowledged the role of serendipity (and of excellent collaboration with other colleagues).

Monoclonal Antibodies from Hybrid Myelomas

Cesar Milstein had set out to answer the question whether amino acid sequence alone controls antibody specificity and, if so, how this control is achieved. Having completed a survey of the structure and evolution of immunoglobulins (36), he began a study of some of Michael Potter's mouse myeloma cell lines cultured in vitro. He intended to investigate whether they would show an unusually high rate of detectable mutation in their Ig product (they did not) or whether there might be evidence for scrambling between the variable and constant regions. To test the latter hypothesis he used an established means—namely Sendai virus—to fuse a mouse myeloma Adj PC5 with an 8-azaguanine resistant rat myeloma line S 210, and examined whether hybrid molecules of Ig were produced (8). Hybrid molecules containing light and heavy chains derived from either parent were detected, but there was no evidence of V-C gene scrambling. For further study he wanted myeloma cell lines that produced Ig with an identified antibody specificity and would grow in continuous culture, but none of his lines combined both properties. When Georges Köhler joined him they decided to try a long shot: Would spleen cells from a mouse immunized with sheep erythrocytes fuse with a well-established 8-azaguanine resistant mouse myeloma line P3, possibly secreting some antibody molecules specific for sheep erythrocytes? The experiment succeeded

Annual Reviews

beyond their best hopes. Not only were antibody-secreting hybrid cells produced, which could be cloned, but also a quite unexpectedly high proportion of the hybrids secreted specific antibody (26). From then on, hybridoma-derived monoclonal antibodies have become exquisitely sharp and popular tools for the identification and preparation of specific antigens. The concept has been extended to fusion of T cells with T-cell lymphomas, with equally important consequences. I have included this as an example of serendipity because the inital fusion experiments were done with no conscious intention to produce monoclonal antibodies and with no clear idea of what the implications of the availability of such antibodies would be. This in no way detracts from their importance or the brilliance of the follow up!

The list could be extended—and readers could surely add examples from their own experience—but it is long enough already to make the point that in immunology, as in other branches of biology, unexpected observations *and the prepared mind* are among the most potent stimulators of important advances. I would add four more, rather obvious points. One is that unless an individual with a prepared mind carries out the experiment personally or is at least closely involved in its execution, the unexpected may not be observed, or if observed may be dismissed as irrelevant. A second is that as many controls as possible need to be done if a plausible but erroneous hypothesis is to be refuted. Third, if the experimental data are largely derived from automated instruments that measure single parameters, only data the instruments are programmed to supply will be available. (This is a warning rather than an argument against instrumentation, since some instruments can provide more information than even the eye can detect.) The fourth, and most important, is that finance for research should always contain a substantial proportion of funds to provide scientists with the security and facilities that will allow them go to in some agreed general direction, but to follow their noses wherever the trail may lead. There was a period, during much of which I was lucky enough to be at work, when in some of the more prosperous countries this was accepted wisdom; but in the current financial climate it may be worth restating.

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Literature Cited

1. Ada, G. L., Byrt, P. L. 1969. Specific inactivation of antigen-reactive cells with ^{125}I-labeled antigen. *Nature* 222:1291–92
2. Askenaze, P. W. 1976. Cutaneous basophil hypersensitivity uncovered in the cell transfer of classical tuberculin hypersensitivity. *J. Immunol.* 117:741–47
3. Blumberg, B. S. 1977. Australia antigen and the biology of hepatitis B. *Science* 197:17–25
4. Borsos, T., Dourmashkin, R. R., Humphrey, J. H. 1964. Lesions in ervthrocyte membranes caused by immune haemolysis. *Nature* 202:251–52
5. Burnet, F. M. 1957. Modification of Jerne's theory of antibody production using the concept of clonal selection. *Aust. J. Sci.* 20:67
6. Burnet, F. M. 1959. *The Clonal Selection Theory of Acquired Immunity.* London: Cambridge Univ. Press
7. Claman, H. N., Chaperon, E. A., Triplett, R. F. 1966. Thymus-marrow cell combinations. Synergism in antibody

Annual Reviews

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production. *Proc. Soc. Exp. Biol. Med.* 122:1167–71

8. Cotton, R. G. H., Milstein, C. 1973. Fusion of two immunoglobulin-producing myeloma cells. *Nature* 244:42–43
9. Dukor, P., Hartmann, K. U. 1973. Hypothesis. Bound C3 as the second signal for B-Cell activation. *Cell. Immunol.* 7:349–56
10. Dourmashkin, R. R., Rosse, W. F. 1966. Morphologic changes in the membranes of red blood cell undergoing hemolysis. *Am. J. Med.* 41:699–710

10a. Erlanger, B. F., Borek, F., Beiser, S. M., Lieberman, S. 1957. Preparation and characterization of conjugates of bovine serum albumin with testosterone and with cortisone. *J. Biol. Chem.* 228:713–27.

11. Gorer, P. A. 1936. Antigenic differences in mouse erythrocytes. *Brit. J. Exp. Pathol.* 17:42–50
12. Gorer, P. A. 1938. Antigenic basis of tumour transplantation. *J. Pathol. Bacteriol.* 36:268–82
13. Humphrey, J. H. 1981. Tolerogenic or immunogenic properties of polysaccharides correlated with cellular localization. *Eur. J. Immunol.* 11:212–20

13a. Humphrey, J. H. 1955. The mechanism of Arthus reactions. I. The role of polymorphonuclear leucocytes and other factors in reversed passive Arthus reactions in rabbits. *Brit. J. Exp. Pathol.* 36: 268–82

14. Humphrey, J. H. 1981. Tolerogenic or immunogenic properties of polysaccharides correlated with cellular localization. *Eur. J. Immunol.* 11:212–20
15. Humphrey, J. H., Dourmashkin, R. R. 1969. The lesions in cell membranes caused by complement. *Adv. Immunol.* 11:75–114
16. Humphrey, J. H., Grennan, D. 1981. Different macrophage populations distinguished by means of fluorescent polysaccharides. Recognition and properties of marginal zone macrophages. *Eur. J. Immunol.* 11:221–28
17. Humphrey, J. H., Jaques, R. 1955. The release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*). *J. Physiol.* 128:9–27
18. Humphrey, J. H., Keller, H-U. 1970. Some evidence for specific interaction between immunologically competent cells and antigen. In *Developmental Aspects of Antibody Formation and Structure*, ed. J. Sterzl, I. Riha, pp. 485–502. New York: Academic

18a. Humphrey, J. H., Yuill, M. E. 1939. Studies in synthetic immunochemistry. IV. Further investigation of O-β-glucosidotyrosyl derivatives of proteins. *Biochem. J.* 33:1826–32

19. Inderbitzin, T. 1956. The relationship of lymphocytes, delayed cutaneous allergic reactions and histamine. *Int. Arch. Allergy* 8:150–59
20. Jerne, N. K., Skovsted, L. 1953. The rate of inactivation of bacteriophage T4R in specific antiserum. *Ann. Inst. Pasteur Paris* 84:73–89
21. Jerne, N. K. 1955. The natural-selection theory of antibody formation. *Proc. Natl. Acad. Sci. USA* 41:849–57
22. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Inst. Pasteur)* 125C;373–89

22a. Kabat, E. A., Mayer, M. M. 1948. *Experimental Immunochemistry.* Springfield, IL: C. C. Thomas

23. Klaus, G. G. B., Abbas, A. K. 1977. Antigen-receptor interactions in the induction of B lymphocyte unresponsiveness. *Curr. Top. Microbiol. Immunol.* 78:31–68
24. Klaus, G. G. B., Humphrey, J. H. 1977. Generation of memory cells. I. The role of C3 in the generation of B memory cells. *Immunology* 33:31–40
25. Klaus, G. G. B., Humphrey, J. H., Kunkl, A., Dongworth, D. W. 1980. The follicular dendritic cell: its role in antigen presentation and the generation of immunological memory. *Immunol. Rev.* 53:3–26
26. Köhler, G., Milstein, C. 1975. Continuous culture of fused cells secreting antibody of predefined specificity. *Nature* 256:495–97

26a. Landsteiner, K. 1945. *The Specificity of Serological Reactions.* Boston: Harvard Univ. Press. Rev. ed.

27. Lederberg, J. 1959. Genes and antibodies. *Science* 129:1649–53

27a. Marrack, J. R. 1938. *The Chemistry of Antigens and Antibodies.* Med. Res. Counc. Spec. Rep. Ser. 230. London: HMSO

28. McDevitt, H. O., Sela, M. 1965. Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. *J. Exp. Med.* 122:517–31
29. McDevitt, H. O., Sela, M. 1967. Genetic control of the antibody response. II. Further analysis of determinant-specific control, and genetic analysis of the response to (H,G)-A—L in CBA and C57 mice. *J. Exp. Med.* 126:969–78
30. McDevitt, H. O., Askonas, B. A., Hum-

Annual Reviews

phrey, J. H., Sela, M. 1966. The localization of antigen in relation to specific antibody-producing cells. I. Use of a synthetic polypeptide (T,G)-A—L labelled with iodine-125. *Immunology* 11:337–51

31. McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H., Snell, G. D. 1972. Genetic control of the immune response. Mapping of the *Ir-1* locus. *J. Exp. Med.* 135:1259–78
32. Medawar, P. B. 1969. *Induction and Intuition in Scientific Thought*. London: Methuen
33. Miller, J. F. A. P. 1961. Immunological function of the thymus. *Lancet* ii: 748–49
34. Miller, J. F. A. P. 1962. Effect of thymectomy on the immunological responsiveness of the mouse. *Proc. R. Soc. Lond. Ser. B*. 156:415–28
35. Miller, J. F. A. P., Mitchell, G. M. 1968. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. *J. Exp. Med.* 128:801–20
36. Milstein, C., Pink, J. R. L. 1970. Structure and evolution of immunoglobulins. *Progr. Biophys. Mol. Biol.* 21:209–63
37. Mitchell, G. M., Miller, J. F. A. P. 1968. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. *J. Exp. Med.* 128:821–37
38. Mitchell, G. M., Humphrey, J. H., Williamson, A. R. 1972. Inhibition of secondary anti-hapten responses with hapten conjugated to type 3 pneumococcal polysaccharide. *Eur. J. Immunol.* 2:460–67
39. Nowell, P. C. 1960. Phytohemagglutinin: An initiator of mitosis in cultures of normal human leucocytes. *Cancer Res.* 20:462–66
40. Osgood, E. E., Krippaehne, M. L. 1955. The gradient tissue culture method. *Exp. Cell Res.* 9:116–27
41. Oudin, J., Michel, M. 1963. Une nouvelle forme d'allotypie des globulines y du sérum de lapin, apparément lièe à la fonction et à la specificité anticorps. *C. R. Inst. Pasteur* 257:805–08
42. Pryjma, J., Humphrey, J. H. 1975. Prolonged C3 depletion by cobra venom factor in thymus-deprived mice and its implication for the role of C3 as an essential second signal for B-cell triggering. *Immunology* 28:569–76
43. Rosenstein, R. W., Musson, R. A., Armstrong, M. Y. K., Konigsberg, W. H., Richards, F. M. 1972. Contact regions for dinitrophenyl and menadione haptens in an immunoglobulin binding more than one antigen. *Proc. Natl. Acad. Sci. USA* 69:877–81
44. Talmage, D. W. 1957. Allergy and Immunology. *Ann. Rev. Med.* 8:239–56
45. Topley, W. W. C. 1933. *An Outline of Immunology*. London: Arnold. viii + 390 pp.

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359

ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395

IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429

COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461

C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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THE HUMAN T-CELL RECEPTOR

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INTRODUCTION

T lymphocytes recognize antigen in the context of membrane-bound products of the major histocompatibility complex (MHC) with exquisite specificity (1–5). This recognition is essential for activation of T cells with cytotoxic effector function as well as immunoregulatory activities. With regard to the former, T cells can lyse specific target cells, including those that have been infected with viruses and carry viral antigens (6–10). Moreover, they regulate the activity of cells within the immune system such as T cells, B cells, and macrophages, as well as hematopoietic stem cells, fibroblasts, osteoclasts, and other cell types extrinsic to the lymphoid system (11–19). Characterization of the T-cell receptor for antigen could be particularly valuable for a molecular understanding of the cellular interactions underlying these activities.

Given that T lymphocytes can recognize antigens in a precise fashion, discriminative surface structures restricted in their expression to individual T-cell clones must exist. The recent development of technologies to generate and continually propagate clonal populations of human T lymphocytes in vitro (20–24) has provided a new basis for identification of such clonotypic recognition determinants. Here, we used antigen-specific clonal human T-cell populations of predefined specificities as immunogens and produced a series of clone-specific and noncrossreactive murine monoclonal antibodies directed at them. These anticlonotypic antibodies identify a novel class of 90KD heterodimers, termed Ti, that are membrane associated with the previously described

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20KD T3 glycoprotein and are present on all mature human T lymphocytes (25, 26).

Since anti-Ti monoclonals were generated against individual clones of functional T lymphocytes, it was possible to characterize both structural and functional features of the Ti molecule and to provide compelling evidence for the notion that each T lymphocyte, regardless of subset derivation, specificity, or regulatory activity, uses an analogous T3-associated Ti heterodimer for antigen recognition.

THE T-CELL SUBSET DERIVATION DETERMINES MHC RESTRICTION OF T-CELL CLONES

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A number of human T cell lineage–restricted surface glycoproteins have been defined by monoclonal antibodies. Thus, each mature T lymphocyte expresses a 20KD glycoprotein, termed T3. The latter appears in late intrathymic ontogeny at the time of acquisition of immunologic competence and plays a central role in T-cell function (27–33). Moreover, two human subsets of mature T lymphocytes were found that exhibit unique regulatory and effector activities. On the basis of their unique 62KD and 76KD membrane markers, these were termed T4+ and T8+, respectively (17–19, 34). The T4+ subset was shown to provide inducer/helper activities for T-T, T-B, and T-macrophage interactions, whereas the T8+ subset principally functioned in a suppressive mode (35, 36). Although both subsets of cells proliferated to alloantigen in mixed lymphocyte cell culture (MLC), the vast majority of cytotoxic effector function was detected in the T8+ population. Moreover, development of cytotoxicity by T8+ cells in general required interactions with T4+ cells or their soluble products. In contrast, only a minor component of cytotoxic effector function resided within the T4+ subset, and this was maximal when T4+ cells alone were sensitized in MLC (36).

To characterize individual cytotoxic effector lymphocytes (CTL) in humans, we developed a strategy to clone and propagate antigen-specific T-cell populations in vitro (24). T lymphocytes were stimulated for five days with the allogeneic B lymphoblastoid cell line Laz 156 and then cloned in soft agar or, alternatively, microtiter plates by limiting dilution. After one month of in vitro expansion with interleukin 2 (IL-2) and frequent restimulation with the alloantigen, cultures were screened for reactivity with anti-T4 and anti-T8 monoclonal antibodies by indirect immunofluorescence on an Epics V cell sorter. Of the initial 22 cultures that showed a homogeneous phenotype in terms of anti-T4 and anti-T8 reactivities, 15 expressed the T8 antigen and 7 expressed the T4 antigen. All 15 T8+ cultures exhibited a high level of cytotoxicity against the stimulating alloantigen Laz 156. In contrast, only 2 of the 7 T4+ cultures killed Laz 156.

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Figure 1 shows representative phenotypes of individual T4+ and T8+ clones as defined by reactivity with a series of monoclonal antibodies and indirect immunofluorescence on an Epics V cell sorter. Virtually all cells

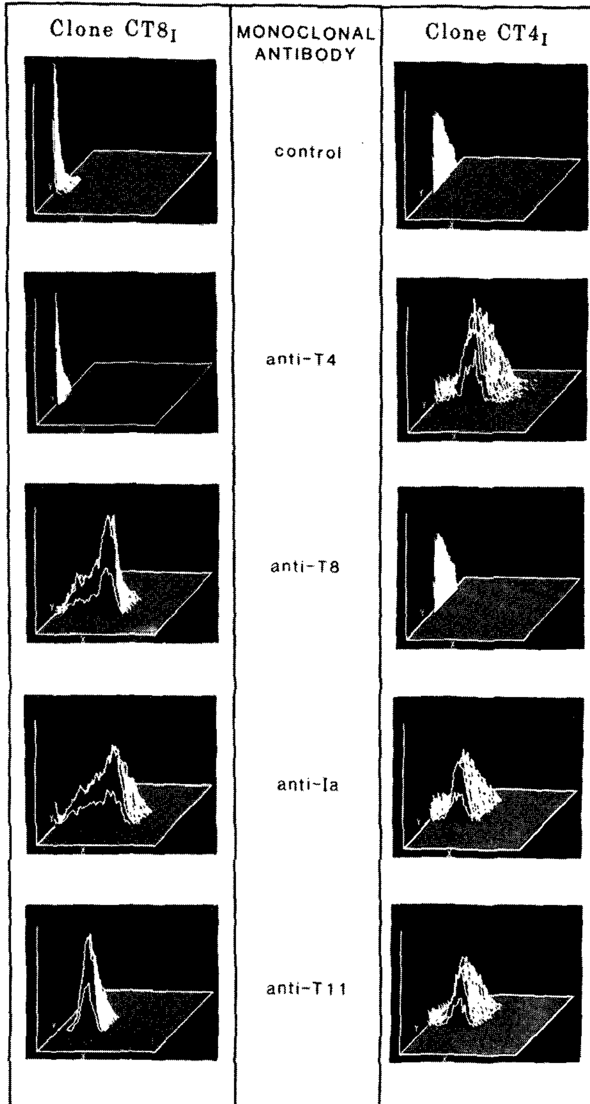

Figure 1 Cytofluorographic analysis. Cytofluorographic analysis of two representative human T cell clones, $CT8_I$ and $CT4_I$, using various monoclonal antibodies and indirect immunofluorescence on an Epics V cell sorter (Coulter Electronics, Hialeah, FL).

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within the T4+ $CT4_I$ clone were anti-T4 reactive and anti-T8 unreactive, whereas the opposite pattern was the case for cells within the T8+ $CT8_I$ clone. Both T4+ and T8+ clones expressed the sheep erythrocyte receptor–associated antigen T11 and were reactive with anti-T3, which is present on mature T lymphocytes. In addition, both T4+ and T8+ clones were reactive with anti-Ia antisera. Dr typing showed that the Ia antigens on these clones were of the original donor genotype and unrelated to the Ia expressed by the allogeneic B lymphoblastoid line Laz 156. This observation is not surprising in light of the fact that human T cells synthesize and express Ia antigens after activation (36). The cytotoxic capacity of individual T4+ and T8+ T cell clones was examined by testing their ability to lyse ^{51}Cr-labeled Laz 156 cells (Figure 2). As shown, both $CT8_I$ and $CT4_I$ were highly cytotoxic for Laz 156. Thus, even at an effector/target ratio of 0.5:1, 10–20% specific killing was observed. In contrast, $HT4_I$ was incapable of killing alloantigens at any ratio tested.

The specificity of T4+ and T8+ clones and subclones was analyzed on a panel of typing cells and by blocking studies of MCH determinants on the stimulating alloantigen with monoclonal antibodies (Table 1). T8+ clones killed targets that shared class I MHC antigens (HLA-A, B) with the original stimulator cells, whereas cytotoxic T4+ clones were directed at class II MHC antigens (Ia-related). Preincubation of the allogeneic target cell with a monoclonal antibody to a nonpolymorphic HLA α-chain determinant inhibited killing by the T8+ clones but did not affect T4+ cytotoxic function. In a recip-

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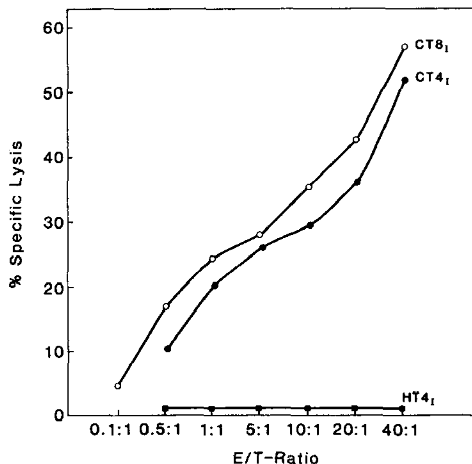

Figure 2 CML function of individual T cell clones. A constant number of ^{51}Cr-labelled target cells was incubated with various numbers of cloned T lymphocytes in a standard 4-hr CML assay.

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Table 1 Inhibitory effects of anti-HLA/Ia antisera on CML of T4+ versus T8+ clones[a]

	$CT4_I$	$CT4_{II}$	$CT8_I$	$CT8_{II}$
Media	100.0[b]	100.0	100.0	100.0
p29,34	9.8	19.0	87.6	107.9
p23,30	54.9	48.7	94.8	110.0
w6/32	96.3	107.0	11.0	2.1
anti-β_2 m	98.7	97.3	96.4	102.0

[a]Final dilutions of the antisera: p29,34—1:250; p23,30—1:20; w6/32—1:50; β2-M—1:20. The effector/target cell ratio utilized was 5:1.
[b]Relative % specific lysis

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rocal fashion, anti-Ia antibodies to common framework structures on the same target cell blocked killing by T4+ but not by T8+ clones. These results indicated that T4+ and T8+ T lymphocytes have receptors for different classes of MHC antigens (T8-class I and T4-class II correlation) (24, 37–39). Analogous findings have been reported from studies with autoreactive T-cell clones as well (9, 10).

EFFECTS OF MONOCLONAL ANTIBODIES DIRECTED AT MONOMORPHIC STRUCTURES ON ANTIGEN-SPECIFIC FUNCTION

The association between the surface phenotype (that is, surface glycoproteins) of CTL and the class of MHC molecules recognized implied that the subset-restricted structures, T4 and T8, might be required to facilitate selective lysis of different target antigens. To determine if individual anti-T4 and anti-T8 antibodies influenced killing function, cytotoxic T-cell clones were preincubated with T cell–specific monoclonal antibody or medium alone prior to the CML assay. As shown in Table 2, anti-$T8_A$ did not diminish the level of killing by MHC class II–restricted T4+ clones (of which $CT4_{II}$ is representative) but markedly decreased cytotoxicity mediated by the MHC class I–restricted T8+ clones ($CT8_{III}$, for example) (24, 40). In contrast, anti-$T4_A$ preincubation resulted in ≥80% reduction of cytotoxicity by the T4+ clones but had no effect on killing by the T8+ clones. In addition, Table 2 indicates that anti-$T3_A$, unlike anti-T4 and anti-T8, inhibits the killing by both T4+ and T8+ clones. Moreover, anti-$T3_A$ is unique among antibodies that define other mature T-cell surface structures since anti-T1 (not shown) and anti-T12 do not inhibit the cytotoxic capacity of these clones even when used in saturating concentrations. This result indicated that the observed inhibition was not simply a function of antibody binding to a clonal effector population.

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Table 2 Target recognition by individual human cytotoxic T cell clones[a]

Clone	Treatment	Related target (Laz 156) + Media	Related target (Laz 156) + Lectin	Unrelated target (Laz 509) + Media	Unrelated target (Laz 509) + Lectin
$CT4_{II}$	Media	47[b]	42	0	40
	Anti-$T3_A$	12	38	0	39
	Anti-$T4_A$	9	36	0	37
	Anti-$T8_A$	48	44	0	41
	Anti-T12	49	43	0	41
$CT8_{III}$	Media	68	52	0	48
	Anti-$T3_A$	14	49	0	47
	Anti-$T4_A$	67	52	0	49
	Anti-$T8_A$	6	46	0	50
	Anti-T12	67	53	0	48
$HT4_I$	Media	0	0	0	0

[a]Prior to the standard 4-hr CML assay employing ^{51}Cr-labelled Laz 156 or Laz 509 target cells, the various effector populations were incubated with one or another monoclonal antibody or media for 30 min at room temperature. The effector/target cell ratio in all experiments was 20:1. Con A was utilized at a final concentration of 25 μg/ml and added with the target cells at the initiation of the CML assay. This concentration of Con A had no effect on the spontaneous ^{51}Cr release.

[b]% specific lysis

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Whether the T3, T4, and T8 surface molecules served as recognition elements or represented components of the lytic mechanism was unknown. To address this question, we utilized the same cloned populations of T4+ and T8+ CTL and examined the ability of monoclonal antibodies to the surface structures (T3, T4, T8) to influence killing under various experimental conditions (41). Because appropriate concentrations of lectin can induce approximation of CTL and target cells in the absence of antigen recognition, it is possible to assess the intrinsic killing capacity of CTL clones even in the presence of monoclonal antibodies that inhibit cytolytic function. We reasoned that if the antibodies bind to a surface structure related to the lytic mechanism itself, then artificial approximation should not be capable of reconstituting effective lysis. However, if these antibodies block structures required for target recognition or appropriate killer-target binding, then lectin approximation should result in effective target lysis.

In the experiment shown in Table 2, cells from the T4+ clone $CT4_{II}$ and the T8+ clone $CT8_{III}$ killed the human B lymphoblastoid line Laz 156 to which they had been sensitized. Thus, at an effector/target ratio of 20:1, killing by $CT4_{II}$ and $CT8_{III}$ was 47% and 68%, respectively. This cytotoxic activity was specific because neither clone mediated the lysis of unrelated targets—e.g. Laz 509. As stated above, incubation of $CT4_{II}$ and $CT8_{III}$ with monoclonal antibody to T3 (anti-$T3_A$) reduced killing by approximately 75%

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(12–14% specific lysis) in both cases. In contrast, the monoclonal antibodies to $T4_A$ (anti-$T4_A$) and $T8_A$ (anti-$T8_A$) selectively inhibited the killing of the clone that expressed the T4 or T8 determinants.

Perhaps more importantly, the inhibitory effects of all three monoclonal antibodies were reversed by culturing CTL and target cells with lectin. In the presence of Con A, the lytic activity of $CT4_{II}$ cells treated with anti-$T3_A$ or anti-$T4_A$ increased from 9–12% to 36–38%. Restoration of lytic activity from 14% and 6%, respectively, to 49% and 46% was also observed after lectin approximation of the target cells and the $CT8_{III}$ clone following preincubation with anti-$T3_A$ or anti-$T8_A$. That the $HT4_I$ helper T-cell clone did not become cytotoxic (Table 2, Figure 2) suggests that killing is a consequence of the cell's functional repertoire rather than the capacity of Con A to bridge two cell surfaces. This observation further stresses the heterogeneity of cells within the human T4+ T-cell subset with respect to the existence of cytotoxic and noncytotoxic populations.

These results imply that at least several surface molecules are important in CML: T3 and T4 molecules on T4+ clones, and T3 and T8 molecules on T8+ clones (41). Since CML is restored by lectin even in the presence of monoclonal antibodies to these molecules, it appears that T3, T4, and T8 are involved in recognition events rather than the lytic mechanism. In further support of this notion is the observation that the target cell specificity of CTL clones is abrogated by lectin approximation. In the presence of Con A, $CT4_{II}$ and $CT8_{III}$ kill unrelated target cells (Laz 509, Table 2). For example, with lectin, $CT4_{II}$ lyses the irrelevant target Laz 509 (37–41% specific lysis). Similar results are seen with $CT8_{III}$ (47–50%). Moreover, under these conditions, the killing of both the irrelevant target Laz 509 and the specific target Laz 156 are comparable.

Antigen-specific CTL clones recognizing autologous B lymphoblastoid lines transformed by Epstein-Barr virus (EBV) are also governed by a series of recognition elements identical to the clones directed at allogeneic targets (9, 10, 42). Specifically, CTL expressing the T8 phenotype recognize the autologous B lymphoblastoid line in the context of class I MHC molecules. Anti-HLA antibodies block CTL effector function at the target level, and anti-T8 antibodies abrogate the ability of these class I–specific killers to kill. In contrast, T4+ CTL recognize Ia (class II) determinants on the autologous lymphoblastoid cell and are inhibited by anti-T4 but not by anti-T8 antibodies. As with the allogeneic CTL, anti-T3 antibody preincubation inhibits the killing ability of both T4+ and T8+ effector T cells. Because these clones fail to lyse autologous B cells not infected with EBV- or PWM-stimulated B-cell blasts, such T4+ and T8+ effectors seem to recognize virally encoded surface glycoproteins in association with class II or class I molecules, respectively. At present, however, these viral proteins have not been identified.

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THE T3 MOLECULAR COMPLEX MODULATES FROM THE T-CELL SURFACE

As demonstrated above, several lineage-specific antigens have been defined on human T lymphocytes. However, in the case of the 20KD T3 surface molecule, its appearance in late intrathymic ontogeny, at the time immunologic competence is acquired, and its critical role in T-lymphocyte function suggested that T3 was closely linked to an important recognition receptor or cell–cell interaction molecule (28–33). Antibodies directed against T3 were unique in the ability to block the induction phase as well as the effector phase of CML, to inhibit T-lymphocyte proliferative responses to soluble antigen, and to be mitogenic for resting T cells. In this latter case, activation was accompanied by release of various T-cell lymphokines, including IL-2 and γ-interferon (43, 44).

Given the central role of the T3 molecule in human T-cell function and the known rapid ligand-receptor modulation that occurs with a variety of hormone and growth-factor receptors, we examined the capacity of anti-T3 to induce modulation of the 20KD T3 molecule. T-cell clones were incubated 18–24 hr at 37°C with one or another monoclonal antibody, and surface antigenic changes were subsequently analyzed by means of indirect immunofluorescence on an Epics V cell sorter. As shown in Figure 3, preincubation of $CT8_{III}$ cells with

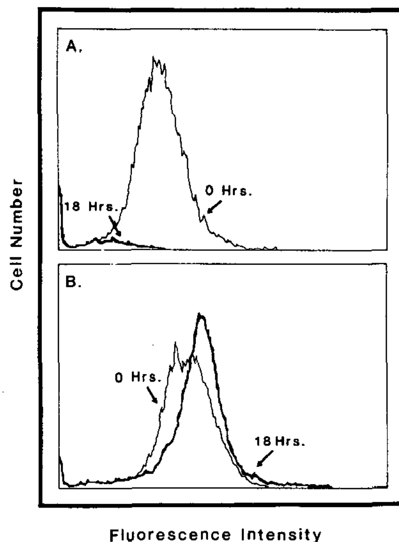

Figure 3 Selective modulation of the T3 antigen from clone $CT8_{III}$. $CT8_{III}$ cells were incubated with monoclonal antibody anti-$T3_A$ for 18 hr at 37°C and subsequently washed extensively. Both untreated and T3 modulated cells (light and dark curves, respectively) were analyzed for reactivity with monoclonal antibodies anti-$T3_A$ (panel A) and anti-$T8_A$ (panel B) using indirect immunofluorescence with goat anti-mouse $F(ab')_2$ FITC on an Epics V cell sorter.

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anti-T3 for 18 hr at 37°C resulted in loss of cell-surface T3 antigen (32, 41). Following modulation, anti-T3 reactivity was similar to that obtained with the unreactive control ascites (data not shown). In contrast, anti-T3-modulated cells were unaffected in their expression of cell-surface T8 antigen.

The capacity of the T3 antigen to modulate was not a result of the subclass of the antibodies used for modulation as opposed to an intrinsic property of the antigens themselves because anti-$T3_A$ (IgM isotype) and anti-$T3_B$ (IgG1 isotype) also induced T3 to modulate. With anti-$T3_A$, modulation was a consequence of external shedding of T3 (32). This could be shown by the loss of immunoprecipitable T3 antigen from lysates of externally ^{125}I–radiolabelled cells following modulation with anti-T3 and by the lack of detectable cytoplasmic or membrane fluorescence after modulation with directly FITC-labelled anti-$T3_A$. Modulation of T3 antigen with either anti-$T3_A$ or anti-$T3_B$ had no effect on the viability of T-cell clones. Furthermore, once anti-T3 or anti-$T3_B$ was removed from cell culture supernatants, T3 antigen was reexpressed within 48 hr (32).

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FUNCTIONAL EFFECTS RESULTING FROM ANTI-T3 ANTIBODY–INDUCED MODULATION OF THE T3 SURFACE COMPLEX ON ANTIGEN-SPECIFIC HUMAN T-CELL CLONES

Although it had already been shown that anti-T3 binding to CTL inhibited their lytic activity (30, 32, 41), one could argue that the inhibitory effects of these monoclonal antibodies on CTL function were indirect and occurred as a consequence of antibody-induced agglutination of cells or steric blockade of still undefined but functionally important surface determinants. However, since anti-T3 led to T3 modulation by selective shedding of both the T3 antigen and the anti-T3 directed toward it without alteration of cell viability or change in the density of other T-cell antigens (including T1, T4, T8, T11, T12, and Ia), it was possible to examine the lytic activity of T3-modulated cells in the absence of surface-bound monoclonal antibody that could affect steric blockade (32, 41).

For this purpose, $CT8_I$ and $CT4_I$ cells were individually modulated with anti-$T3_A$ for 24 hr at 37°C to induce maximal loss of surface T3 antigen, washed extensively, and then examined either immediately for specific cytotoxic T-lymphocyte function (day 0 after modulation) or after 24 and 48 hr (days 1 and 2 after modulation). To rule out a nonspecific effect of modulation on clonal T-cell function, T4 or T8 clones were modulated with anti-T1 in parallel studies. Anti-T3 modulation of T4 and T8 clones markedly reduced cytotoxic effector function to less than 25% of maximal (Table 3). Perhaps more importantly, Table 3 indicates that cytotoxic function increased to approximately 60% of maximal when T4 and T8 clones were tested one day

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Table 3 Modulation of surface T3 antigen abrogates specific CTL effector function by cytotoxic T4 and T8 clones

	% maximal cytotoxicity[a]		
Days post modulation:	0	1	2
$CT8_I$			
Anti-T1 modulation	≧100	≧100	100
Anti-T3 modulation	22	66	100
$CT4_I$			
Anti-T1 modulation	≧100	≧100	100
Anti-T3 modulation	15	61	95

[a] $\%\ \text{maximal cytotoxicity} = \frac{\%\ \text{killing after modulation}}{\%\ \text{killing in unmodulated state}} \times 100$

after modulation and had achieved maximal levels two days after modulation. Given the fact that 48 hr were required to complete T3 antigen reexpression after anti-T3 modulation of T4 and T8 clones, this reestablishment of cytotoxic T-lymphocyte effector function appeared temporally related. The notion that T3 surface expression and clonal cytolytic function parallel one another after modulation is also supported by the finding that partial T3 reexpression one day after modulation, in comparison with cells immediately after modulation (day 0), was associated with increased CTL function.

Since some cytotoxic T-lymphocyte clones display antigen-specific proliferative responses, it was possible to determine whether anti-T3 modulation influenced antigen recognition (32). As shown in Table 4, the anti-$T3_A$-modulated $CT8_I$ clone had a significantly reduced proliferative response to the alloantigen Laz 156 in comparison with the unmodulated clone. In contrast, the modulated clone gave a greater proliferative response to IL-2 than the unmodulated clone. Moreover, in the presence of IL-2, following treatment with anti-T3, the clone's proliferation was equivalent to that manifested by the unmodulated clone stimulated by IL-2 plus Laz 156, suggesting that anti-

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Table 4 Anti-$T3_A$ modulation inhibits antigen-specific proliferation and enhances IL-2 responsiveness

	Proliferation of clone $CT8_I$	
Stimulus	Anti-$T3_A$ modulated	Unmodulated
Media	1,617 ± 370	2,949 ± 33
Laz 156	5,672 ± 522	23,211 ± 1,036
IL-2	20,810 ± 1,121	16,071 ± 398
IL-2 + Laz 156	21,042 ± 1,138	22,530 ± 520

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T3 triggered the cell in a fashion analogous to that of antigen itself. Taken together, these findings suggested that modulated loss of T3 surface molecules interferes with T-cell antigen recognition and that this is a specific effect since IL-2 responsiveness is enhanced and not diminished. Thus, the functional consequences of anti-T3-induced modulation cannot be explained on the basis of a nonlethal diminution in cell responsiveness.

CLONOTYPIC SURFACE STRUCTURES INVOLVED IN ANTIGEN-SPECIFIC HUMAN T-CELL FUNCTION

Given that T lymphocytes recognize antigen in a precise fashion, there had to exist, in addition, discriminative surface-recognition structures unique to individual T-cell clones. To delineate such "clonotypic" molecules, we produced monoclonal antibodies against the human cytotoxic T-cell clones $CT4_{II}$ and $CT8_{III}$ and developed a screening strategy that selects for anticlonotypic antibodies. Thus, we developed a series of noncrossreactive monoclonal antibodies that reacted only with the respective immunizing clone but not with a large number of additional T-cell clones from the same donor. These were termed anti-$Ti_{1A\text{-}B}$ ($CT8_{III}$) and anti-$Ti_{2A\text{-}C}$ ($CT4_{II}$) (25, 26).

Since the unique reactivities of anti-Ti monoclonals with $CT8_{III}$ and $CT4_{II}$ suggested that the surface structures defined were involved in the individual clonal specificity, we determined whether these antibodies could block recognition of antigen. To this end, we incubated $CT4_{II}$ and $CT8_{III}$ effector cells with one or another monoclonal antibody for varying periods prior to assay of the clones' cytolytic and proliferative capacities.

As shown in Table 5, both $CT4_{II}$ and $CT8_{III}$ efficiently lyse ^{51}Cr-labelled Laz 156 target cells (51% and 69%, respectively). In keeping with the above

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Table 5 Inhibitory effects of anticlonotypic monoclonal antibodies on cytotoxicity of clones $CT4_{II}$ and $CT8_{III}$[a]

	$CT4_{II}$	$CT8_{III}$
Medium	56[b]	69
Anti-$T3_A$	9	22
Anti-Ti_{1A}	55	6
Anti-Ti_{1B}	56	17
Anti-Ti_{2A}	7	69
Anti-Ti_{2B}	6	68
Anti-Ti_{2C}	6	69

[a] $CT4_{II}$ and $CT8_{III}$ effector cells were incubated with one or another monoclonal antibody in ascites form at a final dilution of 1:500 or medium for 30 min at room temperature before addition of ^{51}Cr-labelled Laz 156 target cells. The effector/target cell ratio was 30:1.

[b] % specific lysis

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findings, anti-T3 blocked the cytotoxic effector functions of both clones. In contrast, anti-$Ti_{2A\text{-}C}$ selectively inhibited killing by $CT4_{II}$ ($\leq$7%) but not $CT8_{III}$, whereas anticlonotypic antibodies anti-$Ti_{1A\text{-}B}$ blocked killing by the $CT8_{III}$ clone ($\leq$17%) but not $CT4_{II}$.

To examine the effects of anticlonotypic antibodies on the antigen-specific proliferative capacity of clones $CT4_{II}$ and $CT8_{III}$, we performed an additional series of experiments. As shown in Table 6, both $CT4_{II}$ and $CT8_{III}$ cells proliferate to purified IL-2 (4940 cpm and 3394 cpm, respectively) as well as the allogeneic cell line Laz 156 to which they had been originally stimulated (6796 cpm and 4671 cpm, respectively).

Pretreatment of $CT8_{III}$ with anti-Ti_1 or $CT4_{II}$ with anti-Ti_2 markedly reduces antigen-specific proliferative responses (561 cpm and 755 cpm, respectively). That these effects were not simply due to an inactivation of the clones as a result of antibody treatment is clear from the fact that the same anti-Ti-treated clones had augmented proliferative capacities to human IL-2. Moreover, it should be noted that monoclonal antibodies directed at the T4 and T8 surface glycoproteins lacked any inhibitory effect on antigen-specific clonal proliferation of $CT4_{II}$ and $CT8_{III}$.

TI AND T3 ARE ASSOCIATED IN THE T-CELL MEMBRANE

The observation that anti-T3, anti-Ti_1, and anti-Ti_2 all (*a*) inhibited both antigen-specific proliferation and CTL effector function, and (*b*) enhanced IL-2 responsiveness suggested a relationship between the cell-surface structures defined by these antibodies. To determine whether anti-T3-induced modulation produced changes in surface expression of the clonotypic Ti molecules, $CT8_{III}$ and $CT4_{II}$ cells were first incubated with anti-$T3_A$ for 18 hr at 37°C, then washed to remove free monoclonal antibody. Cell reactivity was analyzed

Table 6 Influence of anticlonotypic monoclonal antibodies on proliferative responses of clones $CT8_{III}$ and $CT4_{II}$[a]

	$CT8_{III}$		$CT4_{II}$	
Stimulus	Untreated	Anti-Ti_1 treated	Untreated	Anti-Ti_2 treated
Medium	124[b]	562	653	355
Laz 156	4,671	561	6,796	755
IL-2	3,394	7,359	4,940	12,737

[a] $CT8_{III}$ and $CT4_{II}$ cells were individually treated with clonotypic monoclonal antibodies for 18 hr at 37°C prior to incubation in a standard proliferative assay (3 × 10^4 cells/well).

[b] ^{3}H-TdR uptake (cpm)

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subsequently by indirect immunofluorescence on an Epics V cell sorter with a panel of monoclonal antibodies.

Importantly, anti-T3-induced modulation of T3 also resulted in loss of the anti-Ti_1 and anti-Ti_2 surface epitopes. This was not a nonspecific effect since the T8 or T4 antigen density was uninfluenced by this process (26). In addition, incubation of $CT8_{III}$ or $CT4_{II}$ cells with either anti-Ti_1 or anti-Ti_2 had identical effects: In all cases T3 and the respective Ti molecules comodulated. These results indicated that the molecules defined by anti-T3 and anti-Ti are functionally and phenotypically linked on the cell surface of these clones.

QUANTITATION OF SURFACE EXPRESSION OF T-CELL ANTIGENS

Given the strong evidence that the clonotypic structures are involved in antigen recognition and are membrane associated with T3, it was important to quantitate the number of surface Ti and T3 molecules on $CT4_{II}$, $CT8_{III}$, and resting peripheral blood T lymphocytes. Table 7 shows determinations of binding sites for monoclonal antibodies by quantitative indirect immunofluorescence on an Epics V cell sorter. Comparable results were obtained using ^{125}I-labelled monoclonal antibodies. Three points emerged from this analysis. First, the number of T3 and Ti molecules on each of the clonal populations is similar despite some interclonal variation in the absolute number of T3 and Ti molecules ($CT4_{II}$ = 30,000 molecules; $CT8_{III}$ = 42,000 molecules). This finding suggests a stochastic relationship in which one T3 molecule is linked to one Ti molecule in the cell membrane. Second, on the clonal populations, the

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Table 7 Quantitation of the binding sites for monoclonal antibodies[a]

Cell	Surface molecule	P.Ch.Fl. (R.L.Fl.)	Binding sites/cell
$CT4_{II}$	Ti_2	112 (900)	29,000
	T3	115 (950)	30,000
	T4	165 (3,700)	118,000
$CT8_{III}$	Ti_1	126 (1,300)	42,000
	T3	130 (1,500)	48,000
	T8	180 (5,500)	175,000
T cells	T3	105 (780)	25,000
	T4	118 (960)	30,000
	T8	140 (1,900)	60,000

[a]The number of binding sites for monoclonal antibodies was quantitated by indirect immunofluorescence using an Epics V cell sorter as well as ^{125}I-labelled monoclonal antibodies. Both methods gave identical results. P.Ch.FL. = peak channel fluorescence. R.L.FL. = relative linear fluorescence.

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density of the associative recognition structures T4 and T8 is far greater than T3/Ti (118,000 and 175,000 binding sites per cells, respectively). Third, in contrast to T3 (and presumably Ti) which is expressed to a similar degree on the resting T lymphocytes and T-cell clones, there are three to four times fewer T4 and T8 molecules on resting lymphocytes of the appropriate subset derivation (30,000 and 60,000, respectively) than on the respective activated clonal populations (25).

T-cell activation may thus result in induction and expression of additional associative recognition structures, T4 and T8. This probably offers one explanation for their critical involvement in clonal effector function. In contrast, the number of antigen receptors defined by anti-T3/Ti remains comparable and hence fully expressed in the resting state before antigen binding.

HUMAN CYTOTOXIC T-LYMPHOCYTE CLONES SPECIFIC FOR TUMOR CELLS

Recently a series of clonal lymphocyte populations was established that exhibits cytotoxic activity against tumor cell lines in vitro. Although each individual clone maintained stable phenotype and functional activity for prolonged periods, analysis of surface markers with monoclonal antibodies indicated that considerable heterogeneity existed within this group of tumor-specific cells. Thus, while some of the latter possessed a mature T-cell phenotype, others expressed distinct combinations of T lineage–associated surface structures but lacked the characteristic 20KD T3 molecule. One additional clone did not express any T cell–specific markers or any lineage-related differentiation antigens. Moreover, when investigated for cytotoxic effector function on a large panel of tumor cell lines, each clone had a unique target specificity (45, 46).

Two of these clones, termed JT9 and JT10 (phenotype T3+T8+T4−) and derived from the same donor in independent cloning experiments, have been characterized in further detail. It was assessed whether clone JT9, like the above "classical" CTL clones $CT8_{III}$ or $CT4_{II}$, interacts with targets via class I or class II MHC gene products. As shown in Table 8, preincubation of target cells with anti-HLA or anti-Ia antibodies that were able to block the cytotoxic effector functions of $CT8_{III}$ and $CT4_{II}$, respectively, did not affect lysis by JT9 cells. This finding was not surprising since JT9 killed targets such as K562 that do not express class I or class II alloantigens. The MHC-unrestricted T8+ clone JT9 was not influenced in its lytic capacity by blocking concentrations of anti-T8 monoclonals (not shown), further supporting the hypothesis that the T4 and T8 surface glycoproteins are involved in recognition of HLA and Ia antigens on target cells by classical CTL clones.

Perhaps more importantly, monoclonal antibodies directed against T3 strongly decreased cytotoxicity of JT9, indicative of the central role of the 20KD

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Table 8 Influence of anti-HLA and anti-Ia antisera on effector functions of clone JT9, $CT8_{III}$ and $CT4_{II}$

Effector clone:	JT9			$CT8_{III}$	$CT4_{II}$
Target:	K562	Molt-4	Laz 221	Laz 156	Laz 156
Antiserum					
Medium	81[a]	75	66	72	38
w6/32	89	81	79	22	39
anti-p29/34	90	73	70	66	3

[a] % specific lysis (standard deviation <15%)

glycoprotein in this effector function. In addition, a monoclonal antibody clonotypic for JT9, termed anti-NKTa (47), had a marked inhibitory effect on target-cell lysis. As indicated above for the various Ti molecules, the surface structure defined by anti-NKTa comodulated with T3 following incubation with the former or anti-T3. Given the likely possibility that NKTa was involved in target recognition and killing specificity by JT9 cells, one would expect that clones expressing identical clonotypic surface structures should have identical target specificities. That this is indeed the case is demonstrated in Table 9. A T3+T8+T4− tumor-specific and MHC-unrestricted clone termed JT10, which was established independently from JT9, also reacted with the anti-NKTa anticlonotype and displayed an identical pattern of cytotoxic activity toward a panel of 15 tumor cell lines tested. Note that an additional clone, JT3, which is anti-NKTa unreactive, had a quite distinct target specificity. Taken together, these studies indicated that MHC-unrestricted, tumor-specific T lymphocytes employ Ti-analogous, T3-associated clonotypes (NKT) for target recognition and discrimination (47). Moreover, they suggest that some effector cells with NK-like activity clearly lack a T3-Ti molecular complex and, given their unique killing specificity, must therefore use a different structure for target recognition.

ANTIGEN-LIKE EFFECTS OF MONOCLONAL ANTIBODIES DIRECTED AT T-CELL RECEPTOR STRUCTURES

If anti-Ti monoclonal antibodies define variable regions of the T-cell receptor, then under the appropriate conditions, anti-Ti antibodies might induce clonal T-cell activation in a fashion analogous to that of antigen itself. Because the alloantigens that serve as receptor ligands are membrane bound and likely interact via multi-point surface attachment, and because anticlonotypic monoclonal antibodies, by themselves, were not mitogenic for $CT4_{II}$ and $CT8_{III}$, we investigated the functional effects of purified monoclonal antibodies bound

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Table 9 Cytolytic activity of JT3, JT9, and JT10 cloned cells[a]

Effector cells	Targets														
	K562	KG-1	U937	HL60	Daudi	Laz 221	Nalm-1	Laz 461	Laz 156	Laz 388	Molt 4	HSB	HPB-ALL	JM	CEM
JT3	+[b]	−	−	−	+	−	−	−	−	−	+	+	+/−	+	−
JT9	+	+	+	−	+	+	+	−	−	−	+	+	+	+	+
JT10	+	+	+	−	+	+	+	−	−	−	+	+	+	+	+

[a]JT9 and JT10 express identical NKTa clonotypes, whereas JT3 is NKTa negative.
[b]Specific cytotoxicity: +, ≧45%; +/−, <45%; −, <10%. E/T ratio = 10:1.

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to a solid surface support. Anti-$T3_B$, anti-$T4_A$, anti-$T4_B$, anti-$T8_A$, anti-$T8_B$, anti-Ti_{2A}, and anti-Ti_{1B} were purified and covalently linked to CnBr-activated Sepharose beads. Subsequently, Sepharose-bound antibodies were incubated with one or another T-cell clone, and proliferative responses to these beads, Laz 156, and IL-2-containing media were measured in parallel. As expected from previous studies, $CT4_{II}$ and $CT8_{III}$ proliferated both to irradiated Laz 156 cells as well as to IL-2 containing media but not to the irrelevant B lymphoblastoid target Laz 475 (Figure 4). More importantly, Sepharose anti-Ti_{2A} and anti-Ti_{1B} stimulated a selective proliferation of $CT4_{II}$ and $CT8_{III}$, respectively, which is comparable to that obtained with the allogeneic cell Laz 156. In contrast, neither anti-T4 nor anti-T8 Sepharose-bound antibodies had any mitogenic effect. In addition, Sepharose anti-T3 induced a proliferative response in both clones (48).

From the above results it is clear that $CT8_{III}$ and $CT4_{II}$ can be induced to proliferate to specific antigen, Sepharose-linked anti-T3, or Sepharose coupled with the relevant anti-Ti antibodies in the absence of exogenous lymphokines. To next determine whether such activation might induce endogenous lymphokine production, $CT8_{III}$ or $CT4_{II}$ were incubated for 24 hr at 37°C with either irradiated Laz 156 or Sepharose-linked anti-Ti_{1B}, anti-Ti_{2A}, and anti-T3, respectively. Subsequently, supernatants were harvested and IL-2 activity calculated in units per milliliter. As shown in Table 10, alloantigen stimulation

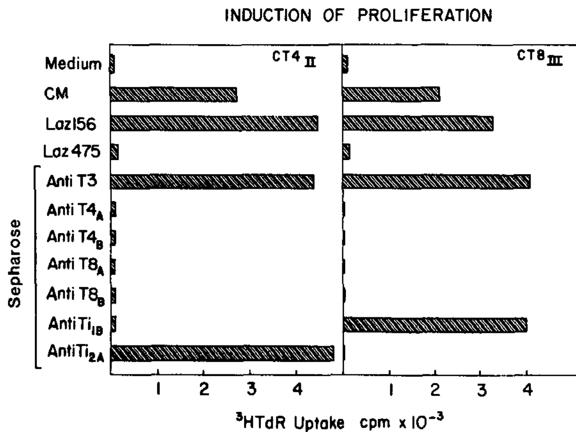

Figure 4 Induction of clonal proliferation by surface-bound monoclonal antibodies. $CT4_{II}$ or $CT8_{III}$ cells (3×10^4/ml) were incubated with medium, Laz 156 cells, Laz 475 cells (3×10^4/well; 5000 rad irradiated), IL-2 containing supernatants (CM) (5% final concentration), or monoclonal antibodies covalently coupled to Sepharose 4B beads. Cultures were individually pulsed after 24 hr with 1 μCi of ^{3}H-TdR and harvested 16 hr later. Values represent means of triplicates. Standard deviations were ≤12%.

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Table 10 Lymphokine secretion by $CT4_{II}$ and $CT8_{III}$[a]

Stimulus	T-cell clone	
	$CT4_{II}$	$CT8_{III}$
Laz 156	1.3[b]	1.2
Sepharose anti-T3	1.6	1.5
Sepharose anti-Ti_{1B}	<0.1	1.8
Sepharose anti-Ti_{2A}	2.0	<0.1

[a]Following incubation of $CT4_{II}$ or $CT8_{III}$ cells (5×10^6/ml) with one or another stimulus for 24 hr, supernatants were harvested, passed through 0.22 μ filters, and subsequently analyzed for IL-2 activity employing an IL-2-dependent human T-cell clone. Purified human IL-2 was used as a reference.
[b]IL-2 (U/ml)

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induced the release of significant amounts of IL-2 from both $CT4_{II}$ and $CT8_{III}$. More importantly, Sepharose-bound anti-T3 and Sepharose-bound anti-Ti directed at the relevant specificity (i.e. anti-Ti_1 for $CT8_{III}$ and anti-Ti_2 for $CT4_{II}$) also induced IL-2 secretion. It should be noted that the same culture supernatants could induce proliferation of $CT8_{III}$ and $CT4_{II}$ as well. Control supernatants from clones and stimulator combinations that did not result in clonal proliferation (i.e. Sepharose-anti-T4 and Sepharose-anti-T8) contained no detectable IL-2 activity. The finding that both the T8+ clone $CT8_{III}$ and the T4+ clone $CT4_{II}$ produce lymphokine upon specific stimulation confirms earlier findings that both human T-cell subsets contain populations capable of secreting IL-2 (49).

More recently, these findings have been extended by investigating a T3+T4+T8− human antigen–specific inducer T-cell clone, termed RW17C, which recognizes ragweed antigen E in the context of an autologous class-II MHC-gene product. A monoclonal antibody, anti-Ti_{4A}, which is clonotypic for RW17C, inhibited antigen-triggered proliferation and helper function for B-cell Ig secretion when added in soluble form. Perhaps more importantly, anti-Ti_4 coupled to Sepharose beads was capable of replacing the requirement for both antigen and MHC in the induction of helper function and clonal proliferation. In contrast, anti-T4 Sepharose failed to initiate proliferation or help (50).

A number of important points emerge from these experiments: (*a*) Antigen, anti-Ti and anti-T3 monoclonal antibodies produce very similar functional effects with regard to clonal proliferation and lymphokine secretion: (*b*) triggering of a single clonally unique epitope appears to be sufficient to induce antigen-specific functions and to substitute for antigen plus MHC determinant; (*c*) multimeric interaction between ligand and antigen receptor is an essential requirement for the initiation of clonal T-cell responses because nonsurface-linked monoclonal antibodies do not mediate these effects; (*d*) the T4 and T8

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surface structures, although critical for MHC-restricted cytotoxic T-lymphocyte (CTL) effector function are not likely involved in induction of clonal proliferation or lymphokine seretion; and (*e*) clonal proliferation to antigen or anticlonotypic antibody is due to endogenous IL-2 secretion. Moreover, given the observation that these clones respond to IL-2-containing media as well, it is clear that a single cell can, under physiologic conditions, both produce and respond to its own lymphokine (autocrine mechanism) (51).

TI: A 90KD DISULFIDE-LINKED HETERODIMER

Since all the above studies demonstrated that there existed a close functional and phenotypic relationship between the 20KD T3 glycoprotein and the Ti clonotype, it was important to define biochemically the surface molecules detected by anti-Ti monoclonal antibodies. Thus, solubilized membrane preparations were obtained from the externally ^{125}I-labelled $CT4_{II}$ and $CT8_{III}$ clones; antigens defined by anti-Ti antibodies were precipitated and electrophoresed on SDS-polyacrylamide gels (25). As shown in Figure 5, the molecule precipitated by anti-Ti_1 from ^{125}I-labelled $CT8_{III}$ cells appears as two bands and consists of a 49KD α chain and a 43KD β chain in reducing conditions (lane a). Moreover, in nonreducing conditions, this structure appears as a single band at ~90KD (lane c). In contrast, anti-Ti_1 does not immunoprecipitate material from the ^{125}I-labelled $CT4_{II}$ clone (lanes f and h). This is not surprising since anti-Ti_1 reacts with $CT8_{III}$ but not with $CT4_{II}$ by indirect immunofluorescence. In a reciprocal fashion, anti-Ti_2 precipitates material from ^{125}I-labelled $CT4_{II}$ (lanes e, g) but not $CT8_{III}$ (lanes b, d). The former appears as two bands of apparent molecular weights 51KD and 43KD on SDS-polyacrylamide gel electrophoresis (PAGE) in reducing conditions (lane e) and 90KD in nonreducing conditions (lane g). Thus, although the Ti_2 and Ti_1 antigens are comparable in molecular characteristics and are derived from T-cell clones of the same individual, they express unique structures that can be defined by noncrossreactive monoclonal antibodies. Although not shown, it should be noted that anti-Ti_4, which is clonotypic for RW17C, precipitated an analogous 90KD heterodimer from its respective clone consisting of a 41KD β and a 52KD α chain (50). The molecular basis for the slight difference in size and labelling intensities among the Ti α and β chains of the three clones is still unclear.

PEPTIDE VARIABILITY EXISTS WITHIN TI MOLECULES OF DIFFERENT T-CELL CLONES

To next determine whether there existed differences in pI (isoelectric point) among the α and β chains of Ti_1 and Ti_2, anti-Ti_1 and anti-Ti_2 immunoprecipitates were subjected to 2-D gel analysis employing isoelectric focusing in

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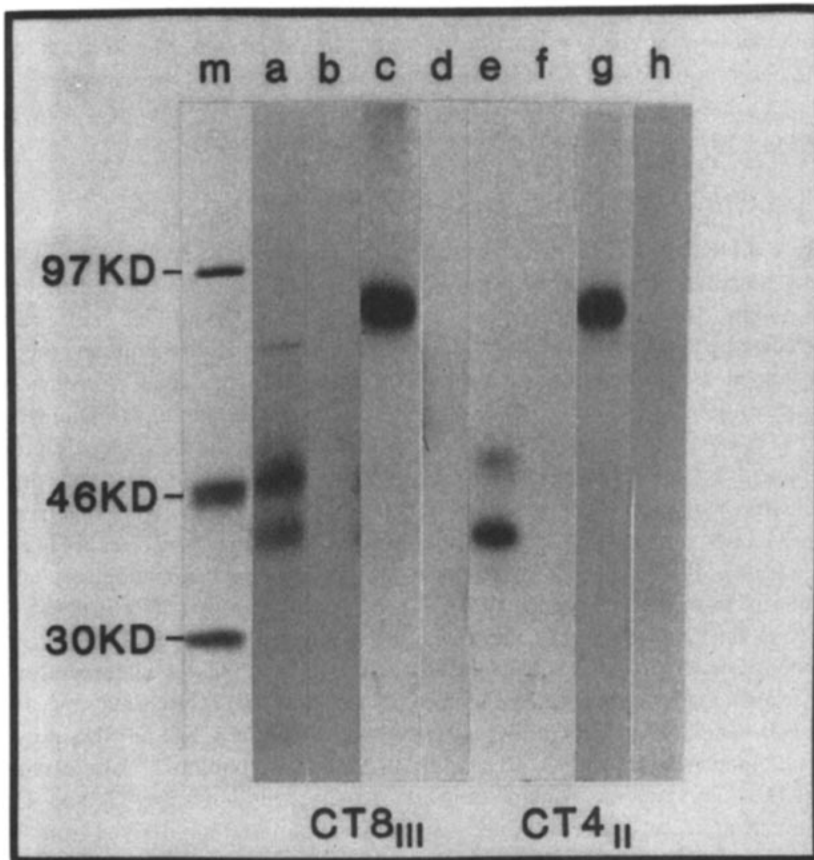

Figure 5 SDS-PAGE of ^{125}I-labelled Ti immune precipitates from $CT8_{III}$ and $CT4_{II}$ clones. SDS-PAGE was performed under reducing (R) and nonreducing (NR) conditions in a 12.5 polyacrylamide gel according to a modification of the Laemmli procedure followed by autoradiography. The following ^{14}C-methylated molecular weight markers (New England Nuclear, Boston, MA) were used (panel m): carbonic anhydrase (mol wt 30,000); ovalbumin (mol wt 46,000); phosphorylase b (mol wt 97,000). Panels a–d: $CT8_{III}$; panels e–h: $CT4_{II}$. a: anti-Ti_{1B} (R); b: anti-Ti_{2A} (R); c: anti-Ti_{1B} (NR); d: anti-Ti_{2A} (NR); e: anti-Ti_{2A} (R); f: anti-Ti_{1B} (R); g: anti-Ti_{2A} (NR); h: anti-Ti_{1B} (NR).

one dimension and SDS-PAGE in the second. As shown in Figure 6, a series of specific spots at 53KD migrate to the acidic side of the gel (mean pI 4.4) in the anti-Ti_2 precipitate from $CT4_{II}$. In contrast, note that the β subunit at 43KD is a more basic protein (mean pI 6.0) than Ti_2 α. A similar relationship

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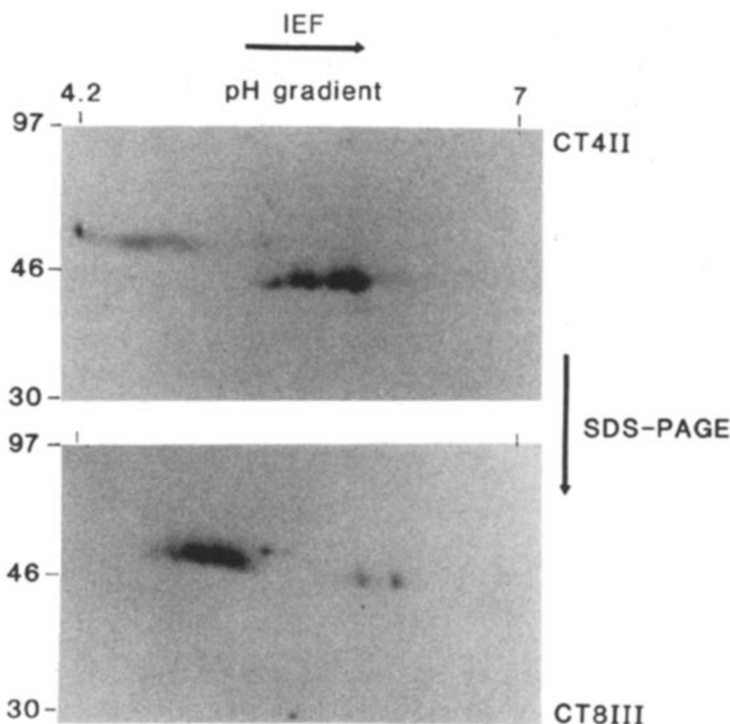

Figure 6 Two-dimensional gel analysis of Ti_2 and Ti_1 immunoprecipitated from $CT4_{II}$ and $CT8_{III}$, respectively. Ti_2 and Ti_1 were immunoprecipitated with the respective anticlonotypic antibodies coupled to Sepharose and analyzed in a 2-D gel system. The pH gradient was detected in a separate IEF gel run in parallel to the same experiment.

is noted between the more acid α and more basic β subunits of Ti_1 on $CT8_{III}$. However, the α subunits of Ti_1 and Ti_2 have distinct pIs (pI 4.4 vs 4.7, respectively) as do the β subunits (pI 6.0 vs 6.2) (52).

The resolution of the α and β subunits from Ti_1 and Ti_2 into a series of spots is likely secondary to differing numbers of sialic acid residues and strongly suggests that both are glycoproteins. The molecular basis for the slight differences in size and labelling intensities among the Ti α chains of the two clones is unclear but consistently demonstrated in both one- and two-dimensional gel analyses (25, 52).

To determine whether Ti_1 and Ti_2 differed in peptide structure, comparative peptide maps were performed on isolated ^{125}I-labelled subunits following

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digestion with proteolytic enzymes. As shown in Figure 7 (A and B), the tryptic peptide maps of the α chains of these two cell types appear very similar. At least one major peptide and one minor peptide (arrow) migrate to an identical position. Mixing experiments supported the conclusion that these two peptides were shared (Figure 7C). In addition, note that a cluster of several peptides with minimal mobility in the chromatographic dimension are likely related. In contrast, the remaining (unmarked) peptides are clearly distinct. Peptide maps made from pepsin digests of the two α clones also supported the notion that the subunits were similar but not identical (data not shown).

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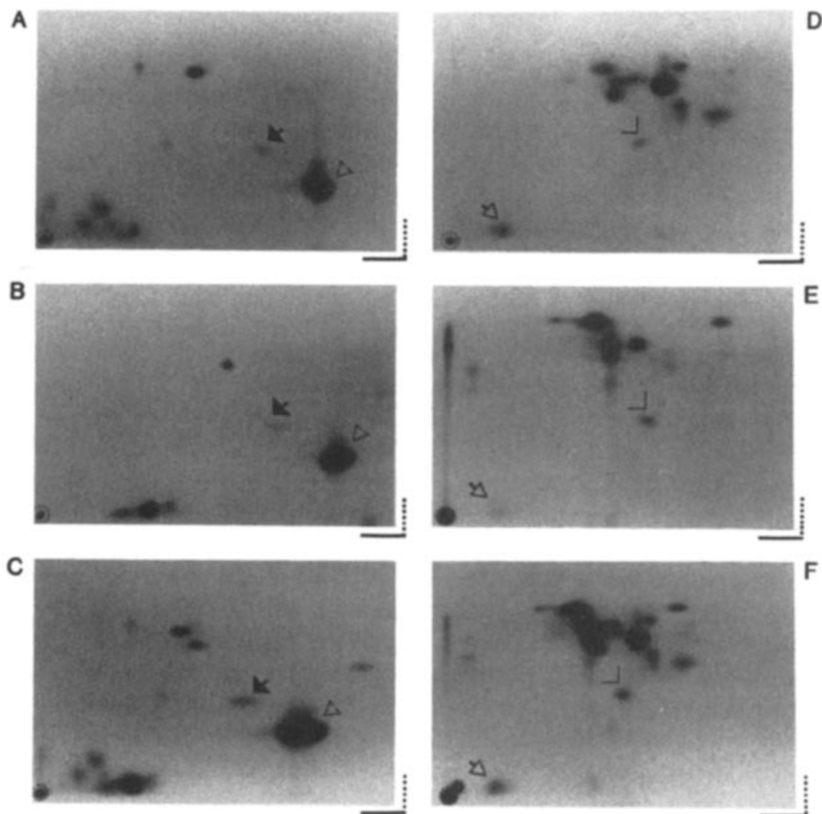

Figure 7 Two-dimensional peptide map comparison of ^{125}I-labelled α and β chains of Ti_1 and Ti_2. α Chains of Ti_1 (A) and Ti_2 (B) were digested with trypsin. C = mix of A + B. β Chains of Ti_1 (D) and Ti_2 (E) were digested with pepsin. F = mix of D + E. Each sample was spotted on a silica gel plate (origin: open circle) and peptides separated by electrophoresis (horizontal dimension: ———) and chromatography (vertical dimension: - - - -).

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The β chains, unlike the α chains, were not well digested by trypsin. Therefore, to make an adequate comparison of the β chains of $CT8_{III}$ and $CT4_{II}$, proteolysis with pepsin was required. As demonstrated in Figure 7 (D and E) and in contrast to the similarity of the Ti α chain, the peptide maps of the two β chains were dissimilar in overall appearance and shared only two minor spots. This was again confirmed in mixing experiments (Figure 7F). It is also important to note that parallel analysis of pepsin digestion of Ti_1 and Ti_2 α and β chains indicated that the individual subunits were comprised of totally distinct peptides and thus bore no precursor-product relationship (52).

The peptide-map comparisons provide unequivocal evidence that the Ti_1 and Ti_2 structures are analogs since they share several peptide fragments. In addition, these results imply that constant domains exist within the Ti α and Ti β subunits. The presence of unique peptides following proteolysis of different Ti molecules, isolated either directly by anticlonotypic monoclonal antibodies as shown here or indirectly by anti-T3 as reported previously (53), supports the notion that variable regions also exist. Based upon the present data, the most likely location of such a "V-region" equivalent would be within the β subunit. Nevertheless, it cannot as yet be ruled out that a second variable region might exist within the α subunit.

The extent of the peptide differences in the antigen receptor of these two clones is considerable but not surprising in view of their different specificities and the fact that they react with noncrossreactive monoclonal antibodies.

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A MODEL OF ANTIGEN RECOGNITION BY T CELLS

A summary of those human T-cell surface molecules involved in antigen recognition is shown in Table 11. These include the 20KD T3 molecules expressed on all T lymphocytes, the subset restricted 62KD T4 and 76KD T8 molecules linked to class II and class I CTL, respectively, and the clonally unique 90KD Ti molecules.

From these studies it is possible to construct a unifying hypothetical model of antigen recognition by T lymphocytes. As shown in Figure 8, each T cell possesses two recognition units on its surface. One structure responsible for antigen discrimination and/or binding consists of clonally unique Ti molecules, each of which is associated with one T3 glycoprotein. Since Ti is comprised of two polypeptides, one or both may be involved in antigen binding, analogous to the heavy and light chains of immunoglobulin.

Depending on the subset derivation of the individual T lymphocyte, the ancillary recognition structure is either T4 or T8. These glycoproteins do not appear to be critical for T-cell activation and may therefore rather be considered to serve as stabilizing elements that mediate the cell–cell contact necessary to allow for efficient target-cell lysis by CTL. In this model, the affinity of

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Table 11 Surface structures involved in antigen recognition by human T lymphocytes

T cell surface molecules	Mol wt	Distribution	Functional effect of monoclonal antibodies to the structure
T3	20,000	All mature T lymphocytes & a minority of thymocytes	1. Inhibits antigen-specific T-cell proliferative responses and cytotoxic effector function of all CTL 2. Enhances IL-2 responsiveness 3. Modulates by external shedding 4. Triggers clonal activation when surface linked
T4	62,000	Majority of thymocytes & 60% of peripheral T lymphocytes	Inhibits CTL effectors directed at class II MHC-gene products
T8	76,000[a]	Majority of thymocytes & 30% of peripheral T lymphocytes	Inhibits CTL effectors directed at class I MHC-gene products
Ti	90,000[b]	Specific for an individual T-cell clone (clonotypic). Similar disulfide-linked heterodimers are expressed on all peripheral T lymphocytes and T3+ thymocytes	1. Identical to anti-T3 effects but inhibits response only of an individual clone with which it reacts 2. Comodulates by external shedding with T3

[a]Nonreduced state; reduces to mol wt 33,000 and 31,000 subunits with 2-mercaptoethanol.
[b]Nonreduced state; reduces to mol wt 41,000–43,000 (α chain) and 49,000–53,000 (β chain).

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two sets of receptors for various ligands would be multiplicative. This might be particularly important for killer-target conjugate formation as well as triggering of primary immune responses prior to clonal selection of high-affinity antigen-responsive cells.

It seems likely that at the clonal level, effector cells exist that display high-affinity Ti/T3 receptors for specific antigen and thus do not require T4 or T8 in order to interact with stimulator/target cells. Indeed, such T-cell clones have been reported recently (10, 37, 46, 54). Given the extent of T-cell diversity at the clonal level, one might in addition expect to find an occasional clone viewing antigen plus MHC-gene product only with its high-affinity Ti/T3

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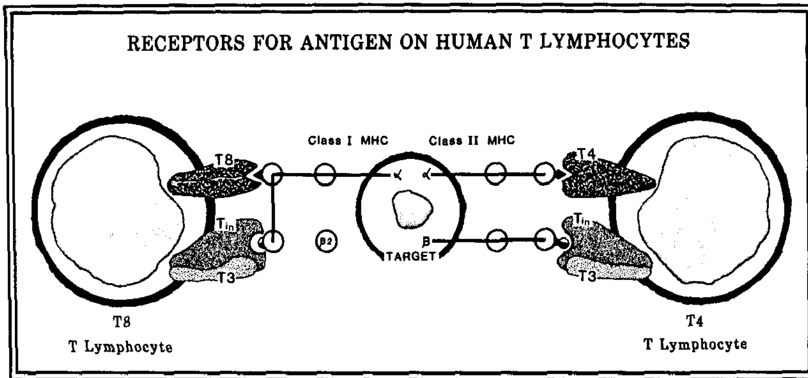

Figure 8 Model of antigen recognition by human T lymphocytes. Each T lymphocyte possesses two types of recognition structures. The T8 and T4 glycoproteins bind to nonpolymorphic regions of class I and class II MHC gene products, respectively. In contrast, T3-Ti recognizes specific antigen in the context of a polymorphic MHC gene product.

antigen receptor and in apparent contradiction to the T4-class II and T8-class I correlation (55).

CONCLUSION

Now that functional human T cell clones are available in a number of laboratories and clonotypic monoclonal antibodies exist that are directed at clones or Ti analogs on tumors of the human T lineage (56), it should be possible to identify the genes that encode constant and variable regions of the α and β subunits of the T-cell antigen receptor. This will in turn provide the basis for understanding the mechanism by which T-cell specificity and receptor diversity are generated and elucidate the relationship of the Ti α and β genes to immunoglobulin and MHC genes.

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Literature Cited

1. Benacerraf, B., McDevitt, H. O. 1972. Histocompatibility-linked immune response genes. *Science* 175:273
2. Schlossman, S. F. 1972. Antigen recognition: the specificity of T cells involved in the cellular immune response. *Transplant. Rev.* 10:97
3. Zinkernagel, R. M., Doherty, P. C. 1975. H-2 compatibility requirement for T cell mediated lysis of target infected with lymphocytic choriomeningitis virus. Different cytotoxic T cell specificities are associated with structures coded in H-2K or H-2D. *J. Exp. Med.* 141:1427
4. Corradin, G., Chiller, J. M. 1979. Lymphocyte specificity to protein antigens. II. Fine specificity of T cell activation with cytochrome c and derived peptides as antigenic probes. *J. Exp. Med.* 149:436
5. Hunig, T., Bevan, M. J. 1981. Specificity of T cell clones illustrates altered self hypothesis. *Nature* 294:460
6. Cerottini, J. C. 1980. Clonal analysis of cytolytic T lymphocytes and their precursors. *Prog. Immunol.* 4:622
7. Doherty, P. C. 1980. Surveillance of self: cell-mediated immunity to virally modified cell surface defined operationally by

Annual Reviews

the major histocompatibility complex. *Prog. Immunol.* 4:563
8. Quinnan, G. V., Kirmani, N., Rook, A. H., Manischewitz, J. F., Jackson, L., Moreschi, G., Santos, G. W., Sarai, R., Burns, W. H. 1982. Cytotoxic T cells in cytomegalovirus infection: HLA-restricted T lymphocyte non-T lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone marrow transplant recipients. *N. Engl. J. Med.* 307:7
9. Wallace, L. E., Rickinson, A. B., Rose, M., Epstein, M. A. 1982. Epstein-Barr virus specific cytotoxic T cell clones restricted through a single HLA antigen. *Nature* 297:413
10. Meuer, S. C., Hodgdon, J. C., Cooper, D. A., Hussey, R. E., Fitzgerald, K. A., Schlossman, S. F., Reinherz, E. L. 1983. Human cytotoxic T cell clones directed at autologous virus-transformed targets: Further evidence for linkage of genetic restriction to T4 and T8 surface glycoproteins. *J. Immunol.* 131:186
11. Gershon, R. K. 1974. T cell control of antibody production. *Contemp. Top. Immunol.* 3:1
12. Cantor, H., Boyse, E. A. 1977. Regulation of cellular and humoral immune responses by T cell subclasses. *Cold Spring Harbor Symposia on Quantitative Biol.* 41:23
13. Cohen, S., Pick, E., Oppenheim, J. J., eds. 1979. *Biology of the Lymphokines.* New York: Academic. pp. 179–95
14. Waldmann, T. A. 1978. Disorders of suppressor immunoregulatory cells in the pathogenesis of immunodeficiency and autoimmunity. *Ann. Int. Med.* 88:226
15. Cantor, H., Gershon, R. K. 1979. Immunological circuits: cellular composition. *Fed. Proc.* 38:2051
16. Lipton, J. M., Reinherz, E. L., Kudisch, M., Jackson, P. L., Schlossman, S. F., Nathan, D. G. 1980. Mature bone marrow erythroid burst forming units (BFU-E) do not require T cells for induction of erythropoietin-dependent differentiation. *J. Exp. Med.* 152:350
17. Reinherz, E. L., Schlossman, S. F. 1980. The differentiation and function of human T lymphocytes: A review. *Cell* 19:821
18. Reinherz, E. L., Schlossman, S. F., 1980. Regulation of the immune response: Inducer and suppressor T lymphocyte subsets in human beings. *N. Engl. J. Med.* 303:370
19. Reinherz, E. L., Schlossman, S. F. 1981. The characterization and function of human immunoregulatory T lymphocyte subsets. *Immunol. Today* 2:69
20. Morgan, D. A., Ruscetti, F. W., Gallo, R. C. 1976. Selective in vitro growth of T lymphocytes from normal human bone marrow. *Science* 193:1007
21. Kurnick, J. T., Gronvik, K. O., Kimura, A. K., Lindblom, J. B., Skoog, V. T., Sjogerg, O., Wigzell, H. 1979. Long-term growth in vitro of human T cell blasts with maintenance of specificity and function. *J. Immunol.* 122:255
22. Bonnard, G. D., Yasaka, K., Maca, R. D. 1980. Continued growth of functional human T lymphocytes: Production of human T cell growth factors. *Cell. Immunol.* 51:390
23. Sredni, B., Tse, H. Y., Schwartz, R. H. 1980. Direct cloning and extended culture of antigen specific, MHC-restricted, proliferating T lymphocytes. *Nature* 283:581
24. Meuer, S. C., Schlossman, S. F., Reinherz, E. L. 1982. Clonal analysis of human cytotoxic T lymphocytes: T4 and T8 effector T cells recognize products of different major histocompatibility regions. *Proc. Natl. Acad. Sci. USA* 79:4395
25. Meuer, S. C., Acuto, O., Hussey, R. E., Hodgdon, J. C., Fitzgerald, K. A., Schlossman, S. F., Reinherz, E. L. 1983. Evidence for the T3-associated 90KD heterodimer as the T cell antigen receptor. *Nature* 303:808
26. Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Hodgdon, J. C., Schlossman, S. F., Reinherz, E. L. 1983. Clonotypic structures involved in antigen specific human T cell function: Relationship to the T3 molecular complex. *J. Exp. Med.* 157:705
27. Reinherz, E. L., Kung, P. C., Goldstein, G., Levey, R., Schlossman, S. F. 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T lineage. *Proc. Natl. Acad. Sci. USA* 77:1588
28. Reinherz, E. L., Hussey, R. E., Schlossman, S. F. 1980. A monoclonal antibody blocking human T cell function. *Eur. J. Immunol.* 10:758
29. van Wauwe, F. P., DeMay, J. R., Goossener, J. G. 1980. OKT3: a monoclonal anti-human T lymphocyte antibody with potent mitogenic properties. *J. Immunol.* 124:2708
30. Chang, T. W., Kung, P. C., Gingras, S. P., Goldstein, G. 1981. Does OKT3 monoclonal antibody react with an antigen recognition structure on human T cells? *Proc. Natl. Acad. Sci. USA* 78:1805
31. Burns, G. F., Boyd, A. W., Beverley, P. C. I. 1982. Two monoclonal anti-human

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Annual Reviews

T lymphocyte antibodies have similar biologic effects and recognize the same cell surface antigen. *J. Immunol.* 124:1451
32. Reinherz, E. L., Meuer, S. C., Fitzgerald, K. A., Hussey, R. E., Schlossman, S. F. 1982. Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell* 30:735
33. Umiel, T., Daley, J. F., Bhan, A. K., Levey, R. H., Schlossman, S. F., Reinherz, E. L. 1982. Acquisition of immune competence by a subset of human cortical thymocytes expressing mature T cell antigens. *J. Immunol.* 129:1054
34. Reinherz, E. L., Morimoto, C., Penta, A. C., Schlossman, S. F. 1980. Regulation of B cell immunoglobulin secretion by functional subsets of T lymphocytes in man. *Eur. J. Immunol.* 10:570
35. Reinherz, E. L., Kung, P. C., Goldstein, G., Schlossman, S. F. 1979. Separation of functional subsets of human T cells by a monoclonal antibody. *Proc. Natl. Acad. Sci. USA* 76:4061
36. Reinherz, E. L., Kung, P. C., Pesando, J. M., Ritz, J., Goldstein, G., Schlossman, S. F. 1979. Ia determinants on human T cell subsets defined by monoclonal antibody: Activation stimuli required for expression. *J. Exp. Med.* 150:1472
37. Biddison, W. E., Rao, P. E., Thalle, M. A., Goldstein, G., Shaw, S. 1982. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens. Evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J. Exp. Med.* 156:1065
38. Krensky, A. M., Clayberger, C., Reiss, C. S., Strominger, J. L., Burakoff, S. J. 1982. Specificity of OKT4+ cytotoxic T lymphocyte clones. *J. Immunol.* 129:2001
39. Meuer, S. C., Schlossman, S. F., Reinherz, E. L. 1982. Differential activation and specificity of human T cell subpopulations. *UCLA Symp. Mol. Cell. Biol.* 24:127
40. Reinherz, E. L., Hussey, R. E., Fitzgerald, K. A., Snow, P., Terhorst, C., Schlossman, . F. 1981. Antibody directed at a surface structure inhibits cytolytic but not suppressor function of human T lymphocytes. *Nature* 299:168
41. Meuer, S. C., Hussey, R. E., Hodgdon, J. C., Hercend, T., Schlossman, S. F., Reinherz, E. L. 1982. Surface structures involved in target recognition by human cytotoxic T lymphocytes. *Science* 218:471
42. Meuer, S. C., Cooper, D. A., Hodgdon, J. C., Hussey, R. E., Morimoto, C., Schlossman, S. F., Reinherz, E. L. 1983. Immunoregulatory human T lymphocytes triggered as a consequence of viral infection: Clonal analysis of helper, suppressor inducer and suppressor effector cell populations. *J. Immunol.* 131:1167
43. von Wussow, P., Plastsoucos, C. D., Wiranowsha-Stewart, M., Stewart, W. E. II. 1981. Human γ interferon production by leukocyte induced with monoclonal antibodies recognizing T cells. *J. Immunol.* 127:1197
44. Palacios, R. 1982. Cloned lines of interleukin 2 producer human T lymphocytes. *J. Immunol.* 129:2586
45. Hercend, T., Meuer, S. C., Reinherz, E. L., Schlossman, S. F., Ritz, J. 1982. Generation of a cloned NK cell line derived from the "null cell" fraction of human peripheral blood. *J. Immunol.* 129:1299
46. Hercend, T., Reinherz, E. L., Meuer, S. C., Schlossman, S. F., Ritz, J. 1983. Phenotypic and functional heterogeneity of human cloned natural killer cell lines. *Nature* 301:158
47. Hercend, T., Meuer, S. C., Brennan, A., Edson, M. A., Acuto, O., Reinherz, E. L., Schlossman, S. F., Ritz, J. 1983. Identification of a clonally restricted 90KD heterodimer on two human cloned natural killer cell lines. *J. Exp. Med.* 158:1547
48. Meuer, S. C., Hodgdon, J. C., Hussey, R. E., Protentis, J. P., Schlossman, S. F., Reinherz, E. L. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988
49. Meuer, S. C., Hussey, R. E., Penta, A. C., Fitzgerald, K. A., Stadler, B. M., Schlossman, S. F., Reinherz, E. L. 1982. Cellular origin of interleukin 2 (IL-2) in man: Evidence for stimulus-restricted IL-2 production by T4+ and T8+ T lymphocytes. *J. Immunol.* 129:1076
50. Meuer, S. C., Cooper, D. A, Hodgdon, J. C., Hussey, R. E., Fitzgerald, K. A., Schlossman, S. F., Reinherz, E. L. 1983. Identification of the antigen/MHC-receptor on human inducer T lymphocytes. *Science* 222:1239
51. Meuer, S. C., Hussey, R E., Cantrelli, D. A., Hodgdon, J. C., Schlossman, S. F., Smith, K. A., Reinherz, E. L. 1984. Triggering of the T3-Ti antigen receptor complex results in clonal T cell proliferation via an interleukin 2 dependent autocrine pathway. *Proc. Natl. Acad. Sci. USA.* In press
52. Acuto, O., Meuer, S. C., Hodgdon, J. C., Schlossman, S. F., Reinherz, E. L. 1983. Peptide variability exists within α and β subunits of the T cell receptor for antigen. *J. Exp. Med.* 158:1368
53. Reinherz, E. L., Meuer, S. C., Fitzger-

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ald, K. A., Hussey, R. E., Hodgdon, J. C., Acuto, O., Schlossman, S. F. 1983. Comparison of T3-associated 49 and 43 kilodalton cell surface molecules on individual human T cell clones: Evidence for peptide variability in T cell receptor structures. *Proc. Natl. Acad. Sci. USA* 80:4104

54. Malissen, B., Rebai, N., Liebeuf, A., Mawas, C. 1982. Human cytotoxic T cell structures associated with expression of cytolysis. I. Analysis at the clonal level of the cytolysis-inhibiting effect of 7 monoclonal antibodies. *Eur. J. Immunol.* 12:739

55. Spits, H., Ijssel, H., Thompson, A., deVries, J. E. 1983. Human T4+ and T8+ cytotoxic T lymphocyte clones directed at products of different class II major histocompatibility complex loci. *J. Immunol.* 131:678

56. Acuto, O., Hussey, R. E., Fitzgerald, K. A., Protentis, J. P., Meuer, S. C., Schlossman, S. F., Reinherz, E. L. 1983. The α and β subunits of the human T cell receptor: Their appearance in ontogeny and biochemical relationship to one another on IL-2 dependent clones and T cell tumors. *Cell* 34:717

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:23-50. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:23-50. Downloaded from arjournals.annualreviews.org
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THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS

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INTRODUCTION

It is now widely accepted by immunologists that antigen recognition by most T lymphocytes occurs through the simultaneous recognition of antigen and a self molecule coded for by genes of the Major Histocompatibility Complex (MHC). This fact has considerably complicated our understanding of antigen recognition by T cells, a situation obvious in the literature of the last decade on the T-cell receptor for antigen. Much effort has gone into characterization of the T-cell receptor based on its presumed similarity to immunoglobulin, the antigen receptor on B lymphocytes. In general, studies have focused on the characterization of antibody-like factors, isolated from T cells, that are involved in antigen recognition and that apparently bind antigen in the absence of MHC products. Described in many reports, these T-cell products have been designated as helper or suppressor factors, react with antiimmunoglobulin antisera, and in some cases have been shown to be ~ 70,000 mol wt proteins with or without disulfide-bonded subunits. These studies have been summarized in recent reviews (1-11) and are not discussed here. Suffice it to say that the relationship between these factors and the receptors on T cells that recognize antigen in association with MHC products is still unknown. Certainly no experiments have conclusively demonstrated any role for immunoglobulin genes in antigen recognition by MHC-restricted T cells (12-17).

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Annual Reviews

In the past year (1983), new evidence has provided a major breakthrough in our understanding of the receptor for antigen on MHC-restricted T cells. These developments began with the efforts of several laboratories to raise antisera to cloned lines of T cells or T-cell hybridomas in the attempt to find antibodies directed toward receptor material on these clones. For example, Infante et al (18) reported production of antisera in mice that stimulated the immunogen T-cell clone (but no other T-cell clones) to proliferate. Subsequently, we published a report on the use of I region–restricted antigen-specific T-cell hybridomas to produce antireceptor antibodies in mice that could specifically block the function of the T-cell hybrids used in immunization (19). Similar results have been reported by others on antisera containing antibodies interfering with antigen recognition by MHC-restricted T-cell clones (20-22).

The next critical development was the isolation by several laboratories of monoclonal antibodies with antireceptor characteristics. In this review we will deal exclusively with the nature of the MHC-restricted T-cell receptor for antigen as defined by these most recently described antibodies. We show that the antireceptor properties of these antibodies include the following: (*a*) They are practically all clone-specific; (*b*) they block or stimulate response of the target T cells in a specific fashion; (*c*) reaction with these antibodies can predict specificity for antigen and MHC of a T cell; (*d*) the antibodies specifically precipitate similar but not identical structures from their respective target T-cell clones; and (*e*) the antibodies toward different T-cell clones recognize structures with both variable and constant peptide regions.

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CLONE-SPECIFIC ANTIBODIES DEFINE THE ANTIGEN RECEPTORS ON MHC-RESTRICTED T CELLS

The antisera raised in mice to antigen receptors on MHC-restricted T cells, as well as the subsequent monoclonal antibodies obtained from fusions of spleen cells from these animals, have in general proved to be specific in their ability to bind the immunogen T-cell clone. One of the first reports of a T-cell clone-specific antibody was by Allison and his co-workers (23). In characterizing tumor antigens on a T-cell lymphoma, C6XL, with monoclonal antibodies directed toward the lymphoma, these investigators discovered one antibody, MAb 124-40, that was highly specific for the C6XL cell line. The antibody 124-40 neither reacted with other lymphomas nor bound to normal lymphoid cells. Meuer et al (24) reported that isolation of two clone-specific antibodies raised toward a cloned line of human cytotoxic T cells (CTL). These monoclonal antibodies, termed anti-Ti_{1A} and anti-Ti_{1B}, were analyzed by indirect immunofluorescence studies for binding to a variety of T-cell types and were found to be highly specific for the immunizing T-cell clone. A third report of

Annual Reviews

a clone-specific monoclonal antibody was made by Fitch and his co-workers (25), who obtained antisera by immunizing mice with a cloned alloreactive CTL line, L3. A monoclonal antibody, 384.5, was produced that showed specific binding only to the immunizing L3 clone.

In our attempts to obtain antireceptor antibodies, we immunized mice with murine T-cell hybridomas [prepared in our laboratory by methods described previously (26-28)] that have defined antigen and MHC specificities. The presence of receptor for antigen on these T-cell hybridomas can be demonstrated by their ability to secrete the lymphokine, Interleukin 2 (IL-2), in response to antigen and antigen-presenting cells bearing the appropriate MHC products (27). Antireceptor antibodies were detected by their ability to interfere with the IL-2 response to antigen/MHC of the immunizing hybridoma. These antibodies were inhibitory and highly specific in their reactions with the immunogen T-cell clones. We then selected mice with high titers of inhibitory antisera for fusions in the attempt to isolate monoclonal antibodies. To date, two such antibodies have been isolated in our laboratory. The first monoclonal antibody, KJ1-26, described elsewhere (29), was shown to be specific for a T-cell hybridoma, DO-11.10, specific for chick ovalbumin (cOVA) and I-A^d. Antibody KJ1-26 bound only to DO-11.10 and a positive subclone, DO-11.10.24; no binding of the antibody could be demonstrated with any other T-cell hybrid tested or with negative variants of DO-11.10, subclones that had lost their ability to make IL-2 in response to cOVA/I-A^d. A second monoclonal antibody, KJ12-98, was isolated later and is described in a more recent publication (30). KJ12-98 was raised toward a T-cell hybridoma, 3DT-52.5 (see 31), which is unusual in being the only T-cell hybrid we have found with specificity for a Class I MHC molecule, D^d. This hybrid is apparently self-reactive, producing IL-2 in response to D^d in the absence of any other defined antigen. In comparing the two antibodies by ELISA assay, it was found that KJI-26 showed specific binding for DO-11.10 and that KJ12-98 bound only to 3DT-52.5; no binding by either antibody could be observed with any of a number of other BALB/c-derived T-cell hybridomas or the tumor parent, BW 5147.

Other monoclonal antibodies specific for cloned lines of T cells or T-cell hybridomas have recently been reported. Samelson & Schwartz (21, 32) reported the isolation of clone-specific antibodies that bind a murine T-cell hybridoma specific for pigeon cytochrome c but do not bind other hybrids with similar specificities. Antisera and a monoclonal antibody specific for cloned helper T-cell lines have been described recently by Kaye et al (22). Another report of clone-specific monoclonal antibodies has come from Bigler et al (33), who immunized mice with a human T-cell leukemia. Most recently, Staerz et al (34) have reported isolation of two monoclonal antibodies specific for a murine CTL clone.

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The clone-specificity indicated by the binding studies using these antibodies suggests that they are directed toward unique and thus idiotypic determinants on the T-cell clones to which they are directed. The demonstration of the antiidiotypic nature of these antibodies is greatly extended in the functional studies described in the next section. From the binding studies alone, however, it is apparent that these antibodies are quite different from those described in earlier studies of antisera directed toward T-cell "allotypes" or "isotypes" (see 1-6). Indeed, as Kaye et al (22) have pointed out, it is surprising that the immunization protocols with cloned T-cell lines have resulted primarily in antibodies showing such great clone-specificity and thus presumably directed toward "variable" regions on T-cell antigen receptors. There do appear, however, to be some exceptions to this rule as a few antireceptor antibodies have now been reported that react with more than one clone of T cells (33, 35). Such antibodies could conceivably be directed toward "constant" or "framework" regions on receptors and are discussed at greater length below.

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ANTIRECEPTOR ANTIBODIES INHIBIT AND/OR STIMULATE FUNCTION OF TARGET T CELLS

As indicated above, the antibodies directed toward antigen receptors on MHC-restricted T-cell clones have in many cases been shown to block effector function of the target T cells. Alternatively, in some instances, these antibodies mimic antigen and MHC products by stimulating response of the target T cells. In our experience, the antisera isolated from mice immunized with antigen-specific, MHC-restricted T-cell hybridomas have always been inhibitory in their effects on IL-2 production by the T-cell hybrids (19). At no time have we observed antisera that showed stimulatory effects, a somewhat surprising result in view of the reports by others (18, 22, 34) of antireceptor antisera with these properties. Our monoclonal antibodies, KJ1-26 and KJ12-98, reflect the properties of the antisera of the mice from which they came in that both antibodies were found to be highly inhibitory, blocking the IL-2 response to antigen/MHC of the target T-cell hybrids over a wide titration range. Unlike the antisera, however, the monoclonal antibodies can, under certain conditions, stimulate IL-2 response of the T-cell hybrids. At high concentrations and in the absence of antigen, the monoclonal antibody KJ12-98 was observed to stimulate IL-2 production of 3DT-52.5 (30). This ability of antibody alone to mimic antigen/MHC was not shared by the other monoclonal antibody, KJ1-26, specific for the T-cell hybrid DO-11.10. On the other hand, if the antibodies were first rendered polyvalent by coupling them to Sepharose beads, they could both stimulate IL-2 production. As shown earlier with KJ1-26-coupled beads (29) and more recently with both antibodies (30), at very low concentrations of antibody-coupled beads, the target T-cell hybrids were stim-

Annual Reviews

ulated to make IL-2 in the absence of antigen/MHC. As previously seen in the binding or inhibition studies with these antibodies, they were found to be specific in their interaction with the target T cells: Beads coupled with KJ1-26 stimulated only the response of DO-11.10; beads coupled with KJ12-98 stimulated only the response of 3DT-52.5.

In almost all cases, the other clone-specific antisera or monoclonal antibodies mentioned above have also been shown to have effects on the functions of their target T cells. The "anti-idiotypic" antisera raised in mice against alloreactive T-cell clones by Infante et al (18) could specifically stimulate both proliferation and IL-2 secretion of the relevant T cells. The clone-specific monoclonal antibodies to receptor material on a human CTL line described by Meuer et al (24) were found to specifically inhibit cytolytic activity of the target clone and had no effect on lysis by other T-cell lines in a panel of 5 CTL clones. In like manner, the antireceptor antibodies specifically inhibited antigen-induced proliferation of the target CTLs. More recently, these authors have described other clone-specific antibodies with similar properties, one toward another normal T-cell clone and three directed toward a human T-cell tumor, REX (36, 37). In one of these reports, studies with the antibodies to normal cells showed that when coupled to Sepharose beads the antibodies could induce proliferation and IL-2 secretion by the target CTL clone (36). This result confirms our observation with the bead-coupled monoclonal antibodies stimulating IL-2 response of the T-cell hybridomas DO-11.10 and 3DT-52.5, providing further evidence that these antibodies in polyvalent form can mimic antigen/MHC. A report by Lancki et al (25) of a clone-specific antibody capable of inhibiting function of the target clone describes the monoclonal antibody, 384.5, which is directed toward a murine cytotoxic T-cell clone, L3. The antibody was found to specifically inhibit cytolysis by L3 and showed no effect on some 90 other cytolytic clones. Samelson & Schwartz (21, 32) have described antireceptor antibodies that specifically inhibit IL-2 production by a T-cell hybridoma, 284, which is B10.A derived and specific for pigeon cytochrome c. The anti-2B4 antibodies inhibited only the antigen/MHC-induced production of IL-2 by the 2B4 hybrid and did not affect activation by Con A, an observation we too made in the case of KJ1-26 antibody inhibition of DO-11.10 response (29).

The ability of clone-specific antibodies to activate T cells has more recently been reported by Kaye et al (22), who have isolated antisera and a monoclonal antibody specific for cloned helper T-cell lines. It was found that the antibodies could induce T cell–dependent B-cell activation and stimulate proliferation of the target T-cell clone, even at quite low concentrations, a finding somewhat different from our observations of antibody substituting for antigen and MHC only at high concentrations or in polyvalent form. Another report of antibodies that both block and activate T cells has been made by Staerz et al (34), who

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have described two monoclonal antibodies, FIG3.1 and F2A11.5, specific for an alloreactive CTL clone, G4, derived from BALB.b mice immunized with H-2^d cells. The antibodies blocked cytotoxic activity of only the G4 clone in chromium release assays; they also had the interesting property of specifically inducing production of immune interferon from this clone. Like the antibody 3D3, reported by Kaye et al (22) and described above, the anti-G4 antibodies at low concentrations could stimulate the target clone, thus mimicking the action of antigen/MHC or mitogen.

The properties of the antibodies just described further illustrate their clone-specificity, but more importantly they provide more direct information about the antigen recognition event in MHC-restricted T cells. The antireceptor antibodies react with T-cell idiotypes either to block the T-cell recognition of antigen and MHC or to mimic antigen/MHC by stimulating a response by T cells. The latter finding that the antibodies under certain circumstances can substitute for *both* the antigen *and* MHC recognition by the T cell is particularly interesting since it suggests that these antibodies react with the T-cell binding molecule for both antigen and MHC—i.e. a single receptor is responsible for recognition of both "ligands" (27, 38, 39).

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ANTIRECEPTOR ANTIBODIES CAN PREDICT T-CELL ANTIGEN AND MHC SPECIFICITY

The evidence reviewed to this point has revealed two important properties of antibodies directed toward T-cell receptors for antigen/MHC: The antireceptor antibodies are nearly all clone-specific, and where T-cell function can be defined they block or activate the target T-cell clone. To further establish the antireceptor characteristics of such antibodies, we designed studies to see whether an antireceptor antibody could detect idiotype on another T-cell clone. This work, described elsewhere (40), is summarized here.

The T-cell hybridoma DO-11.10, to which a clone-specific monoclonal antibody, KJ1-26, was isolated, will produce IL-2 in the presence of cOVA and I-A^d bearing antigen-presenting cells. The hybridoma exhibits a rare fine specificity in its response to several other avian OVAs in the context of H-2^d and to other H-2 haplotypes in the presence or absence of cOVA, a property determined through comparison with over 200 other BALB/c-derived T-cell hybrids, none of which exhibited the same fine specificity pattern as DO-11.10. In previous testing, we had found that the monoclonal antibody KJ1-26 was specific in its reactivity with DO-11.10. If KJ1-26 could be shown to react with any other T-cell hybridoma, would that line prove to have an antigen/MHC receptor with the same specificities as those of the DO-11.10 receptor? Of approximately 400 T-cell hybridomas tested for reactivity with KJ1-26, one hybrid was found that bound the antibody strongly. This hybri-

Annual Reviews

doma, 7DO-286.2, was shown to have a fine specificity pattern identical to that of DO-11.10 in its response to both antigen and H-2 products. Both T-cell hybridomas recognize the same tryptic peptide of cOVA. Thus reaction with the monoclonal antibody KJ1-26 predicted exactly the specificity of a T-cell hybridoma for both antigen and MHC, proving that the monoclonal antibody did indeed react with part or all (probably all) of the receptor(s) for antigen plus MHC on that hybridoma. The fact that this idiotype is relatively rare (only one of about 400 cOVA-specific T-cell hybridomas bore it) suggests that the repertoire of T-cell receptors might be large. The fingerprints of the surface-labeled tryptic peptides derived from receptors on DO-11.10 and 7DO-286.2 are identical, an interesting result the implications of which are discussed in greater detail below.

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ANTIRECEPTOR ANTIBODIES ARE DIRECTED TOWARD DISULFIDE-BONDED HETERODIMERS

The characteristics of antireceptor antibodies to T cells reviewed to this point have been defined by binding and functional studies, but we have also accumulated considerable information on the biochemistry of the molecules recognized by these antibodies. The first report of a product specifically precipitated from T cells by a clone-specific antibody was by Allison et al (23), who showed by two-dimensional SDS-PAGE that the monoclonal antibody 124-40 to the T-cell lymphoma C6XL could precipitate a disulfide-linked glycoprotein with subunits of mol wt 39,000 and 41,000. These authors also analyzed whole-cell lysates and found that disulfide-bonded proteins with molecular weights similar to that of the C6XL molecule existed on other T cells but not on B cells, a result similar to that reported earlier by Goding & Harris (41). Although the clone-specificity of 124-40 indicated that the heterodimer it recognized might be the T-cell antigen receptor, this possibility could not be further investigated because it was not possible to show any functional role for the molecule on C6XL. Meuer et al (24), in their studies with the anti-Ti_{1A} and anti-Ti_{1B} antibodies directed to the human CTL clone $CT8_{III}$, also observed a specifically precipitated product. When subjected to SDS-PAGE, immunoprecipitates of ^{131}I-labeled material from $CT8_{III}$ cells yielded two bands at molecular weights of ~ 49,000 and ~ 43,000. In a later publication (42), these authors showed that the 49,000- and 43,000-dalton structures seen under reducing conditions migrated as a single broad band of 80,000–90,000 daltons when run under nonreducing conditions, suggesting that the molecule recognized by their clone-specific antibodies is a disulfide-linked structure. Similar but not identical results were observed in the SDS-PAGE patterns of immunoprecipitates from the human tumor line REX (37). The anti-Ti_3 antibodies

Annual Reviews

to this line precipitated a 94,000-dalton molecule that reduced to subunits of 53,000 and 44,000 daltons, slightly larger than the bands observed in material from normal human cell clones.

We reported the isolation of a disulfide-bonded structure from the murine T cell hybridoma DO-11.10 (29). This molecule was immunoprecipitated from ^{125}I-labeled extracts of DO-11.10 by the monoclonal antibody KJ1-26, described above. Under nonreducing conditions, the protein precipitated by KJ1-26 migrated at 80,000–90,000 daltons on SDS-PAGE gels; upon reduction, the molecule appeared as a diffuse band of ~ 43,000 daltons. The specificity of the 80,000–90,000-dalton product was clearly demonstrated in a comparison of immunoprecipitates from DO-11.10 and its negative variant DO-11.10.3 (negative subclones of DO-11.10 were identified by their inability to produce IL-2 in response to antigen/MHC); no specific band could be detected in SDS gel analysis of the latter. This specificity was further illustrated in immunoprecipitation studies carried out with antibody-coupled Sepharose beads in which it was shown that the 43,000-dalton band obtained after reduction was specifically precipitated from lysates of DO-11.10 by KJI-26 beads but not by beads coupled to an irrelevant antibody, HOPC-1. When KJI-26 was used to precipitate material from the T-cell hybridoma 7DO-286.2, which bears the same idiotype as DO-11.10, a molecule with properties identical to the product on DO-11.10 was observed (40). We compared the reactivity of a second monoclonal antibody, KJ12-98, directed toward the T-cell hybridoma 3DT-52.5, to that of the first antibody, KJ1-26 (30). As mentioned above, KJ12-98 antibody displayed binding and blocking properties similar to those of KJ1-26 but reacted somewhat differently in its ability to activate the target hybrid at high concentrations of antibody. The immunoprecipitation patterns of KJ12-98 with 3DT-52.5 were also similar to those obtained with KJ1-26 and DO-11.10 but differed in some respects. Whereas KJ1-26 precipitated an 80,000–90,000-dalton molecule from DO-11.10 which reduced to subunits of 43,000 daltons, the KJ12-98 antibody brought down from 3DT-52.5 a slightly smaller protein of 75,000–80,000 daltons, which under reducing conditions appeared as two bands on SDS-PAGE with molecular weights of 43,000 and 40,000.

Biochemical studies from other laboratories have provided further evidence that a disulfide-bonded structure with the molecular weight characteristics described above constitutes the antigen receptor on MHC-restricted T cells. The anti-2B4 antibody to the cytochrome c–specific T-cell hybrid 2B4, described by Samelson & Schwartz (21, 32), precipitated an 85,000–95,000-dalton heterodimer that upon reduction resolves into subunits of 45,000–50,000 daltons and 40,000–44,000 daltons. Again, the product was specific and could not be precipitated from other cell lines. In studies with a clone-specific antibody to a murine helper T-cell clone, Kaye et al (22) have reported preliminary

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biochemical findings of a specific 80,000-dalton product consisting of two 40,000-dalton disulfide-bonded subunits. Bigler et al (33) have published SDS-PAGE data obtained from immunoprecipitates of a human T-cell leukemia line. They observed an approximately 80,000-dalton species under nonreducing conditions that migrated to two bands of ~ 43,000 daltons and ~ 49,000 daltons. Their finding corresponds closely to our observations in the analysis of another human T-cell leukemia, HPB-MLT (43). This line was described by Minowada et al (44) in a study of a whole panel of human T-cell leukemias. The monoclonal antibody T40/25 precipitates from HPB-MLT an 85,000–90,000-dalton dimer with two subunits of 46,000 and 40,000 daltons. Still another disulfide-bonded structure has been reported by Staerz et al (34) in studies of a murine CTL clone, G4. Specific immunoprecipitations from this line yielded an approximately 80,000-dalton protein with two subunits, each of about 42,000 daltons.

From the studies just cited, it can be seen that the clone-specific antibodies to different T-cell lines are precipitating similar molecules, all of which are disulfide-bonded heterodimers. It can also be seen, however, that comparisons between studies on human T cells and those on murine lines reveal certain differences. In all the reports of receptor material isolated from human T-cell clones, the heterodimer precipitated from these lines is made up of subunits of distinctly different molecular weights. On the other hand, data obtained from murine T-cell clones indicate that the T-cell receptor in the mouse is somewhat different; in most cases the subunit structure of the molecule cannot be resolved easily on the basis of molecular weight (30). For example, in our studies of material immunoprecipitated from the murine T-cell hybrid 3DT-52.5, we found that although there appeared to be two bands of slightly different molecular weights (40,000 and 43,000 daltons) under reducing conditions, both chains could vary in molecular weight and therefore could not be distinguished on the basis of size.

Because molecular weight could not be used as a means of separating the two chains, we compared the molecules precipitated from DO-11.10 and 3DT-52.5 by isoelectric focusing (IEF) and nonequilibrium pH gradient electrophoresis (NEPHGE) (30). These experiments showed clearly that the two chains of the receptors from two different murine T-cell hybridomas were heterogeneous in their amino acid composition: DO-11.10 has an acidic (α) subunit with a pI at about pH 5 and a basic (β) subunit with a pI of about pH 7; the two chains from 3DT-52.5 had somewhat different pIs, with the α chain being less acidic than that from DO-11.10 and the β chain being less basic than that from DO-11.10. These results were similar to those obtained by Allison et al (23) in their analysis of the molecule precipitated from the murine T-cell lymphoma C6XL. The material precipitated from C6XL was resolved by IEF into an acidic species with a pI of about pH 5.5 and a basic species

Annual Reviews

with pI of about pH 7, showing that the two subunits could clearly be resolved on the basis of their charge heterogeneity.

In examining the receptor material from human cell lines, we found again that the α and β chains were separable on the basis of pI: Results of a NEPHGE analysis of the human T-cell leukemia HPB-MLT showed that the larger α subunit electrophoresed at an acidic pH of 4.5, and the smaller β chain electrophoresed at about pH 7.0 (43). Similar experiments carried out by Bigler et al (33) with another human T-cell leukemia showed the two chains separated by size but having almost the same pIs. In analysis of normal human T-cell clones, however, Reinherz et al (42) found that lysates from two CTL lines when subjected to isoelectric focusing were resolved into a larger acidic band and a smaller basic species, a result that is in close agreement with our findings on HPB-MLT.

Biochemical studies from a number of laboratories are thus beginning to provide evidence for the structural characteristics of the antigen-specific receptor on MHC-restricted T cells. Molecules detected by clone-specific antibodies, found on both murine and human T-cell lines, are heterodimers linked by disulfide bonds and ranging from ~ 75,000–90,000 daltons. Upon reduction, these proteins resolve into two subunits varying from ~ 38,000–44,000 daltons in the mouse; in the human, one subunit is larger, with an apparent molecular weight of 46,000–49,000. An apparent species difference is suggested by the fact that the two chains found on murine T cells are of about the same molecular weight although distinctly different in their isoelectric points, while the subunits of human T cell receptor material are clearly separable both by size and pI. Usually one chain (the larger in humans) has an acidic pI while the other has a neutral or slightly basic pI. Structural information about these molecules continues to accumulate rapidly. As seen in the studies described below, we are now establishing evidence for constant- as well as variable-peptide regions.

ANTIRECEPTOR ANTIBODIES RECOGNIZE STRUCTURES THAT CONTAIN BOTH CONSTANT- AND VARIABLE-PEPTIDE REGIONS

Peptide Mapping

A more direct approach to examining the variability displayed by T-cell antigen receptors has been through the use of peptide-map analysis of surface-labeled material. Preliminary results indicate that in addition to unique peptide regions, there are some shared amino acid sequences between receptors on different T-cell clones. Acuto et al have recently published studies on the structures detected by clone-specific antibodies to two normal human CTL lines (45) and to a human T-cell tumor line, REX (37). Peptide maps of ^{125}I-labeled

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receptor material digested with trypsin or pepsin indicated that the α and β chains of the normal CTL clone $CT4_{II}$ had several major peptides in common with the α and β chains isolated from the tumor line REX. In addition, there were some peptide differences in both chains from the two lines. McIntyre & Allison (35) have reported findings similar to those discussed above after carrying out comparative peptide analysis of receptor material isolated either from the C6VL murine tumor line or from normal murine thymocytes and T cells, using an xenoantiserum raised to receptor determinants from C6VL. Several peptide regions were found in common between normal T cells and C6VL, but there were also unique peptide regions in maps from the different cell lines.

We have carried out peptide-map analysis to compare receptors isolated from different murine lines and in addition to examine differences between receptors on murine T cells and those on human T cells (34). We first compared maps of surface-labeled receptor material from the two murine hybridomas, DO-11.10 and 7DO-286.2, recognized by the monoclonal antibody KJ1-26. Functional assays had shown that the two hybrids, although independently derived, had identical specificities for antigen and H-2. The receptors from these two hybrids also proved to have identical peptide mapping patterns, suggesting that they may be products of germ line genes. We next examined peptide maps from two other T-cell tumors: 3DT-52.5, another BALB/c-derived hybridoma, and C6VL, the C57BL/6 tumor line. We found that the maps from 3DT-52.5 and C6VL showed considerable peptide differences from the BALB/c hybrids sharing the same idiotype, DO-11.10 and 7DO-286.2. In addition however, and in agreement with the results of Acuto et al (37,45) in studies on human T cells, there were several peptides held in common between the different murine receptors. Two or three major peptides (derived from the β chain) and one minor peptide (derived from the α chain) were found in identical positions on maps from all the murine lines. To compare the murine patterns with the human T-cell receptor, we isolated the α and β chains from the human T-cell leukemia HPB-MLT and peptide mapped the two chains separately. The results showed that the two human T-cell receptor chains have completely different peptide compositions, in keeping with the findings reported by Acuto et al (37, 45). In addition, as might be expected, they show great variations from the chains isolated from murine T-cell lines.

The evidence from all three labs suggests that the T-cell receptor for antigen consists of constant- as well as variable-peptide regions. As indicated previously from isoelectric focusing studies, and more recently by the peptide-mapping data, both chains of the receptor contribute to variability; the results of Acuto et al (37) and our own unpublished data indicate constant- and variable-peptide regions in both chains. That the α and β chains are apparently quite different in their amino acid composition is indicated in the separate

Annual Reviews

peptide-map analyses of the human chains (37, 43), a result we also have recently obtained in the separate analyses of α and β chains from the murine receptor (unpublished data).

Identification of Constant-Peptide Regions with Antisera

The properties of the T-cell receptor for antigen described in this review have been delineated almost exclusively through the use of clone-specific antibodies that recognize only the individual cell lines to which they were raised. If antibodies to T-cell receptors could be obtained with a wider range of reactivity, they could obviously be of much value in comparative studies of receptor material on other cell lines, work that has to date been severely limited by the number of clones available with clone-specific antireceptor antibodies. If, as in the case of immunoglobulin receptors and as indicated by the biochemical data, the T-cell antigen receptor contains both constant- and variable-peptide regions, there should be the potential for obtaining antibodies to both regions. Clone-specific antibodies are likely directed toward idiotypic determinants constructed from variable-peptide regions, and these are the antibodies described in this review as antireceptor antibodies. Antibodies to constant-peptide region sequences of the T-cell receptor, on the other hand, should react with more than one cell line. Some exceptions to the clone-specific antireceptor antibodies have recently been reported. Bigler et al (33) described two antibodies toward a human T-cell leukemia; one antibody was clone-specific, reacting only with the target tumor line, but the other antibody also showed reactivity toward a small population of normal T cells. In the case of the human T-cell receptor, in addition to clonotypic structures, another molecular entity of ~ 20,000 daltons is found on most types of T cells and has been designated T3 (46). Antibodies to T3 react with a range of T cells and also interfere with functional responses, implicating a role for these molecules in recognition of antigen/MHC (24). Because of its wide distribution, apparent functional significance, and comodulation with clone-specific structures, T3 may constitute some constant portion of the T-cell receptor. So far, the analog of T3 in the mouse has not been detected.

Recently, McIntyre & Allison (35) have described an xenoantisera prepared in rabbits that detects receptor material on several murine T-cell lines. This antisera was made by injecting animals with immune complexes consisting of material bound by the clone-specific antibody 124-40 from lysates of the C57BL/6 tumor line C6VL. Electrophoretic analysis showed that the rabbit antisera precipitated the same disulfide-linked molecule from C6VL as did the clone-specific antibody. Moreover, a similar product was not only immunoprecipitated from normal splenic T cells and thymocytes isolated from C57BL/6 mice, but also was found on thymocytes from AKR and BALB/c strains,

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indicating that the molecule was not strain-specific. Further experiments employing IEF and peptide-mapping techniques showed that receptor material isolated from normal T cells contained several peptides in common with the C6VL lymphoma antigen, but there were also unique peptide regions. We have raised antisera by similar means in rats and have recently obtained a monoclonal antibody, KJ16-133, which preliminary results suggest will be useful in isolating receptor material from a variety of T-cell hybridomas and normal T cells from a number of mouse strains (unpublished results).

CONCLUDING REMARKS

Great progress in our understanding of the structure of the antigen-specific MHC-restricted T-cell receptor should occur in the next few years. Studies with the clonotypic antibodies described at length in this review should allow immunologists to define the extent of the T-cell repertoire and to isolate and sequence the proteins involved. These studies should eventually lead to the isolation of the genes involved. Anti–constant region antibodies or anti-framework antibodies should serve the same purposes and also allow us to understand critical events in T-cell maturation better. For example, Zinkernagel and his colleagues (47), and a number of workers since (48-50), have shown that the thymus has a crucial role in controlling the specificities of T cells for self-MHC plus antigen. At present we do not know how this is done nor do we know the meaning of the self-MHC preference of T cells. Anti–constant region antibodies will be used to define the stages at which receptors are expressed and varied on thymocytes and thereby clarify these issues.

Most importantly, it is to be hoped that the types of studies described in this paper will fuse fairly soon with the recent, and as yet unpublished, studies of Davis, Hedrick, and their co-workers. These investigators have described the isolation of a cDNA clone from a T cell–specific cDNA library that appears to be encoded by genes that rearrange between the germ line and mature T cells (51). By comparing similar cDNA clones, Davis and his colleagues have shown that they contain variable- and constant-region sequences with some homology to immunoglobulin. It is likely that this cDNA codes for one of the chains of the T-cell receptor, as described in this review. Future work should establish whether or not this is true.

Finally, the MHC-restricted antigen receptor on T cells, although it does not bear determinants cross-reactive with immunoglobulin or map genetically at least at some immunoglobulin-encoding loci (12-16), is in basic construction strikingly similar to immunoglobulin. Nature has apparently solved the problem of constructing a protein that can recognize an indeterminately large and variable collection of antigens by making it from two disulfide-bonded poly-

Annual Reviews

peptide chains, both of which have variable- and constant-region sequences. Whether the variable parts of these chains will have V, J, and D regions like antibodies remains to be seen.

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Literature Cited

1. Tada, T., Okumura, K. 1979. The role of antigen-specific T cell factors in the immune response. *Adv. Immunol.* 28:1
2. Taussig, M.J. 1980. Antigen-specific T-cell factors. *Immunology* 41:759
3. Cone, R.E. 1981. Molecular basis for T lymphocyte recognition of antigens. *Prog. Allergy* 29:182
4. Owen, F.L., Spurll, G.M. 1981. Evidence for a T cell constant region gene family: characterization of cell surface antigens by immunoprecipitation with alloantisera and monoclonal antibodies. In *Immunoglobulin Idiotypes*, ed. C. Janeway, E.E. Sercarz, H. Wigzell, pp. 419–28. NY: Academic
5. Cramer, M., Reth, M., Grutzmann, R. 1981. T cell V_H versus B cell V_H. See Ref. 4, pp. 429–39
6. Janeway, C.A., Cone, R.E., Rosenstein, R.W. 1982. T cell receptors: through a glass darkly. *Immunol. Today* 3:83
7. Jensenius, J.C., Williams, A.F. 1982. The T lymphocyte antigen receptor—paradigm lost. *Nature* 300:583
8. Marchalonis, J.J., Hunt, J.C. 1982. The antigen receptor of thymus-derived lymphocytes: progress in the characterization of an elusive molecule. *Proc. Soc. Exp. Biol. Med.* 171:127
9. Miller, J.F.A.P., Morahan, G., Walker, I.D. 1983. T-cell antigen receptors: fact and artefact. *Immunol. Today* 4:141
10. Kronenberg, M., Kraig, E., Hood, L. 1983. Finding the T-cell antigen receptor: past attempts and future promise. *Cell* 34:327
11. Webb, D.R., Kapp, J.A., Pierce, C.W. 1983. The biochemistry of antigen-specific T-cell factors. *Ann. Rev. Immunol.* 1:423
12. Kurosawa, Y., von Boehmer, H., Haas, W., Sakano, H., Trauneker, A., Tonegawa, S. 1981. Identification of D segments of immunoglobulin heavy-chain genes and their rearrangement in T lymphocytes. *Nature* 290:565
13. Kronenberg, M., Davis, M.M., Early, P.W., Hood, L.E., Watson, J.D. 1980. Helper and killer T cells do not express B cell immunoglobulin joining and constant region gene segments. *J. Exp. Med.* 152:1745
14. Kraig, E., Kronenberg, M., Kapp, J.A., Pierce, C.W., Samelson, L.E., Schwartz, R.H., Hood, L.E. 1983. T and B cells that recognize the same antigen do not transcribe similar heavy chain variable region gene segments. *J. Exp. Med.* 158:192
15. Kronenberg, M., Kraig, E., Siu, G., Kapp, J.A., Kappler, J., Marrack, P., Pierce, C.W., Hood, L. 1983. Three T cell hybridomas do not contain detectable heavy chain variable gene transcripts. *J. Exp. Med.* 158:210
16. Marrack, P., Kappler, J. 1983. Use of somatic cell genetics to study chromosomes contributing to antigen plus *I* recognition by T cell hybridomas. *J. Exp. Med.* 157:404
17. Roehm, N., McNeil, I., Haskins, K., White, J., Karjalainen, K., Kappler, J.W., Marrack, P. Antigen recognition by helper T cells and hybridomas. In *Factors Regulating Recognition in Cell Mediated Immunity*, ed. J.D. Watson, J. Marbrook. NY: Marcel Dekker. In press

Annual Reviews

18. Infante, A.J., Infante, P.D., Gillis, S., Fathman, C.G. 1982. Definition of T cell idiotypes using anti-idiotypic antisera produced by immunization with T cell clones. *J. Exp. Med.* 155:1100
19. White, J., Haskins, K.M., Marrack, P., Kappler, J. 1983. Use of I region-restricted, antigen-specific T cell hybridomas to produce idiotypically specific anti-receptor antibodies. *J. Immunol.* 130:1033
20. Samelson, L.E., Schwartz, R.H. 1983. T cell clone specific alloantisera that inhibit or stimulate antigen-induced T cell activation. *J. Immunol.* 131:2645
21. Samelson, L.E., Schwartz, R.H. 1983. The use of antisera and monoclonal antibodies to identify the antigen-specific T cell receptor from pigeon cytochrome *c*-specific T cell hybrids. *Immunol. Rev.* 76:59
22. Kaye, J., Porcelli, S., Tite, J., Jones, B., Janeway, C.A. 1983. Both a monoclonal antibody and antisera specific for determinants unique to individual cloned helper T cell lines can substitute for antigen and antigen-presenting cells in the activation of T cells. *J. Exp. Med.* 158:836
23. Allison, J.P., McIntyre, B.W., Bloch, D. 1982. Tumor-specific antigen of murine T-lymphoma defined with monoclonal antibody. *J. Immunol.* 129:2293
24. Meuer, S.C., Fitzgerald, K.A., Hussey, R.E., Hodgdon, J.C., Schlossman, S.F., Reinherz, E.L. 1983. Clonotypic structures involved in antigen-specific human T cell function. *J. Exp. Med.* 157:705
25. Lancki, D.W., Lorber, M.I., Loken, M.R., Fitch, F.W. 1983. A clone-specific monoclonal antibody that inhibits cytolysis of a cytolytic T cell clone. *J. Exp. Med.* 157:921
26. Harwell, L., Skidmore, B., Marrack, P., Kappler, J. 1980. Concanavalin A-inducible interleukin-2-producing T cell hybridomas. *J. Exp. Med.* 152:893
27. Kappler, J.W., Skidmore, B., White, J., Marrack, P. 1981. Antigen-inducible, *H-2*-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and *H-2* recognition. *J. Exp. Med.* 153:1198
28. Marrack, P., Graham, S.D., Leibson, H.J., Roehm, N., Wegmann, D., Kappler, J.W. 1982. Properties of antigen-specific *H-2* restricted T cell hybridomas. In *Isolation, Characterization, and Utilization of T Lymphocyte Clones*, ed. C.G. Fathman, F.W. Fitch, pp. 119–26. NY: Academic
29. Haskins, K., Kubo, R., White, J., Pigeon, M., Kappler, J., Marrack, P. 1983. The Major Histocompatibility Complex–restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149
30. Kappler, J., Kubo, R., Haskins, K., White, J., Marrack, P. 1983. The mouse T cell receptor: comparison of MHC-restricted receptors on two T cell hybridomas. *Cell* 34:727
31. Endres, R.O., Marrack, P., Kappler, J.W. 1983. An IL-2-secreting T cell hybridoma that responds to a self class I histocompatibility antigen in the H-2D region. *J. Immunol.* 131:1656
32. Samelson, L.E., Germain, R.N., Schwartz, R.H. 1983. Monoclonal antibodies against the antigen receptor on a cloned T-cell hybrid. *Proc. Natl. Acad. Sci. USA* 80:6972
33. Bigler, R.D., Fisher, D.E., Wang, C.Y., Rinnooy, Kan, E.A., Kunkel, H.G. 1983. Idiotype-like molecules on cells of a human T cell leukemia. *J. Exp. Med.* 158:1000
34. Staerz, U.D., Pasternack, M.S., Klein, J.R., Benedetto, J.D., Bevan, M.J. 1984. Monoclonal antibodies specific for a murine cytotoxic T lymphocyte clone. *Proc. Natl. Acad. Sci. USA*. In press
35. McIntyre, B.W., Allison, J.P. 1983. The mouse T cell receptor: structural heterogeneity of molecules of normal T cells defined by xenoantiserum. *Cell* 34:739
36. Meuer, S.C., Hodgdon, J.C., Hussey, R.E., Protentis, J.P., Schlossman, S.F., Reinherz, E.L. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988
37. Acuto, O., Hussey, R.E., Fitzgerald, K.A., Protentis, J.P., Meuer, S.C., Schlossman, S.F., Reinherz, E.L. 1983. The human T cell receptor: appearance in ontogeny and biochemical relationship of α and β subunits on IL-2 dependent clones and T cell tumors. *Cell* 34:717
38. Hünig, T.R., Bevan, M.J. 1982. Antigen recognition by cloned cytotoxic T lymphocytes follows rules predicted by the altered-self hypothesis. *J. Exp. Med.* 155:111
39. Heber-Katz, E., Schwartz, R.H., Matis, L.A., Hannum, C., Fairwell, T., Appella, E., Hansburg, D. 1982. Contribution of antigen-presenting cell Major Histocompatibility Complex gene products to the specificity of antigen-induced T cell activation. *J. Exp. Med.* 155:1086
40. Marrack, P., Shimonkevitz, R., Hannum, C., Haskins, K., Kappler, J. 1983. The Major Histocompatibility Complex–restricted antigen receptor on T cells. IV.

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Annual Reviews

An antiidiotypic antibody predicts both antigen and I-specificity. *J. Exp. Med.* 158:1635

41. Goding, J.W., Harris, A.W. 1981. Subunit structure of cell surface proteins: disulfide bonding in antigen receptors, Ly-2/3 antigens, and transferrin receptors of murine T and B lymphocytes. *Proc. Natl. Acad. Sci. USA* 78:4530
42. Reinherz, E.L., Meuer, S.C., Fitzgerald, K.A., Hussey, R.E., Hodgdon, J.C., Acuto, O., Schlossman, S.F. 1983. Comparison of T3-associated 49- and 43-kilodalton cell surface molecules on individual human T-cell clones: evidence for peptide variability in T-cell receptor structures. *Proc. Natl. Acad. Sci. USA* 80:4104
43. Kappler, J., Kubo, R., Haskins, K., Hannum, C., Marrack, P., Pigeon, M., McIntyre, B., Allison, J., Trowbridge, I. 1983. The Major Histocompatibility Complex–restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. *Cell* 35:295
44. Minowada, J., Sagawa, K., Trowbridge, I.S., Kung, P.D., Goldstein, G. 1982. Marker profiles of 55 human leukemia lymphoma cell lines. In *Malignant Melanomas*, ed. S. A. Rosenberg, H.S. Kaplan, pp. 53–74. NY: Academic
45. Acuto, O., Meuer, S.C., Hodgdon, J.C., Schlossman, S.F., Reinherz, E.L. 1983. Peptide variability exists within α and β subunits of the T cell receptor for antigen. *J. Exp. Med.* 158:1368
46. Reinherz, E.L., Meuer, S., Fitzgerald, K.A., Hussey, R.E., Levine, H., Schlossman, S.F. 1982. Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell* 30:735
47. Zinkernagel, R.M., Callahan, G.N., Althage, A., Cooper, S., Klein, P.A., Klein, J. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882
48. Sprent, J. 1978. Restricted helper function of $F_1 \rightarrow$ parent bone chimeras controlled by K-end of H-2 complex. *J. Exp. Med.* 147:1838
49. Fink, P.J., Bevan, M.J. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766
50. Kappler, J.W., Marrack, P. 1978. The role of *H-2* linked genes in helper T-cell function. IV. Importance of T-cell genotype and host environment in *I*-region and *Ir* gene expression. *J. Exp. Med.* 148:1510
51. Marx, J.L. 1983. Likely T cell receptor gene cloned. *Science* 221:1278

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL

David C. Benjamin[1], Jay A. Berzofsky[2], Iain J. East[3], Frank R. N. Gurd[4], Charles Hannum[5], Sydney J. Leach[6], Emanuel Margoliash[7], J. Gabriel Michael[8], Alexander Miller[9], Ellen M. Prager[10], Morris Reichlin[11], Eli E. Sercarz[9], Sandra J. Smith–Gill[12], Pam E. Todd[6], and A.C. Wilson[10]

INTRODUCTION

Proteins are one of the most abundant and diverse classes of antigens to which the immune system can respond. These include transplantation antigens, antigens of infectious and parasitic organisms, and allergens. An understanding of host defense mechanisms and of the ability to distinguish self from nonself requires a knowledge of the structural basis for protein antigenicity. The advent of hybridoma technology (1) to produce monoclonal antibodies, each of which

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binds to a specific area on the protein surface known as an antigenic site, has provided a powerful new tool for investigating antigen structure. Recent studies from a number of laboratories using monoclonal antibodies directed toward structurally defined protein antigens have produced a large body of new information which necessitates a reevaluation of the nature and distribution of the antigenic sites on proteins. Although in this review we emphasize those antigenic sites [antigenic determinants or epitopes (2), terms used interchangeably] recognized by antibodies, we also include limited recent data on the nature of the sites recognized by T cells.

Immunogenicity indicates the ability to elicit an immune response and is the summation of a variety of influences that reflect the previous history of the animal as well as its genetic attributes: the available B-cell repertoire, the activity of T-helper cells and T-suppressor cells, the idiotype network, and the major histocompatibility complex (MHC). In contrast, *antigenicity* merely implies the ability to be recognized by the product of the immune response, i.e. antibodies or immune cells. As has recently been emphasized (3), chemically synthesized peptides may elicit antibodies reactive with determinants on the native protein. The present discussion, however, is limited to studies where antibodies were elicited by immunization with the native protein. While peptides or other derivatives may have been used in such studies to identify antigenic sites reactive with antibody, the sites under discussion are those that are immunogenic on the native molecule.

The fact that antibodies elicited by a native protein often do not react with the denatured form (4,5) and that specific antibodies can be raised against peptides of undefined conformation (3,5–9) led to the definition of two classes of antigenic determinants. *Conformational determinants* were defined as dependent on the native spatial conformation of the protein, while *sequential determinants* were defined as depending only on the amino acid sequence of the corresponding peptide segment (9,10). This operational distinction does not imply different antigenic binding mechanisms. A "conformational" determinant on a native protein can usually be inhibited from interacting with its antibodies by a large molar excess of the corresponding "sequential" peptide. The native conformation is one of many conformations that such a peptide fragment can assume in solution (9–13). *All* determinants are now seen to be conformational in the sense that the antibody combining sites will bind with a measurable affinity only to that population of antigen conformers which presents a complementary constellation of interacting side chains. It follows that antigenic determinants are topographic, i.e. composed of structures on the protein surface. Topographic determinants may be contained within a single *segment* of the amino acid sequence (but not necessarily involving all contiguous residues in the segment), or *assembled* from residues far apart in the amino acid sequence but brought together on the surface by the folding of

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the protein in its native conformation. This latter category probably represents the major group of conformational determinants. Of particular importance in discussions of antigenicity in proteins are the amino acid sequence differences among evolutionary variants of a protein. Such differences often have little or no detectable effect on the backbone conformation but can produce antigenically crucial changes in the nature of the protein surface (14–16).

It must be emphasized that the set of specificities that predominate after immunization with the native protein is clearly not equal to the total potential repertoire. Certain sequences or even individual residues on the surface of proteins have been identified as "immunodominant" sites, i.e. those to which most of the immune response is directed. Several mechanisms could explain immunodominance. One view is that special structural properties intrinsic to certain regions of a protein make these regions immunodominant. An alternative view is that immunodominance of a given region depends on the regulatory mechanisms of the host, including tolerance to structures resembling self, immune response genes, the specificity of T-cell help, and idiotype networks.

Many questions remain, and we explore some in this review. Can all surface structures on a protein molecule be recognized by the immune system as determinants? Are there sites that are intrinsically immunogenic, independent of the responding species? How many of the potential determinants are recognized by an individual's B and T cells? To what extent do homologous proteins in the responding individual affect the expressed repertoire? What is the relationship between the expressed repertoires of B and T cells? How does the expressed T-cell repertoire regulate the B-cell repertoire?

The studies described below have used four structurally well-characterized globular proteins—myoglobin, lysozyme *c*, cytochrome *c*, and serum albumin—as model antigens. Although some different approaches were employed in the study of each protein, several major common conclusions emerge. First, most, if not all, of the surface of a protein may be immunogenic and antigenic and may include multiple, overlapping determinants. Second, most antigenic sites consist of a three-dimensional array of amino acid residues that require the native conformation of the protein for their antigenic integrity. Third, on a given antigen the subset of potential determinants that are immunogenic varies from species to species and depends on the structural differences between that antigen and the host's self proteins, and on regulatory mechanisms that govern interactions among the many subpopulations of cells generating the immune response.

MYOGLOBIN

Sperm whale myoglobin, 153 amino acid residues long, was one of the first proteins to be sequenced (17) and to have its three-dimensional structure deter-

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mined by X-ray crystallography (18,19). Therefore, it was also one of the first to be chosen as a model antigen. Crumpton & Wilkinson (20) first localized some of the antigenic sites using proteolytic fragments of myoglobin to inhibit quantitative precipitin reactions. For rabbit antibodies to sperm whale myoglobin, peptides consisting of residues 15–29 and 147–153 inhibited most strongly. Three other peptide fragments, 56–69, 70–76, and 139–146, gave less inhibition or less consistent inhibition with different antisera. Subsequently, Atassi (21) described "the complete immunochemical anatomy" of myoglobin as consisting of five antigenic sites. Each of these sites, residues 15–22, 56–62, 94–99, 113–119, and 145–151, is composed of six to eight consecutive residues at a bend or exposed corner in the native protein. Sites I and V are contained in the two major sites observed by Crumpton & Wilkinson (20), and these were also observed by a combined solid-phase peptide synthetic and immunoassay procedure in Leach's laboratory (22,23). Site II is contained in the peptide 56–69 found to be weakly antigenic with some antisera by Crumpton & Wilkinson. Besides describing the two new sites III and IV (21), Atassi reported that 99.8% of the antibodies in various antisera could be removed by binding sequentially to these five synthetic sites (24). Thus, he postulated that these sites constituted the only antigenic determinants on sperm whale myoglobin (21,24,25) and that they were the only antigenic sites on myoglobins from any mammalian species, regardless of the amino acid substitutions at these sites (24,25). It is true that spatially homologous regions in a soybean leghemoglobin molecule are immunogenic in spite of the great evolutionary distance between mammalian and plant globins (26). However, the delineation of antigenic sites using small peptides must be reconsidered in the light of the recent findings on general charge and hydrophobic effects described below. Twining et al (25) also postulated that the antibody response to myoglobin is independent of the immunized species, in conflict with earlier views on the role of the responding species (14).

Although the characterization of five antigenic sites of myoglobin constituted a historic step in describing the immunochemical anatomy of a protein, results from several laboratories published in the last few years have produced a new and rather different view of the antigenic structure of myoglobin. These recent results disprove some of the earlier generalizations cited above and lead to a very different concept of protein antigenicity.

1. One of the most important findings is the existence of a number of antigenic sites of myoglobin that are not contained within a single segment of a peptide chain but are *assembled* from several segments. Such sites require the native conformation for their integrity and may not exist in complete form in any single cleavage fragment of the protein. Some specific antibodies will bind peptides containing continuous sequences, albeit with lower affinities than they bind the native structure (27), either because the peptides lack the

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native conformation or because they form only part of a topographic site, or both. The degree to which the affinity is lowered depends on the size and the conformational equilibrium of the peptide (27). However, binding of a peptide to antibodies will siphon the equilibrium over toward the native form, and so the antibodies can still be trapped on an immunoadsorbent carrying the peptide fragment.

Early evidence for assembled topographic sites came from a study of the cross-reactivities of different myoglobins for the fraction of antibodies to beef myoglobin specific for peptide 1–55 of the molecule (28). These antibodies bind with markedly different affinities to beef, sheep, and pig myoglobins in spite of their identity in the 15–22 sequence. These results imply either the involvement of other residues outside of residues 15–22 within this determinant or the existence of another determinant within the 1–55 fragment. Other evidence for the presence of assembled topographic antigenic sites came from Lando et al (29), who found that of the antibodies in each of four antisera to sperm whale myoglobin (from three different species) that bound with high affinity to the native molecule, 30–40% failed to bind to affinity columns of any of the three CNBr (cyanogen bromide) cleavage fragments that make up the whole protein. None of these results could be explained by the existence of five discrete sequential antigenic sites reported earlier (21).

Independent evidence for assembled topographic antigenic sites on myoglobin and of their high frequency of occurrence came from studies of mouse monoclonal antibodies to sperm whale myoglobin, none of which bound to the amino-terminal or carboxy-terminal CNBr fragments (30). Six of those with high affinity (10^8 to 10^9 M^{-1}) (30) were studied in detail, and none bound to any of the three CNBr fragments spanning the whole sequence of the molecule (31). That is, none bound to sequential sites, including sites I–V above. Assignments of antigenic sites could be made for three of these monoclonal antibodies by comparing their relative affinities for 13 to 15 different myoglobins with the sequences of these myoglobins (31). One monoclonal antibody reacted with a site that included Lys 140, a second with a site involving Glu 4, Lys 79, and possibly His 12, and a third with a site involving Glu 83, Ala 144, and Lys 145 (31). The last two sites are clearly assembled topographic sites, in that they include residues far apart in the primary sequence but brought together on the surface of the molecule by the way it folds in the native conformation (Figure 1A,B). Moreover, neither of these sites exists on any single CNBr fragment.

Additional evidence for assembled topographic sites comes from a study of two monoclonal antibodies to human myoglobin (33). Based on comparison of binding to different myoglobins, one monoclonal antibody was found to recognize a site that included residues 34 and 113 or possibly 34 and 53 and the second a site that included residues 74, 87, and 142. Again, these residues

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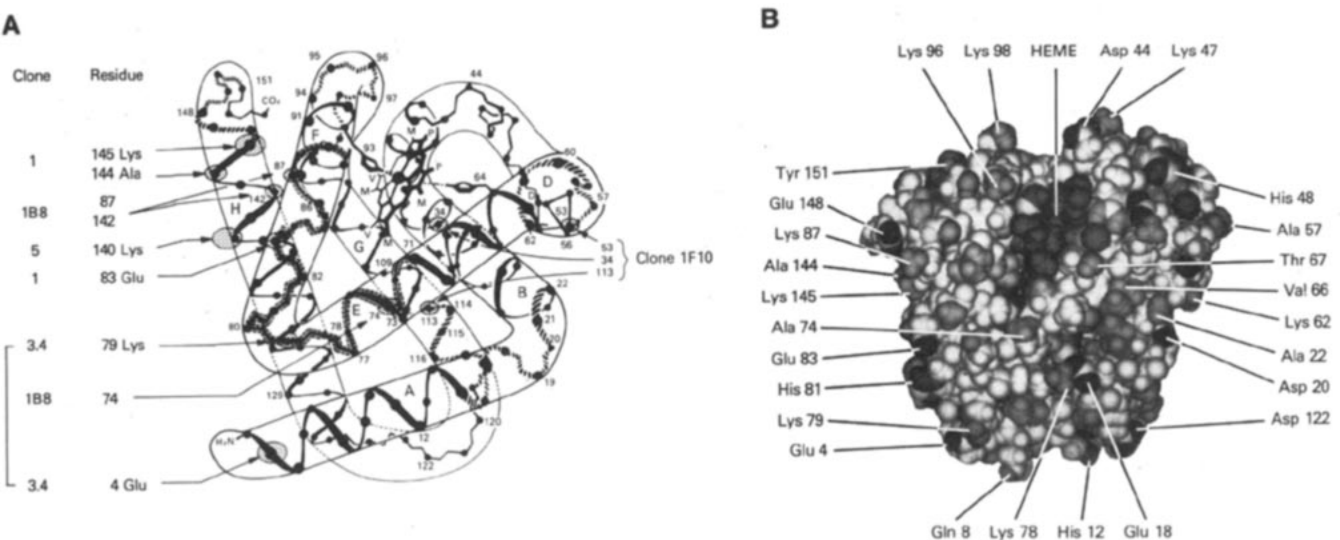

Figure 1 *Panel A:* Line drawing representing the three-dimensional structure of the alpha-carbon backbone of native sperm whale myoglobin [modified from (32) with permission]. The alpha helixes are labeled A through H (the C helix is partly hidden by the heme). Side chains are omitted except for two histidine rings (64 and 93) involved with the heme. The letters P, M, V on the heme indicate the propionic, methyl, and vinyl groups, respectively, of the porphyrin ring. Residues 1 and 2 at the amino-terminus are not shown. Residues recognized by monoclonal antibodies to sperm whale myoglobin (clones 1, 3.4, and 5) (31) and to human myoglobin (clones 1B8 and 1F10) (33) are indicated. Five reported "sequential" sites (21) are indicated by crosshatching, and a sixth region of sequence to which antibodies have been found to bind (residues 72–88) (34) is shown by dashed outlining.

Panel B: Computer-generated space-filling molecular model of sperm whale myoglobin, based on the Takano (19) X-ray diffraction coordinates. This orientation is the same as that in Panel A. The computer method was described by Feldmann et al (35). The carboxyl oxygens are shaded darkest, followed by the heme and aromatic carbons, aliphatic side-chain carbons, noncarboxylic oxygens, primary amino groups, and finally other nitrogens. The protein backbone and the side chains of nonaliphatic residues, except for functional groups, are shown in white. Note that the direction of the helixes is not readily apparent on the surface, in contrast to the backbone drawing in Panel A. [Modified from (31) with permission.]

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require the native conformation to bring them into mutual proximity (Figure 1A,B).

In addition to the majority of antibodies that are specific for the native conformation, there is some evidence for a smaller subpopulation of antibodies with a substantially higher affinity for peptide fragments than for the native protein (12). This previously unrecognized population can be enriched by affinity chromatography on peptide-Sepharose. In a competitive radioimmunoassay with radiolabeled peptide as tracer, these antibodies require 10^5-fold more *native* myoglobin than unlabeled peptide to compete. This population may represent antibodies specific for novel sequential determinants or for the denatured or cleaved protein.

2. A new development in our understanding of the antigenic structure of myoglobin is the realization that almost any part of the surface may be antigenic. (*a*) The studies of Hurrell et al (36) and East et al (28), together with the evidence cited above (29) that 30–40% of antibodies raised in goat, sheep, or rabbit do not bind to any of the three CNBr fragments of sperm whale myoglobin, imply that a substantial proportion of the antibodies in a polyclonal serum are directed to sites that are not defined simply by sequences I–V described earlier (21). Results of pairwise cross-reactivity studies of 13 cetacean myoglobins of known amino acid sequence and rabbit antisera to 12 of them also implied that most of the immunological reactivity was due to regions outside sequences I–V (E. M. Prager, A. C. Wilson, unpublished). (*b*) Of the 12 residues implicated in the binding sites of five monoclonal antibodies to sperm whale or human myoglobin (31,33), only two (residues 113 and 145) are within the original five sites. Most of the residues are not considered close enough in a nearest-neighbor analysis (37) to perturb these five sites; and their effects, therefore, cannot be interpreted as secondary influences on sites I–V. (*c*) A synthetic peptide corresponding to residues 72–88 of beef myoglobin (Figure 1A), containing none of the residues in sites I–V but including four residues, 74, 79, 83, and 87, implicated in the binding of monoclonal antibodies to human or to sperm whale myoglobin (31,33), bound 16% of the antibodies in a rabbit antiserum to beef myoglobin, consistent with the surface area of beef myoglobin it occupies (34). Certain monoclonal antibodies to beef myoglobin also bind to this peptide (D. Dorow, K. Haynes, P-t. Shi, P. E. E. Todd, S. J. Leach, manuscript in preparation), presumably because (unlike the five small peptides referred to earlier) it corresponds structurally to a substantial surface area in the intact myoglobin. Interestingly in retrospect, two of the chymotryptic peptides that bound to one of Crumpton & Wilkinson's (20) rabbit antisera to sperm whale myoglobin were the fragments 70–76 and 77–89. Therefore, it is clear that this area may represent another substantial antigenic region of myoglobins. (*d*) Other sites outside this area and outside the five earlier sites have been identified; for example, the synthetic peptide

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25–55 of beef myoglobin reacts with polyclonal as well as with two out of eight monoclonal antibodies to beef myoglobin (D. Dorow et al, cited above). Together, these peptides and the other sites implicated above cover a large part of the surface of the molecule.

3. Another new development in defining the antigenic structure of myoglobin is the finding that the five original sites (21) are not always recognized. In one study (38) high-titer antisera to sperm whale myoglobin raised in goats, sheep, and several strains of high-responder mice were tested for their ability to bind to synthetic peptides corresponding to residues 56–63 (site II) and 93–102 (site III). No binding to either peptide could be demonstrated either by competitive or direct binding assays over a wide concentration range. Thus, these sites are not recognized by all antisera. In fact, a general problem with the use of small peptides to define antibody specificity has come to light in a recent study (P-t. Shi, J. Riehm, P. E. E. Todd, S. J. Leach; manuscript submitted) using short synthetic peptides attached to solid-phase resins and measuring binding of ^{125}I-labeled antibodies. Athough peptides corresponding to previously reported sequential sites I–V (21) of beef myoglobin bound antibodies raised to beef myoglobin, they also bound antibodies to staphylococcal nuclease nearly as well. Moreover, the antibodies to myoglobin also bound to short unrelated peptides in a manner that implicated lysine and aromatic residues as main structures determining binding. It was concluded that the observed binding of antibodies to short and unstructured peptides of two to seven residues is of limited biological significance. Biological specificity can be assessed only with peptides that represent larger areas of the protein antigen surface and that have demonstrable conformational preferences.

4. A significant observation, which necessitates revision of the earlier postulate that the same sites are recognized regardless of the species immunized (24,25), is that antibodies to beef myoglobin raised in sheep have a fine specificity very different from that of antibodies to beef myoglobins raised in rabbits, dogs, and chickens (H. M. Cooper, I. J. East, P. E. E. Todd, S. J. Leach, manuscript submitted). This difference in response is explained by the fact that sheep and beef myoglobin differ by only six residues, whereas beef myoglobin differs substantially from the myoglobins of the other three responding species. The interpretation of such data, in common with those for cytochrome *c* responses (16), is that immunogenicity depends significantly on the difference between the immunogen and the responder's homologous protein, and is not an inherent property of the antigen alone.

5. T-cell and antibody responses to myoglobin have quite different fine specificities. For instance, antisera raised to sperm whale myoglobin in several strains of mice cross-react extensively with horse myoglobin and vice versa (39). In contrast, T cells immune to sperm whale myoglobin in three strains (B10.S, B10.D2, B10.GD) do not cross-react with horse myoglobin, and T

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cells immune to horse myoglobin in at least one strain (B10.S), which is a high responder to both myoglobins, do not cross-react with sperm whale myoglobin (40,40a). Moreover, an immunodominant site recognized by T lymphocytes from these three strains was identified, centering on residue 109. The small difference between Glu 109 of sperm whale myoglobin and Asp 109 of horse myoglobin appears to be critical for T-cell recognition (40). This specificity has been confirmed with monoclonal populations of myoglobin-specific T cells from B10.D2 mice grown in long-term tissue culture (40a). So far, no laboratory has identified any antibodies that bind to this site. Thus, sites immunogenic for T-lymphocyte responses are not necessarily the same as those immunogenic for antibody production. At the very least, the frequency distribution is skewed quite differently for the two types of responses, and the number of sites seen by T cells may be far more limited than the number recognized by antibodies.

A second, minor site around Lys 140 has been identified that is recognized by several clones of T cells from B10.D2 mice (I. J. Berkower, H. Kawamura, L. A. Matis, F. R. N. Gurd, J. A. Berzofsky, manuscript in preparation). This site does coincide with the site recognized by one monoclonal antibody (31). Interestingly, the MHC of antigen-presenting cells (such as macrophages or dendritic cells) determines which antigenic site is stimulatory for T cells. All T-cell clones studied that recognized myoglobin in association with the *I–A*-subregion-encoded Ia antigen of antigen-presenting cells were specific for the Glu 109 site, whereas all of those restricted to the *I–E*-encoded Ia molecule were specific for the Lys 140 site. Thus, the major histocompatibility antigens play a major role in this skewing or limitation of the T-cell repertoire.

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LYSOZYME *c*

Chicken lysozyme *c* from hen egg-white (HEL) has long served as a prototype protein for investigating the specificity of immune recognition. HEL is a small globular protein (129 residues), unusually stable in solution, whose mode of enzymatic action has been extensively studied (41) (Figure 2). In addition, its three-dimensional crystalline structure has been determined to a high degree of precision (48). The presence of four disulfide bonds makes it likely that the structure of HEL in solution is close to that in the crystalline state. X-ray analysis of human lysozyme, which differs from HEL at 52 amino acid residues, shows that the peptide backbone is highly conserved evolutionarily (49). Thus, it is reasonable to assume that the many sequenced bird lysozymes, which are more closely related to HEL, have very similar three-dimensional structures, as has already been demonstrated for turkey lysozyme (50). However, local changes due to radical amino acid substitutions as well as subtle long-range effects are not excluded by present analyses.

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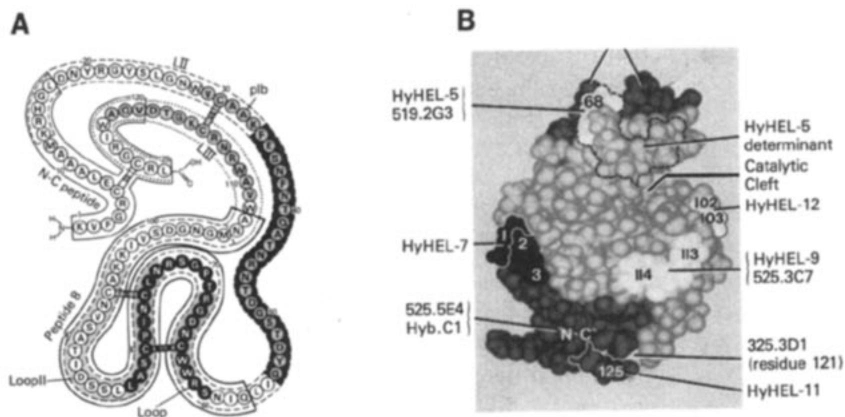

Figure 2 A: Primary structure of chicken lysozyme (HEL) with eight peptides that have been shown to be antigenic when tested against anti-HEL [reviewed in (42)]: N–C peptide, solid outline; LII, dashed outline; plb, stippled; a continuous region (amino acids 34–54) within plb, black box; peptide 8, heavy black outline; the Loop, black with white lettering; Loop II, stippled box; and LIII, dotted outline.

B: Space-filling model of HEL, computer generated as described previously (35,43). The Loop and N–C peptide are dark grey, with residues 1–3 black. Specific residues recognized by monoclonal antibodies are colored or outlined in white; a hypothesized unit determinant for antibody HyHEL–5 is outlined in a dotted black line. HyHEL antibodies are from the Smith-Gill laboratory (43; S. J. Smith-Gill, T. B. Lavoie, C. R. Mainhart, manuscript submitted). Antibody Hyb.C1 is from Fujio's laboratory (44). All other antibodies are from the Sercarz laboratory (45–47).

Several important issues have been addressed by the study of anti–HEL antibodies. One set of questions focuses on the definition of antigenic structure of the native HEL molecule. Since such studies necessarily utilize the host immune response as an assay or measure of antigenicity, it has therefore been necessary to consider a second set of questions, focusing on the nature of the repertoire of antibodies in the responding host and on the regulatory mechanisms that limit the total expressed repertoire to a skewed set of specificities that become dominant in the course of the immune response.

That the antigenicity of HEL is dependent upon intact conformational structure is supported by the observation of little or no cross-reactivity between native and denatured lysozymes (51). Purified peptides such as the N–C terminal peptide (see Figure 2A) can react with both polyclonal and monoclonal antibodies to HEL but with a far lower affinity than does HEL itself (42 and references therein; 44,51–53). The majority of antibodies do not react with any nondisulfide peptides. These studies support the conclusion that most antigenic determinants on HEL are *assembled topographic determinants*.

An antigenic region known as the "Loop," which includes residues 64–80 (see Figure 2A), has been studied extensively by the use of purified disulfide peptides. Whole goat, rabbit, and mouse antisera to native lysozymes (anti-

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lysozyme), as well as anti-lysozyme fractionated into antibodies reactive with only Loop (anti-Loop) or with all regions except the Loop, were tested against evolutionary variants of lysozyme as well as against Loop peptides. Several important conclusions concerning antigenicity of HEL have emerged from these studies (42 and references therein; 54): (*a*) The Loop participates in the antigenic structure of lysozyme, and position 68 has a major role in its specificity; (*b*) the ability of evolutionarily related lysozymes to compete with Loop for binding to anti-lysozyme or anti-Loop correlates highly with sequence changes in the Loop, while the ability to compete with native lysozymes does not; (*c*) not all residues contribute equally to antigenicity; (*d*) antigenicity of the Loop depends upon intact conformational structure which is maintained by a disulfide bond between residues 64 and 80; (*e*) the region-specific anti-Loop response in mice is under genetic control and does not necessarily correspond to the total anti-lysozyme response (55).

The nature of individual determinants recognized by monoclonal antibodies has recently been studied (43,44,45,47). One such monoclonal, described in detail by Smith-Gill et al (43), is sensitive to substitution of Arg 68 by Lys and is insensitive to a variety of amino acid substitutions in other parts of the lysozyme molecule. The authors postulate that a determinant that includes Arg 45, Arg 68, Thr 47, and Asp 48 is being recognized. This determinant is distinct from but overlaps the Loop, and is part of an anti-parallel β-sheet that was implicated previously as an antigenic region using polyclonal antibodies (53).

The question of how much of the surface of HEL is antigenic has been controversial. Most of the HEL surface was implicated in its antigenicity by an extensive study of Wilson, Prager, and colleagues (e.g. 54,56–59), which showed that the cross-reactivity of rabbit antisera to lysozymes correlated with the number of amino acid sequence differences among 15 evolutionarily related bird lysozymes tested. Another study (60) reported that in large antibody excess, five molecules of rabbit anti–HEL bound to a single HEL molecule. Based on size considerations, a maximum of six immunoglobulin molecules could theoretically bind HEL simultaneously (61). These results suggest that all faces of the HEL surface contain at least one antigenic site.

Atassi and his colleagues (62,63) used "surface-simulated peptides" to mimic surface determinants on the molecule. Three peptides were able to interact at low affinity with goat antibodies to native HEL and could absorb entirely the reactivity of these antisera. Thus, they suggested that "the precise and entire antigenic structure of native lysozyme" had been solved for all hosts. They proposed that only three antigenic sites (I—residues 5, 7, 13, 14, 125; II—33, 34, 113, 114, 116; III—62, 87, 89, 93, 96, 97) exist in HEL and, further, that all these sites are "discontinuous"—i.e. *assembled*. Their postulate of three sites contrasts with the numerous studies discussed above suggesting that

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a much larger proportion of the surface may be involved in antigenicity. Notably their sites do not include Arg 68, the Loop, and several segmental antigenic regions previously demonstrated (Figure 2A).

This view of limited antigenicity disagrees with that of a more encompassing "multideterminant" structure (4,58,59,64) suggested by previous studies discussed above. Recent reexaminations of the antigenic structure of HEL as defined by the total number of different specificities that can be detected in panels of monoclonal antibodies from several strains of inbred mice (43–45,47,47a) also suggest that the Atassi structure is incomplete. Four lines of evidence from these studies support the conclusion that the capacity for antibody response to HEL is very broad, consisting of many different clonotypes that bind distinct, but often overlapping, determinants including in aggregate most, if not all, of the HEL surface. (*a*) Studies of reactivity with panels of evolutionary variants of lysozyme, chemically modified lysozyme, and peptides have allowed the identification of specific residues and regions as critical to the binding of specific monoclonal antibodies. Antibodies specific for determinants containing the following residues have been identified: 1, 68, 121, 125, 113–114, 19–21, and 102–103. Additionally, monoclonal antibodies have been identified that bind to the N–C region, LII, or LIII (43,44,47,47a) (Figure 2B). (*b*) Most monoclonal antibodies to HEL have individually distinct patterns of fine specificity (47,47a). For example, when each of 44 monoclonal antibodies from A-strain mice were tested for reactivity with a panel of 10 different lysozymes, they could be divided into at least 18 fine-specificity groups, and only 3 pairs of antibodies with very similar profiles were found. (*c*) Most monoclonal antibodies had distinct profiles of competitive binding to HEL, and the complementation groups derived from these profiles revealed complex patterns of overlap (45,47a). These results also suggest that many individual subsites or determinants are recognized by specific antibodies binding within large regions that themselves overlap and include most of the HEL surface. (*d*) Studies on the isoelectric focusing patterns, heavy-chain variable-region gene rearrangements, and N-terminal amino acid sequences of the heavy and light chains of the antibodies themselves indicate that the antibodies express a great diversity of heavy- and light-chain variable-region genes (45,47a); such structural diversity is consistent with a broad pattern of determinant recognition.

Though the repertoire of immune responses to HEL, as represented by monoclonal antibodies, is very diverse, the actual repertoire expressed in the serum represents a skewed distribution of the total potential repertoire, such that some specificities are rare and others predominate. For example, one A-strain mouse monoclonal antibody (1G11) competed for binding to HEL with all other A-strain monoclonal antibodies tested, as well as with polyclonal antisera. The other monoclonal antibodies each competed with some, but not

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all, of the remaining antibodies, indicating that although many unique determinants are recognized, they are confined to a single face of HEL (45). However, that the apparently predominant specificities may differ among strains is indicated by complementation studies using a BALB/c hybridoma, HyHEL–5; while HyHEL–5 overlaps with most of the A-strain monoclonals tested, including 1G11 (45), it shows little overlap with most of the other BALB/c hybridomas (47a).

Further, the specificity repertoire distribution of the monoclonal antibodies differs from that found in individual antisera during both primary and secondary antibody responses (47). Thus, the "hybridoma" repertoire taken as a whole, although itself selected, may reveal a broader spectrum of the available repertoire than do serum antibodies from a given individual or inbred strain at the height of the in vivo response. For example, recent studies employing $H\text{–}2^a$ mice indicated that at least 75% of secondary response antibodies to HEL could be adsorbed on immobilized N–C peptide (52). Most such antibodies (90–95%) reacted equally well with HEL and with lysozyme lacking its three amino-terminal residues (AP–HEL) (46). In contrast, half the early primary response antibody does not recognize AP–HEL (46). These results suggest an ordered progression in recognition of different determinants on HEL, probably determined by regulatory mechanisms.

Several interesting observations have been made in studies of the role of T cells in the immune response to HEL. (*a*) While most antibodies to HEl do not react with nondisulfide peptides, segmental peptides suffice to trigger T-cell proliferative activity in all cases explored (52,61). (*b*) Mice of each MHC haplotype recognize a characteristic determinant or a small set of determinants on lysozyme. For example, $H\text{–}2^b$ helper cells seem to select a single site within the tryptic peptide containing amino acids 74–96, $H\text{–}2^d$ T cells strongly prefer a site including amino acids 113 and 114, while $H\text{–}2^a$ T cells recognize three sites but in a clearly hierarchical way (65). (*c*) The restriction of the T-helper cell repertoire can influence the population of B cells selected for expression in the antibody response (61,65). (*d*) Not only the antigen-specific but also the idiotype-recognizing T cells influence the specificity of the emerging B-cell population (66). (*e*) In addition to being highly restricted, the repertoires of suppressor and helper T cells inducible by peptide fragments seem to be nonoverlapping in the case of lysozyme (52,67). Suppressor T-cell-inducing reactivity in $H\text{–}2^b$ mice is circumscribed to a single area on the HEL molecule, at the amino terminus (46,67).

In summary, studies designed to examine total antibody repertoire, such that rare as well as predominant specificities are detected (as in cases of large antibody excess or with large numbers of monoclonal antibodies), are consistent with the hypothesis that most, if not all, of the HEL surface is potentially antigenic, consisting of multiple, overlapping determinants that may be rec-

Annual Reviews

ognized in some hosts. In contrast, studies designed to sample the predominantly expressed specificities in individual hosts (or inbred strains) have led to the conclusion that the immune response for any given individual is skewed such that antibodies recognizing a limited number of antigenic regions on the surface of HEL will predominate. The latter result depends upon the species and genetic make-up of the host, and reflects the interplay of regulatory circuits operating in that individual's immune system.

CYTOCHROME *c*

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Cytochrome *c* is a small heme protein consisting of a single polypeptide chain of 103 to 111 residues, long found to be a poor immunogen (14,15). It was only when glutaraldehyde-polymerized forms were employed that consistent and relatively strong antibody responses were obtained, making it possible to utilize the proteins of many different species to study in detail the specificities of both antibodies and T cells raised against any one of them.

Mitochondrial cytochomes *c* present a particularly attractive model system for the immunological study of globular proteins (14,15). The protein is easy to prepare, it is present in all eukaryotes, over 100 of them have had their amino acid sequences determined (68–70), and X-ray crystallographic studies have shown that all maintain the same polypeptide backbone spatial structure, the so-called *cytochrome c fold* (68,71). Differences in amino acid sequence among mitochondrial cytochromes *c* are, in effect, reflected only in local differences in surface topography resulting from the variation in side chains. This argument is particularly strong for cytochromes *c* that differ by less than 10% in amino acid sequence, with the variant residues having side chains at the surface of the protein, an ideal situation for the study of topographic antigenic determinants.

The first isolation in pure form of an antibody population directed toward a single site on cytochrome *c* was performed with rabbit antisera directed against human cytochrome *c* (72). Rhesus monkey cytochrome *c* differs from the human protein only at threonine 58, an external residue on the "back" surface of the molecule. Adsorption of anti-human antisera with the monkey protein left a population of antibodies that bound only to those cytochromes *c* with an isoleucine 58, such as the human or kangaroo proteins. Less conclusive, indirect evidence indicated that there may be three other antigenic determinants on human cytochrome *c* with respect to the rabbit host (14,15,73), in conformity with the stoichiometry of four specific Fab′ fragments binding simultaneously to the human protein, as determined by fluorescence quenching titrations (74).

Urbanski & Margoliash (16), using sequential adsorption of antisera on insolubilized cytochromes *c*, isolated three single-site antibody populations

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from rabbit antisera raised against either mouse or guanaco cytochromes *c*, as well as from ascitic fluid of mice immunized against guanaco or rabbit cytochromes *c*. The three variable positions to consider were 44, 62, and 89, at which the rabbit protein carries valine, aspartic acid, and aspartic acid; the mouse protein, alanine, aspartic acid, and glycine; and the guanaco protein, valine, glutamic acid, and glycine. Thus, in every case the immunogen differs from the host cytochrome *c* at only two residues, and in every case, antibodies specific for both of the corresponding regions were isolated. Remarkably, a third antibody population that bound to the area of aspartic acid 62, a residue shared by the immunogen and the host proteins, was present in both the rabbit antiserum to mouse cytochrome *c* and the mouse antiserum (ascitic fluid) to the rabbit protein. Farr assays showed that the guanaco protein, with a glutamic acid at 62, interacted more strongly with these antibodies than did the mouse and rabbit proteins, which carry aspartic acid at that position. This observation served to locate the binding domain of the third antibody population. The mechanism for the production of anti-self antibodies in this case, and the role that regulatory influences may play in their elicitation, are unknown. However, a simple speculation (16) was that the clones responsible for the antibodies were the products of B cells directed against the glutamic acid 62–containing surface and had not been eliminated during ontogeny because of their weak reactivity with the self-protein, with an aspartic acid 62, yet could be activated by the large doses of the cross-reacting cytochrome *c* used as immunogen.

Jemmerson & Margoliash (13) fractionated completely the rabbit antibody response to horse cytochrome *c*, isolated seven subpopulations of antibodies directed against three sites on the molecule, and showed that these comprised the totality of the horse cytochrome *c*–specific antibodies (Figure 3). Four of the populations bound a complex determinant affected by the sequence variations at residues 89 and 92, two populations bound the region of residue 44, and one that of residue 60. Competitive binding assays with a series of cytochromes *c* of varying similarity to the horse protein revealed that residues in the vicinity of the immunodominant side chains were also involved in antibody binding. These results correlate well with studies of rabbit anti-horse cytochrome *c* antisera by Berman & Harbury (76) and by Eng & Reichlin (77). The latter authors employed affinity adsorption techniques and observed the same three immunogenic areas on the protein.

The rabbit antibody response to pigeon cytochrome *c* was analyzed by Hannum & Margoliash, employing competitive plate-binding assays (C. H. Hannum, E. Margoliash, unpublished). Antibodies were found to be directed against four sites on the pigeon molecule, representing all seven of the sequence variations with rabbit cytochrome *c*, confirming fluorescence quenching stoichiometry calculations.

The first observation of a T-cell response to a cytochrome *c* was reported

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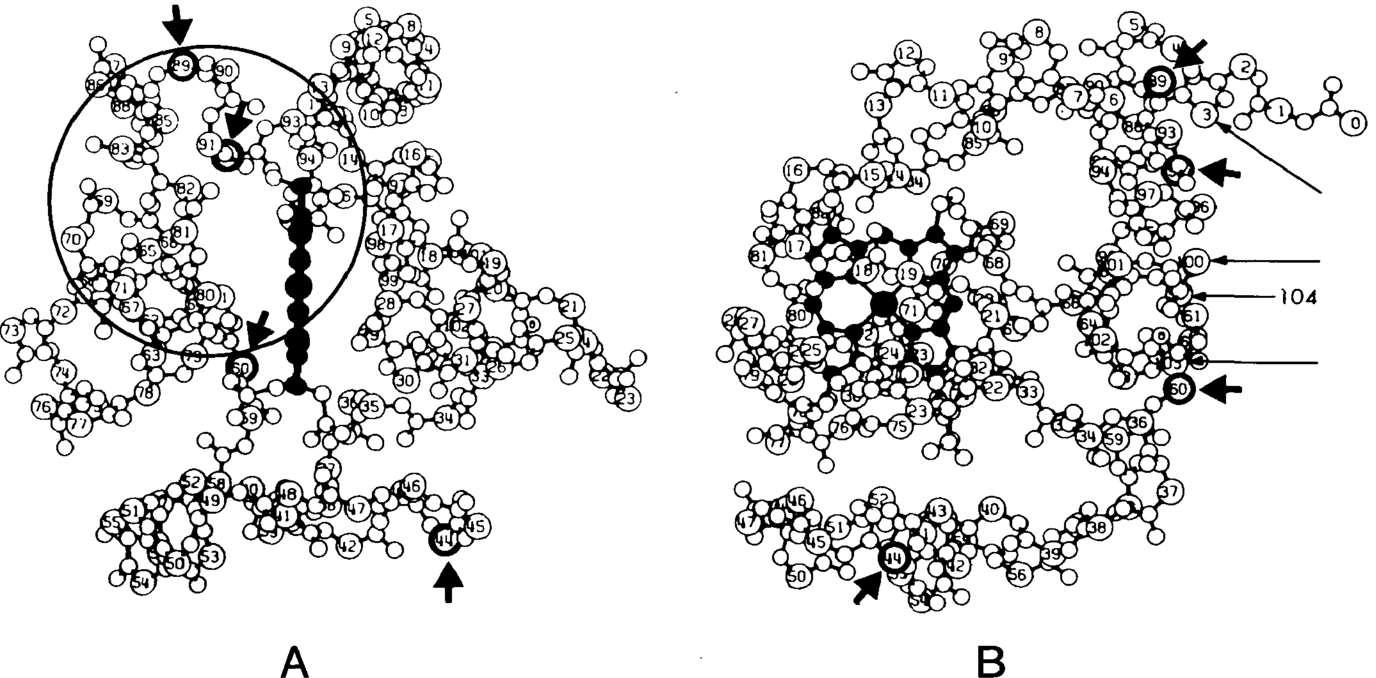

Figure 3 Diagrams of the backbone conformations of cytochrome *c*. *Panel A* is a view from the "front," with the solvent-accessible edge of the heme prosthetic group in darkened atoms. The large circle is the enzymic interaction domain. *Panel B* is a view from the righthand side, in which the square structure of the heme (darkened atoms) is seen in full. In both panels, the larger numbered circles are the α carbons. The α carbons of the residues that were shown to be immunodominant for rabbit anti-horse cytochrome *c* antibodies are indicated by heavy circles and heavy arrows. Three of the four immunodominant residues that constitute the major mouse anti-pigeon cytochrome *c* determinant (residues 3, 100, and 103) are indicated by thin arrows. The fourth, residue 104, is not shown, as the X-ray crystallographic conformation employed is taken from an electron density map of tuna cytochrome *c* (75), which has only 103 residues. However, the thin arrow

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by Reichlin & Turk (78), who induced delayed hypersensitivity in guinea pigs. The responses displayed the typical pattern of cross-reactivities to a variety of cytochromes *c* similar to that observed with rabbit antibodies. Fragments of the immunogen were ineffective. Contrary to the antibody response, however, guinea pigs immunized with a cytochrome *c* polymer displayed stronger hypersensitivity to polymers than to monomers, while the opposite was observed for animals primed with a monomer. Thus, T cells, unlike B-cell products (79,80), apparently differentiate between the single antigenic determinants of the monomeric protein and the clustered determinants of a polymer. Subsequent work by Wolff & Reichlin (81) suggested that the specificity of the guinea pig T cells was at least as sensitive to the amino acid substitutions on cross-reacting antigens as were rabbit antibodies. Similarly, Corradin & Chiller (82) found that mouse T cells primed with either beef or horse cytochrome *c* proliferated in response to either the native or fragmented forms of the immunogen, and that single residue substitutions could be recognized. One such site occurred at residue 89.

A comprehensive examination of both B- and T-cell responses to the same cytochrome *c* was carried out with B10.A mice immunized with the monomeric pigeon protein. Using a proliferative T-cell assay, Solinger et al (83) mapped the genes governing responsiveness to monomeric pigeon cytochrome *c* to both the *I–A* and *I–E* subregions of the mouse major histocompatibility complex. Subsequent experiments with monoclonal antibodies have shown that such a response indeed requires the Ia molecule $I\text{–}A^{k}_{e\beta}{:}I\text{–}E^{k}_{\alpha}$ or the hybrid Ia molecule $I\text{–}A^{s}_{e\beta}{:}I\text{–}E^{k}_{\alpha}$ (84,85). Cross-reactivity experiments with a large variety of cytochromes *c* and their cyanogen bromide fragments showed that the region of the molecule responsible for T-cell activation contains the glutamine at position 100 and the carboxyl-terminal lysine at position 104, with the possible added involvement of isoleucine 3 (86). An analysis of the B-cell response of the same mice to pigeon cytochrome *c* showed that the majority of the antibodies bound to the region of residues 3, 100, 103, and 104, a complex determinant that, although not identical to the major T-cell determinant, completely overlaps with it (C. H. Hannum, L. A. Matis, R. H. Schwartz, E. Margoliash, unpublished). These residues are all located within a few angstroms of one another and form an assembled topographic determinant containing segments of the carboxyl-terminal and amino-terminal α-helixes (Figure 3). It was also clearly demonstrated that all the antibodies are directed against those regions of the molecule where pigeon cytochrome *c* differs from the mouse protein. A comparison of the concentrations of pigeon and mouse cytochromes *c* that produce 50% inhibition of binding allows the calculation of the contribution of the variant side chains to the binding energy of the antibody-antigen interaction, amounting to about −3 kcal/mole (87). If the average affinity constant, K_a, for pigeon cytochrome *c* is about 10^8 M^{-1},

Annual Reviews

then the −3 kcal/mole would constitute some 30% of the total energy of interaction. The remaining energy is apparently provided by the interaction with the surface of the protein surrounding the immunodominant residues, areas identical on both immunogen and host proteins. This is probably the basis for the extensive cross-reactivities always observed when antibodies raised against a particular cytochrome *c* are tested with heterologous cytochromes *c*, including the host protein.

It should be noted that whereas antibodies raised in rabbits and mice to native horse or pigeon cytochromes *c* showed much lower affinities for peptide fragments of the immunogens (13,87), T cells from mice primed with horse, beef, or pigeon cytochrome *c* responded as well, and sometimes better, to the fragmented forms in proliferation assays (82,83,86). This is in contrast to the failure of guinea pigs to display delayed hypersensitivity following challenge with fragments of the horse protein (78). The differences between B- and T-cell reactivities with immunogen fragments could reflect a smaller T-cell receptor interaction domain, which makes it possible for peptide segments to react as well as they do in the native immunogen.

The one result that varies from all others was reported by Atassi (88), who found that antibodies raised in two rabbits against beef cytochrome *c* all bound to an insolubilized synthetic peptide comprising residues 42–50 of beef cytochrome *c*, a segment spanning one of the four variant residues between the beef and rabbit proteins, namely proline 44. Two other insolubilized synthetic peptides covering the other three variant residues did not bind any of the antibodies. It is not obvious why antibodies directed against the other potential determinants on beef cytochrome *c* were either not generated or not detected in these experiments, when, as discussed above, in every other case examined, rabbits produced antibodies to every one of the areas of the protein containing residues that differed from those in rabbit cytochrome *c*.

That cytochromes *c* identical to that of the homologous host protein are also capable of eliciting an antibody response became evident when rabbits injected with acetylated γ-globulin-conjugated (89) or glutaraldehyde-polymerized rabbit cytochrome *c* (90) yielded rabbit cytochrome *c*–specific antibodies in amounts averaging about 10% of the response to heterologous cytochromes *c*. Fractionation by affinity adsorption, and analysis for specificity by competitive binding assays (90), showed that these antibodies are not directed against all or many sites on the self immunogen, but rather against three areas that contain the segments of the protein that have varied most recently in the course of the evolutionary descent of mammalian cytochromes *c*. Jemmerson & Margoliash have speculated (90) that such remarkable antibody specificities represent a breakdown of self-tolerance resulting from the stimulation of cross-reactive clones, the repertoire for which has not yet been eliminated by the evolutionary selective process that may govern the retention of such autoimmune poten-

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tialities. Whether this or some other process is involved in these phenomena has not yet been examined.

In summary, cytochrome *c* can elicit antibodies in rabbits and appropriate strains of mice that react to every one of the areas in which the immunizing cytochrome *c* has one or more side-chain differences from the cytochrome *c* of the host. Furthermore, antigenic determinants responsible for T-cell proliferation appear to have the same general specificities. However, in some cases antibodies have also been obtained, albeit with great difficulty, to areas of the immunizing protein identical to those of the host protein. The mechanisms underlying such self-immunity phenomena have not been examined.

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SERUM ALBUMIN

Serum albumin (Figure 4) is a single polypeptide chain of approximately 582 amino acids. Its three structural and functional domains are phylogenetically related (91) and are independent folding units (93). Although homology in general structure exists among domains, the amino acid sequence and function of each domain have diverged (91). For example, there is at most 25% sequence identity between any two domains and no more than 10% among all three. Most of this identity among all three domains (about $\frac{12}{19}$ conserved residues) centers around the disulfide bonds, which are highly conserved throughout evolution (91). Indeed, $\frac{18}{19}$ residues shared among the domains of bovine serum albumin (BSA) are shared between it and human serum albumin (HSA). Serum albumin has long been a standard tool for the immunologist, the immunochemist, the protein biochemist, and the evolutionary biologist, who uses albumin antisera to estimate the genetic relatedness and times of divergence of species. Yet until recently, little has been known about its chemical and physical structure, not to mention its detailed antigenic structure or the control of immune responses to this protein.

There seems little doubt that serum albumin is a multideterminant antigen for which each determinant is unique, i.e. each occurs only once in the albumin molecule. This concept of multiple, distinct, antigenic determinants on serum albumin is supported by a vast literature spanning three decades beginning with the pioneer work on human serum albumin by Lapresle (94). In these early studies, Lapresle demonstrated that various enzymes would degrade HSA into several antigenically distinct fragments. Porter and his group (95,96) have shown that fragments from BSA and HSA bear only a portion of the total antigenic determinants present on the intact molecule. Using a variety of techniques Weigle (97) and others (98–100) have shown many distinct antigenic determinants on BSA. Other studies on antibody synthesis by single cells (101), on acquired immunological tolerance to albumin (102), and on

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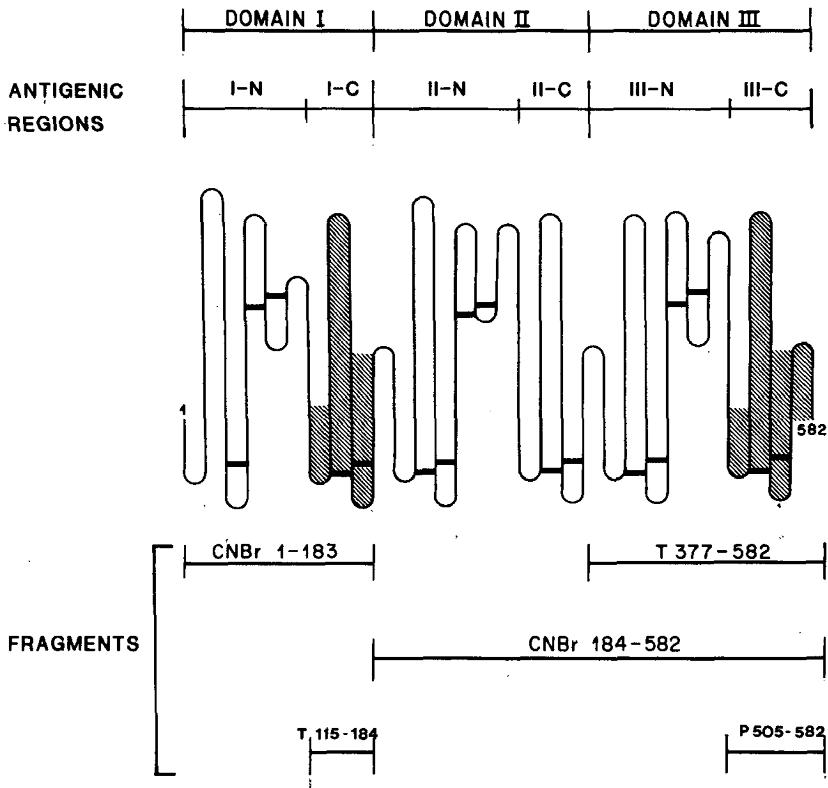

Figure 4 Diagrammatic representation of the structure of bovine serum albumin as adapted from Brown (91). Antigenic regions and antigenically active fragments are as described by Benjamin & Teale (92) and references therein.

control of immune responses to BSA by restricted populations of T cells (103) support this multi-distinct-determinant nature of albumin.

A more recent series of studies on BSA (92,104 and references therein; 105,106) and HSA (107) has yielded essentially identical results. Using polyclonal antisera to intact albumin and antigenically active fragments of albumin, each laboratory has demonstrated albumin to be composed of several antigenic regions (see Figure 4), each of which has one or more distinct determinants. Some evidence of cross-reactivity among domains, involving a small proportion of the antibodies, has occasionally been found (105,107). Similar results have been obtained with T-cell responses to BSA and fragments representing the domains and subdomains of the molecule (108). Thus, the

Annual Reviews

determinants on serum albumin are multiple and distinct at both the B-cell and the T-cell level.

Also recently, a series of studies was initiated using monoclonal antibodies and T-cell clones (104,109; L. D. Wilson, R. L. Riley, D. C. Benjamin, manuscript submitted; D. C. Benjamin, K. DeCourcy, manuscript submitted). Two hundred hybridoma cell lines producing antibody to BSA were established, and 64 randomly chosen clones have been fully characterized. In no instance did a monoclonal antibody react with more than one domain or subdomain. The 64 monoclonal antibodies could be placed into 25 groups based on their cross-reactivity with a panel of 10 mammalian albumins, suggesting a minimum of 25 different determinants. If, in addition to cross-reactivity patterns, the domain or subdomain specificity of each monoclonal antibody was considered, then a minimum of 33 determinants could be demonstrated. In other words, two or more monoclonal antibodies may have the same cross-reactivity pattern but be specific for distinct regions of the BSA molecule and thus define two, or more, distinct determinants rather than one as defined by cross-reactivity alone. Thirteen of the 64 monoclonal antibodies define 13 nonoverlapping antigenic determinants as determined by competitive inhibition assays. The remaining 51 monoclonal antibodies competitively inhibit the binding of one or more of these 13 monoclonals in a manner consistent with the existence of multiple overlapping antigenic determinants. These studies have been partially confirmed in at least one other laboratory (110). In addition, long-term T-cell lines specific for BSA are exquisitely specific for a single subdomain (109; L. D. Wilson, R. L. Riley, D. C. Benjamin, manuscript submitted). One might argue that during production of monoclonal antibodies or T-cell clones those specific for minor determinants were preferentially isolated. However, the fact that not a single monoclonal reagent has been found that cross-reacts among domains makes that possibility highly unlikely.

These results contrast with those described by Atassi and his colleagues (111,112), who have suggested that serum albumin contains only two determinants, that these two determinants are present on the carboxy-terminal double-disulfide loop of a domain, and that they are shared among domains, i.e. two determinants repeated three times on the entire molecule. However, these conclusions are inconsistent with other reports from their laboratory showing that: (*a*) sera taken early after immunization do detect multiple determinants (113); (*b*) although the reaction of late antisera (day 398 after immunization) with BSA is inhibited almost entirely (80–100%) by fragments representing single domains, using immunoadsorbent techniques a maximum of 50% of the anti-BSA was reactive with any one fragment (112); and (*c*) in cross-reactivity studies (100) these same late antisera showed varying degrees of cross-reactivity with a series of heterologous albumins, demonstrating multiple (not two) distinct determinants on BSA.

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Further doubt is cast on the suggestion of only two determinants by the fact that BSA probably differs from the albumin of the responding rabbit by many residues. Rabbit and cow diverged about 80 million years ago, which is about the time cow, human, and rat diverged from one another (114). Assuming a constant rate of albumin evolution (114), the rabbit-cow difference can be estimated from the known cow, human, and rat sequences (115,116), whose average pairwise difference is 160 amino acid residues. Given the assumption discussed above for cytochrome *c* that the responding animal will produce antibodies to all areas of the antigen different from its own protein, the calculation of White et al (59) that essentially all differences between the antigen and a similar protein from another animal are immunologically detectable (see the discussion section, below), and the fact that similarities among all three albumin domains are to a great extent conserved throughout evolution, we would predict many more than two determinants.

Certain other papers have been cited as supporting the repeating determinant hypothesis (111,112). For example, Peters et al (105) have suggested six distinct antigenic regions defined by distinct determinants. They have also shown that a portion of their antibody cross-reacts with fragments representing different domains. For instance, antibody reactive with fragment P307–582 (a peptic fragment containing amino acid residues 307–582) was partially inhibited in its reaction with BSA by fragments CNBr1–183 and P1–306, suggesting that the amino-terminal and carboxy-terminal portions of BSA share determinants. In direct contradiction to this result, they found that fragment P1–385 does not inhibit the same reaction although it would be expected to bear all the determinants present on the previous two inhibitory fragments. In addition, their studies show that antibody to native BSA reactive with P1–306 is not significantly inhibited by fragments from the carboxy-terminal half of BSA.

Thus, the evidence from many laboratories, published over three decades, supports the hypothesis that serum albumin contains multiple, distinct, sometimes overlapping antigenic determinants that do not repeat from domain to domain.

Serum albumin has been used more extensively than any other protein for immunological estimation of the extent of genetic divergence among species (e.g. 114,117). Albumins from thousands of pairs of vertebrate species have been compared immunologically (chiefly by quantitative micro-complement fixation—cf 114,118), and the results have been used to establish approximate time scales for the genealogical trees by which these species are related, thereby permitting a more quantitative approach to the study of evolution than was possible before. The evidence now presented for the existence of multiple determinants on albumin provides additional, strong justification for the use of albumin antisera for quantitative evolutionary studies.

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DISCUSSION—A MULTIDETERMINANT-REGULATORY MODEL

Currently there are opposing views of what constitutes a determinant on a protein antigen. One proposal is that proteins contain a very limited number of sites that are intrinsically immunogenic, irrespective of the host responding to the antigen (21,24,25,62,63,88,111–113). A contrasting view is that most of the accessible surface of any globular protein is potentially immunogenic, that one can define which sites are immunogenic only with respect to a particular responding individual, and that the total antigenic structure of a protein is the sum of all sites recognized by a large variety of responding individuals and species. The evidence summarized above strongly supports the second view and requires that we extend what has been called the "multideterminant hypothesis" (4,58,59,64) to encompass the following concepts: (*a*) The surface of a protein is essentially a continuum of potential antigenic sites; and (*b*) the structural differences between the antigen and self-protein as well as the host's immunological regulatory mechanisms are the important factors influencing the outcome of the immune response. We term this extended hypothesis the "multideterminant-regulatory model."

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Antigenic determinants are defined by the specificities of the antibodies to that antigen. Because the complexity of the antibody response varies with the degree of antigen foreignness (16), as well as type of antibody studied (i.e. polyclonal versus monoclonal), varying views of the definition of an antigenic determinant have arisen. Determinants have historically been considered to be discrete, nonoverlapping portions of the surface structure, and to consist of a definable, finite number for a given protein in a given host (119). For highly conserved proteins such as cytochrome *c*, polyclonal antisera can be fractionated into distinct, nonoverlapping specificities that correspond to sites of sequence variation. On the other hand, experiments designed to examine the full repertoire of monoclonal antibody specificities to protein antigens have yielded complex patterns of overlap, and these support the hypothesis that the surface of a protein molecule is a complex array of overlapping antigenic determinants. From the present data, one cannot exclude any surface elements from the antigenic structure of a globular protein. Therefore, any antigenic determinant can only be operationally defined as that region of the protein surface bound by a particular antibody molecule, and this may be termed the *unit antigenic determinant*.

The multideterminant-regulatory model predicts that nearly all evolutionary substitutions would directly affect immunological cross-reactivity. Table 1 summarizes experiments in which rabbits were immunized with a particular protein from one organism and the polyclonal antisera then tested for immunological cross-reactivity with evolutionary variants of that protein. A measure

Annual Reviews

of immunological distance derived from the observed degree of cross-reactivity (56,64,114,118,120) shows a statistically highly significant linear correlation with the number of amino acid differences between each pair of proteins tested (Table 1). From the square of the coefficient one estimates that about 80% of the variation in the immunological cross-reactivity is ascribable to variation in the number of amino acid differences. A similar correlation exists for other, less extensively studied proteins (59,114). More convincing statistical data stem from experiments with small proteins differing by only one to five amino acid substitutions per 100 residues, which can routinely be distinguished with the quantitative micro-complement fixation technique (118) and polyclonal antisera. Table 2, which shows the results of an analysis of 14 independent pairs of closely related proteins, indicates that if the fraction of potentially antigenic residues were small, e.g. if F were 0.15, there would be little chance (P <0.0005%) that every pair of these proteins would be antigenically nonidentical. By contrast, if F were 0.80, the chance of every pair being nonidentical would be high (P = 48%). The observation that every pair tested is nonidentical is thus consistent with F being in the vicinity of 0.80. Most amino acid substitutions are, therefore, immunologically detectable. Direct evidence for this conclusion comes from experiments with highly conserved small proteins such as cytochrome *c* (discussed in detail above); in a host whose own cytochrome *c* differed by only one or a few residues from the immunogen, every amino acid substitution could elicit antibodies.

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Table 1 Dependence of immunological distance on the number of differences in amino acid sequence for 290 pairs of proteins

Protein (and reference)	Number of Pairs Tested	Correlation Coefficient[a]
Lysozyme *c* (58)[b]	106	0.95
Ribonuclease (64)	60	0.92
Myoglobin[c]	78	0.87
Cytochrome *c* (56)	23	0.87
Azurin (120)	13	0.85
Serum Albumin[d]	10	0.96

[a]Correlation coefficients were calculated from least-squares lines relating the number of positions in the amino acid sequence at which these proteins differ to the degree of immunological difference measured with polyclonal antibodies produced in rabbits. The quantitative microcomplement fixation technique (118) was used in all cases except for cytochrome *c*, done by macrocomplement fixation, and serum albumin, where the quantitative precipitin method as well as micro-complement fixation was employed.

[b]E. M. Prager, A. C. Wilson, unpublished work on additional bird, reptile, and mammal lysozymes.

[c]E. M. Prager, A. C. Wilson, unpublished work on 13 whale, dolphin, and porpoise myoglobins and antisera to 12 of them.

[d]E. M. Prager, A. C. Wilson, V. M. Sarich, unpublished calculations. The immunological comparisons are from references 97 and 121 and V. M. Sarich, unpublished measurements. The complete sequences of cow, human, and rat albumins were considered along with partial sequences (164 to 469 amino acids) of sheep, pig, horse, baboon, and mouse albumins. We thank J. R. Brown, T. Peters, Jr., and A. Dugaiczyk for personal communications and summaries concerning published and unpublished sequences.

Annual Reviews

Table 2 Immunological detectability of amino acid substitutions in small monomeric globular proteins of known three-dimensional structure[a]

Protein	Number of Independent Pairs Tested	Detectability of Substitutions		
		Expected		Observed
		F = 0.15	F = 0.80	
Lysozyme *c*	4	2.1	77	100
Ribonuclease	4	3.2	79	100
Myoglobin	5	1.4	79	100
Azurin	1	48.0	100	100
All Proteins	14	0.00045	48	100

[a]Following the method of White et al (59), the expected values in the table indicate the probability in percent that pairs of closely related proteins (differing by 0.7–8.5% in sequence) will be immunologically distinguishable if *F*, the fraction of antigenic residues in the molecule, is 0.15 or 0.80. For each protein the product of the probabilities for the indicated number of independent pairs is given. The experimentally observed values, likewise given in percent, are the number of distinguishable pairs found relative to the number examined. The data and calculations are from references 58, 59, 64, and 120 and from footnotes *b* and *c* to Table 1; all data were obtained with the micro-complement fixation technique (118).

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Some evolutionary substitutions may influence antigenicity through long-range effects, either conformational or electrostatic (122,123), as observed in allosteric proteins such as hemoglobin. Even for hemoglobin, however, less than 5% of such substitutions appear to cause long-range effects (124). For monomeric globular proteins, most evolutionary substitutions probably have only local effects on structure and function (31,33,43,124a,124b). Indeed, for several proteins discussed above, substitutions that markedly affect the binding of a monoclonal antibody to one site do not affect the binding of a second monoclonal antibody to an adjacent site (31,33,43; S. J. Smith-Gill, C. R. Mainhart, T. B. Lavoie, manuscript submitted) and vice versa. The long-range hypothesis would have predicted both sets of substitutions to perturb the binding of both antibodies, and this does not appear to occur.

The use of monoclonal antibodies has allowed the delineation of antigenic structure at a level of precision not previously possible. One can now study the specificity of each monoclonal antibody individually and thus avoid the ambiguity present in whole antisera or even antisera fractionated into populations directed to single antigenic sites. While the specificity of polyclonal antibodies for cross-reacting proteins is generally related to the number of amino acid differences or "evolutionary distance" between the two proteins (56–59,64,114,118,120; Table 1), the specificity of a monoclonal antibody is not, because a monoclonal antibody recognizes only a single site and not an average of a number of determinants summed. The sensitivity with which single amino acid substitutions can be detected is much greater with monoclonal antibodies than with antisera in which a host of other antibodies not affected by the substitution may swamp out any effects on overall binding.

Annual Reviews

This sensitivity has allowed the delineation of boundaries of determinants (43), i.e. an amino acid substitution that affects the binding of antibody A but not B is not within the unit determinant recognized by antibody B (31,33,43). However, the precision of these boundaries is limited and dependent upon the availability of related proteins with amino acid sequence changes at each relevant border of the site. Such an analysis can be done only with difficulty using heterogeneous antisera that have been fractionated into more homogeneous populations.

Similarly, studies of competition between pairs of monoclonal antibodies for binding simultaneously to the same monomeric protein antigen often allow the grouping of antibodies into groups such that members of each group compete with one another but not with members of another group (45,125,126). This approach has allowed definition of boundaries of nonoverlapping unit determinants for myoglobin (125), lysozyme (45; S. J. Smith-Gill, C. R. Mainhart, T. B. Lavoie, manuscript submitted), and serum albumin (104,109; D. C. Benjamin, K. DeCourcy, manuscript submitted). However, these competition experiments have also frequently suggested complex patterns of overlap. For instance, numerous cases of three antibodies, e.g. called A, B, and C, interacting in a pattern such that A competes with B, B competes with C, but A does not compete with C, have been found with HEL (45; S.J. Smith-Gill, C.R. Mainhart, T. B. Lavoie, manuscript submitted), and BSA (109; D. C. Benjamin, K. DeCourcy, manuscript submitted). Such complex patterns have allowed the conclusion, on the one hand, that most of the antibodies in the secondary response to HEL by A-strain mice recognize unit antigenic determinants on a single face of the HEL surface, each antibody with its distinct pattern of fine specificity (45), and on the other hand, that most, if not all, of the BSA surface is recognized by mouse monoclonal antibodies (104,109; D. C. Benjamin, K. DeCourcy, manuscript submitted). Observation of these complex patterns of overlap is consistent with the existence of overlapping determinants and with the multideterminant-regulatory hypothesis. In three of the four protein system discussed in this review, monoclonal antibodies were found that react with overlapping determinants that in aggregate cover large portions, if not all, of the protein surface. In the case of the pigeon cytochrome *c*, a significant portion of the binding energy is due to invariant residues adjacent to the variant residue used to localize the site (87). Because a single amino acid residue may be contained in several overlapping unit determinants, the number of discrete determinants is probably greater than the number of surface residues. As a result, a number of overlapping determinants probably have not yet been distinguished from each other. These observations exclude any hypothesis that restricts the number of determinants to a few discrete sites on the surface of a protein antigen (21,62,63,88,111–113).

Important examples illustrating many of the above points are the immune

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responses to two surface glycoproteins of the influenza virus (126–129). The antigenic determinants of the neuraminidase molecule are overlapping and cluster on the distal surface of the molecule, forming a "nearly continuous surface across the top" (127). The antigenic determinants of the hemagglutinin protein similarly cluster on the distal end (126,128,129). In one study (128), 104 distinct fine specificities could be distinguished among antibodies that recognized four antigenic regions delineated by Wiley et al (126) at which antibody binding could select for viral mutations. It was estimated that the total repertoire included a minimum of 1500 fine specificities (128). In another study (129), 125 monoclonal antibodies raised to the hemagglutinin of influenza virus H3N2 cross-reacted with 15 other strains in a way that permitted mapping of 10 overlapping regions covering the entire accessible surface.

Thus, a nearly limitless specificity spectrum of antibody reactivity can potentially be elicited. However, the entire spectrum is rarely, if ever, seen in any individual. The pertinent question is what determines which of the many specifically reactive clones are expressed. Elimination or anergy of self-reactive clones would severely reduce this spectrum, especially in those instances where the immunogen is derived from a source closely related to the responding species. In the case of cytochrome *c*, a slowly evolving protein, the limited sequence differences between each cytochrome *c* and that of the responding host (2–10%) restricts the number of determinants detectable in any host. However, the fact that most substitutions that occur are immunologically detectable in some host supports the hypothesis that virtually all the surface is immunogenic. The relative simplicity of the response to closely related heterologous cytochromes *c* allowed the separation of antibody populations directed to simple or complex topographic determinants and even of several subpopulations reacting with subsites of a single complex determinant (13,16). These not only gave definitive evidence that the sequence variations between antigen and the homologous host protein were responsible for immunogenicity, but also provided the first demonstration of an overlapping distribution of determinants.

When the immune response appears to be directed predominantly toward particular antigenic regions, this may reflect limitation of the specificity repertoire by the regulatory mechanisms operative in that individual at the time of immunization. For instance, MHC-linked genes have been found to control the fine specificity of antibody and/or T-cell responses in mice and guinea pigs to a variety of protein antigens, including myoglobin (130,131), serum albumin (104,108,109,132; L. D. Wilson, R. L. Riley, D. C. Benjamin, manuscript submitted), lysozyme (133–135), cytochrome *c* (82,83), insulin (136–138), and staphylococcal nuclease (139,140). Furthermore, the predominant determinants recognized by T cells may be different from and frequently fewer than those predominant determinants recognized by antibodies in the same

Annual Reviews

individual (40,40a,52,134). The antigenic sites utilized by antigen-specific helper T cells in a particular individual can influence the specificity of the subsequent antibody response to that antigen (52,61,67,141). Last, the expressed repertoire of an individual may be regulated by idiotypic networks (66,142).

Studies with polyclonal antibodies have suggested that protein conformation is critical to the integrity of antigenic determinants (4,5,8,27–29,51). A colorful example of the conformational specificity of polyclonal antisera is that of antibodies that distinguish the conformation of apomyoglobin from that of holomyoglobin (119). When brown holomyoglobin was reacted with antibodies to apomyoglobin, the precipitate was white. Thus, the antibody shifted the equilibrium toward a form in which the heme was excluded.

The analysis of individual determinants recognized by monoclonal antibodies has shown that a given antigenic site may include amino acids quite distant in the primary sequence that are brought close together on the surface during folding of the polypeptide chain (31,33,36,43,62). This is to be expected because an antibody sees a continuous surface, as in the space-filling models shown in Figures 1B and 2B.

The lower affinity usually observed for binding of peptide fragments of an antigen to an antibody raised against the native antigen (27,29) may be due either to the absence in the peptide of a portion of the determinant, to conformational differences between the peptide and the native molecule, or to the absence in the peptide of long-range effects (e.g. electrostatic) that may exist in the native protein (122,123). Thus, if a peptide that contains only a portion of the antigenic determinant is presented to the antibody, even if it were in the same conformation as in the native protein, the binding should be much weaker. However, in general, binding of anti-protein antibodies to short and unstructured peptides should be interpreted with caution, since recent experiments (P-t. Shi, J. Riehm, P. E. E. Todd, S. J. Leach, manuscript submitted), discussed in detail in the section on myoglobin, above, have demonstrated that such binding may be of limited biological significance, but rather dependent on hydrophobicity and charge.

In contrast to studies with antibodies, T cells elicited by immunization with a native protein frequently have been found to react equally well when challenged with either the native or denatured forms of that antigen or its peptide fragments (131,134,137,143,144). The molecular mechanism(s) of this cross-reactivity remains to be elucidated.

CONCLUSIONS

The surface of a protein antigen consists of a complex array of overlapping potential antigenic determinants; in aggregate these approach a continuum. Most determinants depend upon the conformational integrity of the native

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molecule. Those to which an individual responds are dictated by the structural differences between the antigen and the host's self-proteins and by host regulatory mechanisms, and are not necessarily an inherent property of the protein molecule reflecting restricted antigenicity or limited antigenic sites.

Literature Cited

1. Köhler, G., Milstein, C. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256:495
2. Jerne, N. K. 1960. Immunological speculations. *Ann. Rev. Microbiol.* 14:341
3. Lerner, R. A. 1982. Tapping the immunological repertoire to produce antibodies of predetermined specificity. *Nature* 299:592
4. Landsteiner, K. 1936. *The Specificity of Serological Reactions*. Springfield, IL: Charles C. Thomas
5. Brown, R. K., Delaney, R., Levine, L., Van Vunakis, H. 1959. Studies on the antigenic structure of ribonuclease. I. General role of hydrogen and disulfide bonds. *J. Biol. Chem.* 234:2043
6. Shinka, S., Imanishi, M., Miyagawa, N., Amano, T., Inouye, M., Tsugita, A. 1967. Chemical studies on antigenic determinants of hen egg white lysozyme. I. *Biken J.* 10:89
7. Gerwing, J., Thompson, K. 1968. Studies on the antigenic properties of egg-white lysozyme. I. Isolation and characterization of a tryptic peptide from reduced and alkylated lysozyme exhibiting haptenic activity. *Biochemistry* 7:3888
8. Young, J. D., Leung, C. Y. 1970. Immunochemical studies on lysozyme and carboxymethylated lysozyme. *Biochemistry* 9:2755
9. Sela, M. 1969. Antigenicity: some molecular aspects. *Science* 166:1365
10. Sela, M., Schechter, B., Schechter, I., Borek, F. 1967. Antibodies to sequential and conformational determinants. *Cold Spring Harbor Symp. Quant. Biol.* 32:537
11. Furie, B., Schechter, A. N., Sachs, D. H., Anfinsen, C. B. 1975. An immunological approach to the conformational equilibrium of staphylococcal nuclease. *J. Mol. Biol.* 92:497
12. Lando, G., Reichlin, M. 1982. Antigenic structure of sperm whale myoglobin. II. Characterization of antibodies preferentially reactive with peptides arising in response to immunization with the native protein. *J. Immunol.* 129:212
13. Jemmerson, R., Margoliash, E. 1979. Topographic antigenic determinants on cytochrome *c*. Immunoadsorbent separation of the rabbit antibody populations directed against horse cytochrome *c*. *J. Biol. Chem.* 254:12706
14. Reichlin, M. 1975. Amino acid substitution and the antigenicity of globular proteins. *Adv. Immunol.* 20:71
15. Urbanski, G. J., Margoliash, E. 1977. The antigenicity of cytochrome *c*. In *Immunochemistry of Enzymes and Their Antibodies*, ed. M. R. J. Salton, p. 203. New York: Wiley
16. Urbanski, G. J., Margoliash, E. 1977. Topographic determinants on cytochrome *c*. I. The complete antigenic structures of rabbit, mouse, and guanaco cytochromes *c* in rabbits and mice. *J. Immunol.* 118:1170
17. Edmundson, A. B. 1965. Amino-acid sequence of sperm whale myoglobin. *Nature* 205:883
18. Kendrew, J. C., Dickerson, R. E., Strandberg, B. E., Hart, R. G., Davies, D. R., Phillips, D. C., Shore, V. C. 1960. Structure of myoglobin. A three-dimensional Fourier synthesis at 2Å resolution. *Nature* 185:422
19. Takano, T. 1977. Structure of myoglobin refined at 2.0Å resolution. I. Crystallographic refinement of metmyoglobin from sperm whale. *J. Mol. Biol.* 110:537
20. Crumpton, M. J., Wilkinson, J. M. 1965. The immunological activity of some of the chymotryptic peptides of sperm-whale myoglobin. *Biochem. J.* 94:545
21. Atassi, M. Z. 1975. Antigenic structure of myoglobin: The complete immunochemical anatomy of a protein and conclusions relating to antigenic structures of proteins. *Immunochemistry* 12:423
22. Smith, J. A., Hurrell, J. G. R., Leach, S. J. 1977. A novel method for delineating antigenic determinants: Peptide synthesis and radioimmunoassay using the same solid support. *Immunochemistry* 14:565
23. Todd, P. E. E., East, I. J., Leach, S. J. 1982. The immunogenicity and antigenicity of proteins. *Trends Biochem. Sci.* 7:212
24. Twining, S. S., David, C. S., Atassi, M. Z. 1981. Genetic control of the immune response to myoglobin. IV. Mouse antibodies in outbred and congenic strains

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against sperm-whale myoglobin recognize the same antigenic sites that are recognized by antibodies raised in other species. *Mol. Immunol.* 18:447

25. Twining, S. S., Lehmann, H., Atassi, M. Z. 1980. The antibody response to myoglobin is independent of the immunized species. Analysis in terms of replacements in the antigenic sites and in environmental residues of the cross-reactions of fifteen myoglobins with sperm-whale myoglobin antisera raised in different species. *Biochem. J.* 191:681
26. Hurrell, J. G. R., Smith, J. A., Leach, S. J. 1978. The detection of five antigenically reactive regions in the soybean leghemoglobin a molecule. *Immunochemistry* 15:297
27. Sachs, D. H., Schechter, A. N., Eastlake, A., Anfinsen, C. B. 1972. An immunological approach to the conformational equilibria of polypeptides. *Proc. Natl. Acad. Sci. USA* 69:3790
28. East, I. J., Todd, P. E., Leach, S. J. 1980. On topographic antigenic determinants in myoglobins. *Mol. Immunol.* 17:519
29. Lando, G., Berzofsky, J. A., Reichlin, M. 1982. Antigenic structure of sperm whale myoglobin: I. Partition of specificities between antibodies reactive with peptides and native protein. *J. Immunol.* 129:206
30. Berzofsky, J. A., Hicks, G., Fedorko, J., Minna, J. 1980. Properties of monoclonal antibodies specific for determinants of a protein antigen, myoglobin. *J. Biol. Chem.* 255:11188
31. Berzofsky, J. A., Buckenmeyer, G. K., Hicks, G., Gurd, F. R.N., Feldmann, R. J., Minna, J. 1982. Topographic antigenic determinants recognized by monoclonal antibodies to sperm whale myoglobin. *J. Biol. Chem.* 257:3189
32. Dickerson, R. E. 1964. X-ray analysis and protein structure. In *The Proteins*, ed. H. Neurath, 2:603. New York: Academic, 2nd ed.
33. East, I. J., Hurrell, J. G. R., Todd, P. E. E., Leach, S. J. 1982. Antigenic specificity of monoclonal antibodies to human myoglobin. *J. Biol. Chem.* 257:3199
34. Leach, S. J. 1983. How antigenic are antigenic peptides? *Biopolymers* 22:425
35. Feldmann, R. J., Bing, D. H., Furie, B. C., Furie, B. 1978. Interactive computer surface graphics approach to study of the active site of bovine trypsin. *Proc. Natl. Acad. Sci. USA* 75:5409
36. Hurrell, J. G. R., Smith, J. A., Todd, P. E., Leach, S. J. 1977. Cross-reactivity between mammalian myoglobins: Linear vs spatial antigenic determinants. *Immunochemistry* 14:283
37. Kazim, A. L., Atassi, M. Z. 1980. Nearest-neighbour analysis of myoglobin antigenic sites. Nearest-neighbour residues whose replacement can alter the environment of binding-site residue(s) and thus change their characteristics and binding capability. *Biochem. J.* 191:673
38. Berzofsky, J. A., Buckenmeyer, G. K., Hicks, G., Killion, D. J., Berkower, I., Kohno, Y., Flanagan, M. A., Busch, M. R., Feldmann, R. J., Minna, J., Gurd, F. R. N. 1983. Topographic antigenic determinants detected by monoclonal antibodies to myoglobin. In *Protein Conformation as Immunological Signal*, ed. F. Celada, V. Schumaker, E. E. Sercarz, p. 165. New York: Plenum
39. Berzofsky, J. A., Buckenmeyer, G. K., Hicks, G. 1982. Genetic control of the immune response to myoglobins. VI. Distinct *Ir* genes for different myoglobins: Complementing genes in *I–A* and *H–2D* for equine myoglobin. *J. Immunol.* 128:737
40. Berkower, I., Buckenmeyer, G. K., Gurd, F. R. N., Berzofsky, J. A. 1982. A possible immunodominant epitope recognized by murine T lymphocytes immune to different myoglobins. *Proc. Natl. Acad. Sci. USA* 79:4723

40a. Berkower, I., Matis, L. A., Buckenmeyer, G. K., Gurd, F. R. N., Longo, D. L., Berzofsky, J. A. 1984. Identification of distinct predominant epitopes recognized by myoglobin-specific T cells under control of different *Ir* genes and characterization of representative T cell clones. *J. Immunol.* 132:1370.

41. Osserman, E. F., Canfield, R. E., Beychok, S., eds. 1974. *Lysozyme*. New York: Academic
42. Arnon, R. 1977. Immunochemistry of lysozyme. In *Immunochemistry of Enzymes and Their Antibodies*, ed. M. R. J. Salton, p. 1. New York: Wiley
43. Smith-Gill, S. J., Wilson, A. C., Potter, M., Prager, E. M., Feldmann, R. J., Mainhart, C. R. 1982. Mapping the antigenic epitope for a monoclonal antibody against lysozyme. *J. Immunol.* 128:314
44. Kobayashi, T., Fujio, H., Kondo, K., Dohi, Y., Hirayama, A., Takagaki, Y., Kosaki, G., Amano, T. 1982. A monoclonal antibody specific for a distinct region of hen egg-white lysozyme. *Mol. Immunol.* 19:619
45. Miller, A., Ch'ng, L.-K., Benjamin, C., Sercarz, E., Brodeur, P., Riblet, R. 1983. Detailed analysis of the public idiotype of anti-hen egg-white lysozyme antibodies. *Ann. NY Acad. Sci.* 418:140
46. Wicker, L. S., Benjamin, C. D., Katz, M. E., Sercarz, E. E. Miller, A. 1982.

Annual Reviews

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The similar and highly restrictive epitope specificity of Ts and primary B cells. *Fed. Proc.* 41:302 (Abstr.)

47. Metzger, D. W., Ch'ng, L.-K., Miller, A., Sercarz, E. E. 1983. The lysozyme-specific B-cell repertoire: I. Specificity repertoire of anti-hen eggwhite lysozyme antibodies probed with hybridomas. *Eur. J. Immunol.* In press

47a. Smith-Gill, S. J., Mainhart, C. R., Lavoie, T. B., Rudikoff, S., Potter, M. 1984. V_L–V_H expression by monoclonal antibodies recognizing avian lysozyme. *J. Immunol.* 132:963

48. Phillips, D. C. 1974. Crystallographic studies of lysozyme and its interactions with inhibitors and substrates. See Ref. 41, p. 9

49. Banyard, S. H., Blake, C. C. F., Swan, I. D. A. 1974. The high resolution X-ray study of human lysozyme: A preliminary analysis. See Ref. 41, p. 71

50. Sarma, R., Bott, R. 1977. Crystallographic study of turkey egg-white lysozyme and its complex with a disaccharide. *J. Mol. Biol* 113:555

51. Thompson, K., Harris, M., Benjamini, E., Mitchell, G., Noble, M. 1972. Cellular and humoral immunity: A distinction in antigenic recognition. *Nature New Biol.* 238:20

52. Harvey, M. A., Adorini, L., Miller, A., Sercarz, E. E. 1979. Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype. *Nature* 281:594

53. Takagaki, Y., Hirayama, A., Fujio, H., Amano, T. 1980. Antibodies to a continuous region at residues 38–54 of hen egg white lysozyme found in a small fraction of anti-hen egg white lysozyme antibodies. *Biochemistry* 19:2498

54. Ibrahimi, I. M., Eder, J., Prager, E. M., Wilson, A. C., Arnon, R. 1980. The effect of a single amino acid substitution on the antigenic specificity of the loop region of lysozyme. *Mol. Immunol.* 17:37

55. Mozes, E., Maron, E., Arnon, R., Sela, M. 1971. Strain-dependent differences in the specificity of antibody responses toward lysozyme. *J. Immunol.* 106:862

56. Prager, E. M., Wilson, A. C. 1971. The dependence of immunological cross-reactivity upon sequence resemblance among lysozymes. I. Micro-complement fixation studies. *J. Biol. Chem.* 246:5978

57. Prager, E. M., Wilson, A. C. 1971. The dependence of immunological cross-reactivity upon sequence resemblance among lysozymes. II. Comparison of precipitin and micro-complement fixation results. *J. Biol. Chem.* 246:7010

58. Ibrahimi, I. M., Prager, E. M., White, T. J., Wilson, A. C. 1979. Amino acid sequence of California quail lysozyme. Effect of evolutionary substitutions on the antigenic structure of lysozyme. *Biochemistry* 18:2736

59. White, T. J., Ibrahimi, I. M., Wilson, A. C. 1978. Evolutionary substitutions and the antigenic structure of globular proteins. *Nature* 274:92

60. Fujio, H., Kishiguchi, S., Shinka, S., Saiki, Y., Amano, T. 1959. Immunochemical studies on lysozyme. I. Comparative studies of lysozyme and lysozyme methyl ester. *Biken J.* 2:56

61. Cecka, J. M., Stratton, J. A., Miller, A., Sercarz, E. 1976. Structural aspects of immune recognition and its consequences for antibody specificity. *Eur. J. Immunol.* 6:639

62. Atassi, M. Z. 1978. Precise determination of the entire antigenic structure of lysozyme: Molecular features of protein antigenic structures and potential of 'surface-simulation synthesis'—a powerful new concept for protein binding sites. *Immunochemistry* 15:909

63. Atassi, M. Z., Lee, C.-L. 1978. The precise and entire antigenic structure of native lysozyme. *Biochem. J.* 171:429

64. Prager, E. M., Welling, G. W., Wilson, A. C. 1978. Comparison of various immunological methods for distinguishing among mammalian pancreatic ribonucleases of known amino acid sequence. *J. Mol. Evol.* 10:293

65. Katz, M. E., Miller, A., Krzych, U., Wicker, L., Maizels, R., Clarke, J., Shastri, N., Oki, A., Sercarz, E. 1983. Hierarchical relationships among epitopes: Only certain potentially utilizable epitopes on protein antigens are actually presented in a particular haplotype. In *Ir Gene: Past, Present, and Future*, ed. C. W. Pierce, S. E. Cohen, J. A. Kapp, B. D. Schwartz, D. C. Schreffler, p. 311. Clifton, NJ: Humana

66. Benjamin, C. D., Miller, A., Sercarz, E. E. 1983. Idiotypic encounters regulate the quality, quantity and specificity of the antibody response to lysozyme. *Fed. Proc.* 42:417 (Abstr.)

67. Adorini, L., Harvey, M. A., Miller, A., Sercarz, E. E. 1979. Fine specificity of regulatory T cells. II. Suppressor and helper T cells are induced by different regions of hen egg-white lysozyme in a genetically nonresponder mouse strain. *J. Exp. Med.* 150:293

68. Margoliash, E. 1972. The molecular variations of cytochrome *c* as a function of the evolution of species. *Harvey Lect.* 66:177

69. Brautigan, D. L., Ferguson-Miller, S., Margoliash, E. 1978. Mitochondrial

Annual Reviews

Annu. Rev. Immunol. 1984.2:67-101. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

cytochrome *c*: preparation and activity of native and chemically modified cytochromes *c*. *Meth. Enzymol.* 53:128

70. Schwartz, R. M., Dayhoff, M. O. 1978. Cytochromes. In *Atlas of Protein Sequence and Structure,* Vol. 5 (Suppl. 3), ed. M. O. Dayhoff, p. 29. Washington DC: Natl. Biomed. Res. Found.
71. Dickerson, R. E. 1980. Cytochrome *c* and the evolution of energy metabolism. *Sci. Amer.* 242(3):136
72. Nisonoff, A., Reichlin, M., Margoliash, E. 1970. Immunological activity of cytochrome *c*. II. Localization of a major antigenic determinant of human cytochrome *c*. *J. Biol. Chem.* 245:940
73. Margoliash, E., Nisonoff, A., Reichlin, M. 1970. Immunological activity of cytochrome *c*. I. Precipitating antibodies to monomeric vertebrate cytochromes *c*. *J. Biol. Chem.* 245:931
74. Noble, R. W., Reichlin, M., Gibson, Q. H. 1969. The reactions of antibodies with hemeprotein antigens. The measurement of reaction kinetics and stoichiometry by fluorescence quenching. *J. Biol. Chem.* 244:2403
75. Swanson, R., Trus, B. L., Mandel, N., Mandel, G., Kallai, O. B., Dickerson, R. E. 1977. Tuna cytochrome *c* at 2.0 Å resolution. I. Ferricytochrome structure analysis. *J. Biol. Chem.* 252:759
76. Berman, P. W., Harbury, H. A. 1980. Immunochemistry of cytochrome *c*. Identification of antigenic determinants through the study of hybrid molecules. *J. Biol. Chem.* 255:6133
77. Eng, J., Reichlin, M. 1979. Fractionation of rabbit anti-horse cytochrome *c*—I. Specificity of the isolated fractions. *Mol. Immunol.* 16:225
78. Reichlin, M., Turk, J. L. 1974. Specificity of antigen receptors for cytochrome *c* in delayed hypersensitivity. *Nature* 251:355
79. Margoliash, E., Reichlin, M., Nisonoff, A. 1968. The immunological properties of cytochrome *c*. In *Structure and Function of Cytochromes,* ed. K. Okunuki, M. D. Kamen, I. Sekuzu, p. 269. Tokyo: Univ. Tokyo Press
80. Reichlin, M., Nisonoff, A., Margoliash, E. 1970. Immunological activity of cytochrome *c*. III. Enhancement of antibody detection and immune response initiation by cytochrome *c* polymers. *J. Biol. Chem.* 245:947
81. Wolff, M. L., Reichlin, M. 1978. Antigenic specificity of T cell receptors for cytochrome *c* on pig lymphocytes. *Immunochemistry* 15:289
82. Corradin, G., Chiller, J. M. 1979. Lymphocyte specificity to protein antigens. II. Fine specificity of T-cell activation with cytochrome *c* and derived peptides as antigenic probes. *J. Exp. Med.* 149:436
83. Solinger, A. M., Ultee, M. E., Margoliash, E., Schwartz, R. H. 1979. T-lymphocyte response to cytochrome *c*. I. Demonstration of a T-cell heteroclitic proliferative response and identification of a topographic antigenic determinant on pigeon cytochrome *c* whose immune recognition requires two complementing major histocompatibility complex–linked immune response genes. *J. Exp. Med.* 150:830
84. Hansburg, D., Hannum, C., Inman, J. K., Appella, E., Margoliash, E., Schwartz, R. H. 1981. Parallel cross-reactivity patterns of 2 sets of antigenically distinct cytochrome *c* peptides: Possible evidence for a presentational model of *Ir* gene function. *J. Immunol* 127:1844
85. Matis, L. A., Hedrick, S. M., Hannum, C., Ultee, M. E., Lebwohl, D., Margoliash, E., Solinger, A. M., Lerner, E. A., Schwartz, R. H. 1982. The T lymphocyte response to cytochrome *c*. III. Relationship of the fine specificity of antigen recognition to major histocompatibility complex genotype. *J. Immunol.* 128:2439
86. Ultee, M. E., Margoliash, E., Lipkowski, A., Flouret, G., Solinger, A. M., Lebwohl, D., Matis, L. A., Chen, C., Schwartz, R. H. 1980. The T lymphocyte response to cytochrome *c*—II. Molecular characterization of a pigeon cytochrome *c* determinant recognized by proliferating T lymphocytes of the B10.A mouse. *Mol. Immunol.* 17:809
87. Hannum, C., Ultee, M., Matis, L. A., Schwartz, R. H., Margoliash, E. 1982. The major B and T cell determinant on pigeon cytochrome *c* in B10.A mice. In *Immunobiology of Proteins and Peptides—II,* ed. M. Z. Atassi, p. 37. New York: Plenum
88. Atassi, M. Z. 1981. Immune recognition of cytochrome *c*. I. Investigation by synthesis whether antigenic sites of polymeric cytochrome coincide with locations of sequence differences between the immunizing and host cytochromes. *Mol. Immunol.* 18:1021
89. Nisonoff, A., Margoliash, E., Reichlin, M. 1967. Antibodies to rabbit cytochrome *c* arising in rabbits. *Science* 155:1273
90. Jemmerson, R., Margoliash, E. 1979. Specificity of the antibody response of rabbits to a self-antigen. *Nature* 282:468.
91. Brown, J. R. 1976. Structural origins of mammalian albumin. *Fed. Proc.* 35:2141
92. Benjamin, D. C., Teale, J. M. 1978. The antigenic structure of bovine serum albumin. Evidence for multiple, different,

Annual Reviews

Annu. Rev. Immunol. 1984.2:67-101. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

domain-specific antigenic determinants. *J. Biol. Chem.* 253:8087

93. Teale, J. M., Benjamin, D. C. 1976. Antibody as an immunological probe for studying the refolding of bovine serum albumin. II. Evidence for the independent refolding of the domains of the molecule. *J. Biol. Chem.* 251:4609
94. Lapresle, C. 1955. Étude de la dégradation de la sérumalbumine humaine par un extrait de rate de lapin. II. Mise en évidence de trois groupements spécifiques différents dans le motif antigènique de l'albumine humaine et de trois anticorps correspondants dans le sérum de lapin anti-albumine humaine. *Ann. Inst. Pasteur* 89:654
95. Porter, R. R. 1957. The isolation and properties of a fragment of bovine serum albumin which retains the ability to combine with rabbit antiserum. *Biochem. J.* 66:677
96. Press, E. M., Porter, R. R. 1962. Isolation and characterization of fragments of human serum albumin containing some of the antigenic sites of the whole molecule. *Biochem. J.* 83:172
97. Weigle, W. O. 1961. Immunochemical properties of the cross-reaction between anti-BSA and heterologous albumins. *J. Immunol.* 87:599
98. Timpl, R., Furthmayr, H., Wolff, I. 1967. Antigenic determinant analysis of bovine serum albumin. *Int. Arch. Allergy Appl. Immunol.* 32:318
99. Kamiyama, T. 1977. Immunological cross-reactions and species-specificities of bovine, goat and sheep serum albumins. *Immunochemistry* 14:85
100. Sakata, S., Atassi, M. Z. 1979. Immunochemistry of serum albumin. VI. A dynamic approach to the immunochemical cross-reactions of proteins using serum albumins from various species as models. *Biochem. Biophys. Acta* 576:322
101. Benjamin, D. C., Weigle, W. O. 1970. Frequency of single spleen cells from hyperimmune rabbits producing antibody of two different specificities. *J. Immunol.* 105:537
102. Benjamin, D. C., Weigle, W. O. 1970. The termination of immunological unresponsiveness to bovine serum albumin in rabbits. I. Quantitative and qualitative response to cross-reacting albumins. *J. Exp. Med.* 132:66
103. Ferguson, T. A., Peters, T. Jr., Reed, R., Pesce, A. J., Michael, J. G. 1983. Immunoregulatory properties of antigenic fragments from bovine serum albumin. *Cell. Immunol.* 78:1
104. Benjamin, D. C., Daigle, L. A., Riley, R. L. 1983. The antigenic structure of bovine serum albumin: T-cell, B-cell, and Ia-determinants. See Ref. 38, p. 261
105. Peters, T. Jr., Feldhoff, R. C., Reed, R. G. 1977. Immunochemical studies of fragments of bovine serum albumin. *J. Biol. Chem.* 252:8464
106. Kamiyama, T. 1977. Immunological cross-reactions and species-specificities of cyanogen bromide cleaved fragments of bovine, goat and sheep serum albumins. *Immunochemistry* 14:91
107. Doyen, N., Pesce, A. J., Lapresle, C. 1982. Immunochemical cross-reactivity between cyanogen bromide fragments of human serum albumin. *J. Biol. Chem.* 257:2770
108. Daigle, L. A., Riley, R. L., Benjamin, D. C. 1982. MHC genetic control of immune responses to bovine albumin. *Fed. Proc.* 41:294 (Abstr.)
109. Benjamin, D. C., DeCourcy, K., Riley, R. L., Wilson, L. D. 1983. Clonal analysis of the antigenic structure and genetic control of immune responses to bovine serum albumin. *Fed. Proc.* 42:1237 (Abstr.)
110. Krieger, N. J., Pesce, A., Michael, J. G. 1983. Immunoregulation of the anti-bovine serum albumin response by polyclonal and monoclonal antibodies. *Cell. Immunol.* 80:279
111. Habeeb, A. F. S. A., Atassi, M. Z. 1976. A fragment comprising the last third of bovine serum albumin which accounts for almost all the antigenic reactivity of the native protein. *J. Biol. Chem.* 251:4616
112. Sakata, S., Atassi, M. Z. 1979. Immunochemistry of serum albumin. V. A time-dependent examination of the antibody response to bovine serum albumin by the activity of its third domain. *Mol. Immunol.* 16:451
113. Sakata, S., Reed, R. G., Peters, T. Jr., Atassi, M. Z. 1979. Immunochemistry of serum albumin. VIII. The antigenic reactivity of the third domain of bovine serum albumin resides in the last subdomain. A dynamic examination of the change of antibody affinity and specificity. *Mol. Immunol.* 16:703
114. Wilson, A. C., Carlson, S. S., White, T. J. 1977. Biochemical evolution. *Ann. Rev. Biochem.* 46:573
115. Peters, T. Jr. 1975. Serum albumin. In *The Plasma Proteins,* ed. F. W. Putnam, 1:133. New York: Academic
116. Sargent, T. D., Yang, M., Bonner, J. 1981. Nucleotide sequence of cloned rat serum albumin messenger RNA. *Proc. Natl. Acad. Sci. USA* 78:243
117. Carlson, S. S., Wilson, A. C., Maxson, R. D. 1978. Do albumin clocks run on time? *Science* 200:1183

Annual Reviews

Annu. Rev. Immunol. 1984.2:67-101. Downloaded from arjournals.annualreviews.org
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118. Champion, A. B., Prager, E. M., Wachter, D., Wilson, A. C. 1974. Microcomplement fixation. In *Biochemical and Immunological Taxonomy of Animals*, ed. C. A. Wright, p. 397. London: Academic
119. Crumpton, M. J. 1974. Protein antigens: the molecular bases of antigenicity and immunogenicity. In *The Antigens*, ed. M. Sela, 2:1. New York: Academic
120. Champion, A. B., Soderberg, K. L., Wilson, A. C., Ambler, R. P. 1975. Immunological comparison of azurins of known amino acid sequence: Dependence of cross-reactivity upon sequence resemblance. *J. Mol. Evol.* 5:291
121. Sarich, V. M., Wilson, A. C. 1966. Quantitative immunochemistry and the evolution of primate albumins: Microcomplement fixation. *Science* 154:1563
122. Al Moudallal, Z., Briand, J. P., Van Regenmortel, M. H. V. 1982. Monoclonal antibodies as probes of the antigenic structure of tobacco mosaic virus. *EMBO J.* 1:1005
123. Gurd, F. R. N., Friend, S. H., Rothgeb, T. M., Gurd, R. S., Scouloudi, H. 1980. Electrostatic stabilization in sperm whale and harbor seal myoglobins. Identification of groups primarily responsible for changes in anchoring of the A helix. *Biophys. J.* 32:65
124. Perutz, M. F., Imai, K. 1980. Regulation of oxygen affinity of mammalian haemoglobins. *J. Mol. Biol.* 136:183
124a. Smith-Gill, S. J., Rupley, J. A., Pincus, M. R., Carty, R. P., Scheraga, H. A. 1984. Experimental identification of a theoretically predicted "left-sided" binding mode for $(GlcNAc)_6$ in the active site of lysozyme. *Biochemistry* 23:993
124b. Hornbeck, P. V., Wilson, A. C. 1984. Local effects of amino acid substitutions on the active site region of lysozyme: a comparison of physical and immunological results. *Biochemistry* 23:998
125. Kohno, Y., Berkower, I., Minna, J., Berzofsky, J. A. 1982. Idiotypes of anti-myoglobin antibodies: Shared idiotypes among monoclonal antibodies to distinct determinants of sperm whale myoglobin. *J. Immunol.* 128:1742
126. Wiley, D. C., Wilson, I. A., Skehel, J. J. 1981. Structural identification of the antibody-binding sites of Hong Kong influenza haemagglutinin and their involvement in antigenic variation. *Nature* 289:373
127. Colman, P. M., Varghese, J. N., Laver, W. G. 1983. Structure of the catalytic and antigenic sites in influenza virus neuraminidase. *Nature* 303:41
128. Staudt, L. M., Gerhard, W. 1983. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. I. Significant variation in repertoire expression between individual mice. *J. Exp. Med.* 157:687
129. Underwood, P. A. 1982. Mapping of antigenic changes in the haemagglutinin of Hong Kong influenza (H3N2) strains using a large panel of monoclonal antibodies. *J. Gen. Virol.* 62:153
130. Berzofsky, J. A. 1978. Genetic control of the immune response to mammalian myoglobins in mice. I. More than one *I*-region gene in *H-2* controls the antibody response. *J. Immunol.* 120:360
131. Berzofsky, J. A., Richman, L. K., Killion, D. J. 1979. Distinct *H-2*-linked *Ir* genes control both antibody and T cell responses to different determinants on the same antigen, myoglobin. *Proc. Natl. Acad. Sci. USA* 76:4046
132. Riley, R. L., Wilson, L. D., Germain, R. N., Benjamin, D. C. 1982. Immune responses to complex protein antigens. I. MHC control of immune responses to bovine albumin. *J. Immunol.* 129:1553.
133. Hill, S. W., Sercarz, E. E. 1975. Fine specificity of the H-2 linked immune response gene for the gallinaceous lysozymes. *Eur. J. Immunol.* 5:317
134. Maizels, R. M., Clarke, J. A., Harvey, M. A., Miller, A., Sercarz, E. E. 1980. Epitope specificity of the T-cell proliferative response to lysozyme: Proliferative T-cells react predominantly to different determinants from those recognized by B-cells. *Eur. J. Immunol.* 10:509
135. Katz, M. E., Maizels, R. M., Wicker, L., Miller, A., Sercarz, E. E. 1982. Immunological focusing by the mouse MHC: Mouse strains confronted with distantly related lysozymes confine their attention to very few epitopes. *Eur. J. Immunol.* 12:535
136. Keck, K. 1975. Ir-gene control of immunogenicity of insulin and A-chain loop as a carrier determinant. *Nature* 254:78
137. Barcinski, M. A., Rosenthal, A. S. 1977. Immune response gene control of determinant selection. I. Intramolecular mapping of the immunogenic sites on insulin recognized by guinea pig T and B cells. *J. Exp. Med.* 145:726
138. Kapp, J. A., Strayer, D. S., Robbins, P. F., Perlmutter, R. M. 1979. Insulin-specific murine antibodies of limited heterogeneity. I. Genetic control of spectrotypes. *J. Immunol.* 123:109
139. Berzofsky, J. A., Schechter, A. N., Shearer, G. M., Sachs, D. H. 1977. Genetic control of the immune response to staphylococcal nuclease. III. Time-

Annual Reviews

course and correlation between the response to native nuclease and the response to its polypeptide fragments. *J. Exp. Med.* 145:111

140. Schwartz, R. H., Berzofsky, J. A., Horton, C. L., Schechter, A. N., Sachs, D. H. 1978. Genetic control of the T lymphocyte proliferative response to staphylococcal nuclease: evidence for multiple MHC-linked *Ir* gene control. *J. Immunol.* 120:1741
141. Berzofsky, J. A. 1983. T–B reciprocity. An Ia-restricted epitope-specific circuit regulating T cell–B cell interaction and antibody specificity. *Surv. Immunol. Res.* 2:223
142. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Inst. Pasteur)* 125C:373
143. Gell, P. G. H., Benacerraf, B. 1959. Studies on hypersensitivity. II. Delayed hypersensitivity to denatured proteins in guinea pigs. *Immunology* 2:64
144. Berzofsky, J. A. 1980. Immune response genes in the regulation of mammalian immunity. In *Biological Regulation and Development*, ed. R. F. Goldberger, 2:467. New York: Plenum

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

Annu. Rev. Immunol. 1984.2:67-101. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:67-101. Downloaded from arjournals.annualreviews.org
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TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS[1]

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INTRODUCTION

Cells oncogenically transformed in vitro, or taken from in vivo tumors, display distinctively different profiles and structures in cell-surface carbohydrates from those of nontransformed progenitor cells. Changes in carbohydrate structure in tumor cells are essentially quantitative rather than qualitative—i.e. a smaller

[1] *Abbreviations and nomenclature*. Nomenclature of glycolipids follows the recommendations of the Nomenclature Committee, International Union of Pure and Applied Chemistry (125). **Cer** = ceramide (N-fatty acyl sphingosine); **LacCer** = Galβ1→4Glc→Cer; **Gg_3** = "asialo GM_2", GalNAcβ1→4Galβ1→4Glc→Cer; **Gg_4** = "asialo GM_1", Galβ1→3GalNAcβ1→4Galβ1→4Glc→Cer; **Gb_3** = "CTH" Galα1→4Galβ1→4Glc→Cer; **Gb_4** = "globoside", GalNAcβ1→3Galα1→4Galβ1→4Glc→Cer; **IV^3NeuAcα2→3GalGb$_4$** = sialosylgalactosylgloboside = NeuAcα2→3Galβ1→3GalNAcβ1→3Galα1→4Galβ1→4Glc→Cer; **Forssman antigen** = GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glc→Cer; **Lc_3** = GlcNAcβ1→3Galβ1→4Glc→Cer; **nLc_4** = Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **Lc_4** = Galβ1→3GlcNAcβ1→3Galβ1→4Glc→Cer; **nLc_6** = Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **nLc_8** = Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **iLc_8** = Galβ1→4GlcNAcβ1→3[Galβ1→4GlcNAcβ1→6]Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **Le^a** = Galβ1→3[Fucα1→4]GlcNAcβ1→R; **Le^b** = Fucα1→2Galβ1→3[Fucα1→4]GlcNAcβ1→R; **X** = Galβ1→4[Fucα1→3]GlcNAc→R; **Y** = Fucα1→2Galβ1→4[Fucα1→3]GlcNAc→R; **III^3FucnLc$_4$** = Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1]4Glc→Cer; **Y_2** = V^3FucnLc$_6$ = Galβ1→4[Fucα1→3]Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **Z_1** = $VIII^3$FucnLc$_8$ = Galβ1→4[Fucα1→3] GlcNAcβ1→3Galβ1→ 4GlcNAcβ1→3Galβ1→ 4GlcNAcβ1→3Galβ1→ 4Glc→Cer; **Z_2** = VII^3V^3Fuc$_2$nLc$_8$ = Galβ1→4[Fucα1→3]GlcNAcβ1→3Galβ1→4[Fucα→3]GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer; **III^3V^3Fuc$_2$nLc$_6$, $III^3V^3VII^3$Fuc$_3$nLc$_8$**, see Table 3. Abbreviations of the ganglio-series gangliosides, such as GM_3, GM_2, GM_1, GM_{1a}, GM_{1b}, GD_2, GD_3, GD_{1a}, GD_{1b}, GT_{1b}, follow the system of Svennerholm (126).

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quantity of the structure observed in tumor cells is generally found in normal cells. Nevertheless, the organizational profiles of tumor cell surface carbohydrates may be so different that they can be recognized by immunological methods as tumor-associated antigens. Although the biological significance of tumor-associated carbohydrate changes are still largely unknown, they may reflect developmental arrest or a frozen program of differentiation—i.e. they may play a vital role in the process of cell recognition during development and differentiation.

Two general approaches have been undertaken to identify the tumor-associated carbohydrate antigens. The first includes a systematic chemical characterization of carbohydrate composition and structure, followed by antibody production to an abnormal component if such a structure is detected. The antibodies, either polyclonal or monoclonal, serve in verifying the specificity of such glycolipids in tumor cells. The antibodies, if properly chosen for immunoglobulin class and affinity, may inhibit the tumor growth in vivo. This first approach has been the major classical procedure in establishing tumor-associated glycolipid antigens in experimental animal tumors and in some human cancers; typical examples are listed in Table 1.

The second approach has evolved from recent observations that many "tumor-specific" monoclonal antibodies are directed to carbohydrate antigens. A number of glycolipid antigens in a variety of human cancers have been identified using this technique. This approach, however, does not always pick up the unique antigen with a novel chemical structure. Rather, the monoclonal antibody may define a common glycolipid structure present in a relatively small quantity in normal cells and cryptic under normal conditions. (See below, the section on Factors Affecting Carbohydrate Tumor Antigens.)

This review concisely surveys (*a*) the general biochemical characteristics of the carbohydrate changes associated with oncogenic transformation; (*b*) the chemical basis of carbohydrate tumor-associated antigens that occur in various types of experimental and human cancers, particularly those defined by monoclonal antibodies; and (*c*) the factors affecting expression of carbohydrate antigens.

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BIOCHEMICAL FEATURES OF CARBOHYDRATE CHANGES IN TUMORS

Two chemically distinctive groups of carbohydrates show drastic changes in transformed cells or tumor cells: (*a*) carbohydrates bound to ceramides (glycosphingolipids or glycolipids) inserted in the lipid bilayer; and (*b*) carbohydrates that are bound to cell-surface proteins (glycoproteins), including integral membrane proteins such as glycophorin and peripheral membrane proteins such as fibronectin.

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Table 1 Glycolipid tumor antigens established by classical chemical analysis followed by immunochemical characterization

Antigen	Structure	Observation
1. Lactoneotetraosyl-ceramide (nLC_4) in hamster NIL/py tumor (5,12)	Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer	a. NIL/py cells and derived tumors had nLc_4 in contrast to NIL and normal hamster tissues which did not contain nLc_4 in appreciable amounts (5; K. Watanabe, T. Matsubara, and S. Hakomori, unpublished observation) b. Sera of hamsters bearing NIL/py tumors had antibodies to nLc_4 (12)
2. Gangliotriaosylceramide (Gg_3) in mouse sarcoma (KiMuSV sarcoma in Balb/C) and mouse lymphoma (L–5178 in DBA/2) (4,13)	GalNAcβ1→4Galβ1→ 4Glcβ1→1Cer	a. KiMuSV sarcoma (in Balb/C) (4) and L–5178 lymphoma (in DBA/2) (13) contain a large quantity of Gg_3; normal tissues and organs of these mice did not contain Gg_3 in appreciable amount (4,13) b. Monoclonal IgG_3 antibody to Gg_3 inhibited the lymphoma growth in DBA/2 mice (13). Syngeneic immunization by L–5178 lymphoma results in anti-Gg_3 antibody response
3. Blood group A-like antigen in human cancer of blood group O or B individuals (111–113)	GalNAcα1→3Hex→HexNAc→Hex→Hex→Cer (113) GalNAcα1→3(Fucα1→2)Hexβ1→4(Fucα1→2) HexNAc→Hex→Hex→Cer (111)	a. Gastric, colonic, and hepatic adenocarcinoma of blood group of O or B individuals contain glycolipids that weakly inhibit A-hemagglutination b. Antigens were partially identified as having atypical A-determinants
4. Forssman antigen in human cancer (59–62,113)	GalNAcα1→3GalNAcβ1→Hex→Cer (113) GalNAcα1→3GalNAcβ1→3Galα1→4Galβ1→4Glcβ1→1Cer (59)	a. Various human cancer tissues derived from Forssman-negative tissue contain Forssman glycolipid antigen (59)
5. Blood group P1, P-like antigen in the tumor of the patient with pp genotype (115–117)	Galα1→4Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer (P1) GalNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glc→Cer (P-like) (117)	a. Background, see text and references 115 and 116 b. The tumor tissue of the blood group pp individual had P-like and P1 glycolipid, the structures of which are identified as above

Annual Reviews

Changes in Glycolipid

Three types of changes in glycolipids have been observed in a large variety of transformed cells as well as in tumor cells in vivo, including human cancer: (*a*) blocked synthesis, with or without accumulation of precursors (for review see 1–3;11); (*b*) neosynthesis due to the activation of glycosyl transferases that were inactive in progenitor cells (4–8; for a review, 11), and (*c*) organizational change of glycolipids at the cell surface—i.e. a loss of crypticity (5,9–11). Tumor-associated glycolipids often contain an aberrant ceramide composition (for a review, see 11) that may contribute to the change of glycolipid organization or orientation in membranes. Thus, tumor cells are characterized by the accumulation of precursor glycolipids or "neoglycolipids" that are highly exposed at the cell surface and are recognized by immunological methods as distinctive tumor-associated antigens (4,5,12,13) (see the section below on Glycolipid Tumor-Associated Antigens).

Changes in Glycoprotein

Major changes in the carbohydrate chains of glycoprotein in tumors can be summarized in three categories: (*a*) the presence of glycopeptides with asparagine-linked oligosaccharide that have a larger molecular weight than the corresponding fractions derived from nontransformed progenitor cells (14,15); tumor-associated glycopeptide fractions have been partially characterized as having a larger antennary structure due to increased branching at the mannosyl core—i.e. an increase in Galβ1→4GlcNAcβ1→Man branches (16–18), and the appearance of a bisecting GlcNAcβ1→4Man structure (18); (*b*) the increase of O-glycosylated mucin-type structures, particularly the appearance of a densely glycosylated region of the protein with sialosyl di- to tetrasaccharides (19–21); and (*c*) the presence in the peripheral region of side chains in either N- or O-glycosidic-linked structures showing either incomplete synthesis or neosynthesis (18,22) similar to that demonstrated in glycolipids. This peripheral structure in the side chain of glycoproteins has the same carbohydrate structure as in the lacto-series structure of glycolipids (see the section below on Shared Antigen Between Glycoprotein and Glycolipid).

Enzymatic Basis of Carbohydrate Changes in Glycolipids and in Glycoproteins

That synthesis of various glycolipids is blocked by suppression of glycosyl transferase activity has been demonstrated in a number of transformants induced by various tumor viruses and chemical carcinogens (1–3). In contrast, relatively few examples elucidating the enzymatic basis of "neosynthesis" exist. Enhanced activity has been demonstrated in enzymes responsible for neosynthesis of Gg_3 in 3T3 cells transformed by Kirsten strain murine sarcoma virus

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(6), for neosynthesis of fucosyl GM_1 in precancerous livers of rats fed with the chemical carcinogen (FAA), and in rat hepatoma (8). The presence of a highly branched multiantennary core structure in N-linked carbohydrates of transformed cells must be ascribable to the appearance of an aberrant GlcNAc transferase that makes the GlcNAcβ1→4Man structure.

In general, a specific enzyme defect, or a specific enhancement of an enzyme activity responsible for the changes in glycolipid carbohydrate chain, can be demonstrated (1–3); however, some transformants have a clear chemical change, but the enzyme activities responsible for such carbohydrate change cannot be demonstrated. For example, no positive correlation was found between CMP-sialic acid:LacCer sialyltransferase activity and the chemical level of GM_3 in chick embryonic fibroblasts infected with temperature-sensitive mutants of Rous sarcoma virus (24; K. Itaya and S. Hakomori, unpublished observation). It is therefore possible that carbohydrate structures are not only controlled by the quantity and quality of glycosyltransferases (i.e. at the level of transcription) but also are regulated by the assembly and organization of multiglycosyltransferase complexes in membranes (i.e. at the level of membrane organization) (23), which may be controlled by posttranslational modification of glycosyltransferase through glycosylation and phosphorylation.

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Cell-Biological Significance of Carbohydrate Changes

No direct correlation has yet been demonstrated between cell-surface carbohydrate changes and the expression of an oncogene; however, carbohydrate changes may occur as a consequence of a series of cascade mechanisms triggered by oncogene activation. Changes in glycolipid (10,24,25) and shifts in core structures of glycopeptides (26) were observed in cell lines transformed by temperature-sensitive mutants of tumor viruses at permissive temperature (26) and in cells transfected-transformed by human cancer DNAs (10,27). The last finding strongly suggests that single or multiple human cancer oncogenes may trigger the mechanism leading to changes in cell-surface carbohydrate.

Although the evidence is fragmentary, many of these changes in carbohydrate structure reflect a developmental arrest and/or a frozen program of differentiation—i.e. the same or closely related structure and organization are expressed at the cell surfaces at certain stages of development and differentiation (for reviews see 11,28,29). The aberrant cell-surface carbohydrate may be instrumental in the failure of "functional cell contact" and "cell communication" (30,31), thus providing the invasive and infiltrative properties of tumor cells.

The changes in carbohydrate structure in glycolipids may also alter fluidity and rigidity of membranes (32,33,34) and may affect the functions of such membrane proteins as receptors for cell growth factors (35) and membrane

Annual Reviews

enzymes (36,37); however, no strong evidence for such events has yet been furnished. Similarly, changes in carbohydrate structures may greatly affect conformation of membrane proteins; thus, the rate of metabolic turnover (38) and the antigenicity of proteins (39,40) can be altered by carbohydrates.

GLYCOLIPID TUMOR-ASSOCIATED ANTIGENS

Glycolipid Antigens in Experimental Tumors

The presence of lactoneotetraosylceramide (nLc_4) in hamster embryonic fibroblasts transformed by polyoma virus (NIL/py) and its complete absence in progenitor cells (NIL) initially served as a model of "neosynthesis" (5). The same glycolipids accumulate in NIL/py tumors grown in hamsters, while various normal tissues of hamsters have very little nLc_4. Sera of hamsters bearing NIL/py tumor had the enhanced antibody titer directed to nLc_4 (12). Mouse 3T3 cells transformed by Kirsten strain murine sarcoma virus contained a high quantity of gangliotriaosylceramide (Gg_3) (4). The sarcoma developing after inoculation of such cells into Balb/c mice also accumulated a large quantity of this glycolipid. However, Gg_3 was undetectable in various normal cells and tissues of host Balb/c mice except for a trace quantity in lymphoid cells and spleen. Similarly, L–5178 lymphoma cells grown in DBA/2 mice were characterized by the accumulation of Gg_3, whereas various normal tissue and cells did not contain it in detectable quantities (13). Subsequently, two monoclonal antibodies directed to Gg_3, one IgM and another IgG_3, were established (41). Inoculation of L–5178 lymphoma into DBA/2 mice, followed by administration of IgG_3 (but not IgM) monoclonal antibodies, completely prevented subsequent lymphoma growth (13). More recently, WKA rats hyperimmunized with a syngeneic fibrosarcoma (KMT–17) were used for producing monoclonal antibodies by fusing their spleen cells with a mouse myeloma. Two monoclonal antibodies showing cytotoxicity against KMT–17 sarcoma were found directed to glycolipids. One monoclonal was directed to Gb_3 and/or α-galactosyl-nLc_4, and the other was directed to nLc_4 (42).

Vesicular Stomatitis Virus (VSV) obtained from cell line transformed by SV40 acquires a tumor-specific transplantation antigen which causes rejection of SV40 tumors. A glycolipid fraction prepared from VSV of SV40 tumor and incorporated into liposomes was shown to be immunogenic and capable of suppressing tumor growth. Antiserum directed to liposomes containing the polar glycolipid fraction and absorbed with normal hamster tissue specifically reacts with the SV40 transformed cells (43). These models clearly indicate that certain glycolipids are indeed tumor-associated antigens and can be useful as targets for the immunotherapy of certain tumors.

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Glycolipid Human Cancer Antigens Defined by Monoclonal Antibodies

Many monoclonal antibodies directed to human tumor cells or tissues that show a distinctive reactivity to the specific type of human cancer have been identified as being directed to glycolipids. For convenience, the chemical properties of glycolipid tumor-associated antigens will be discussed according to three classes of basic structures: ganglio-, globo-, and lacto-series.

GANGLIO-SERIES ANTIGENS IN HUMAN CANCERS

Human melanoma The antigen defined by a human melanoma-specific IgG_3 antibody (R24) (44) as well as by an IgM antibody (4.2) (45) was identified as a GD_3 ganglioside (46,47). The antigen was characterized as having a much higher proportion of long-chain fatty acids (C22 and C24) than does brain ganglioside (47). This glycolipid is relatively abundant in retina (48) and kidney (49); nevertheless, the reactivity of the antibody was highly restricted to human melanoma cells and tissues. Normal melanocytes, naevus, and normal kidney did not react with the antibody; normal retina was not tested (45). An association of GD_3 with human melanoma had previously been claimed by Portoukalian et al (50).

Oncofetal antigens defined by human monoclonal antibodies Two human monoclonal antibodies against oncofetal antigens (OFA–I–1 and OFA–I–2) were produced by transforming peripheral blood B-lymphocytes of melanoma patients by Epstein-Barr virus (51). The antibody to OFA–I–1 reacts with a variety of human cancer cells including those from melanoma, brain tumors, breast cancers, and tumors of several other types. The antigen was identified as GM_2 (52). Another antibody to OFA–I–2 defines an antigen on neuroectodermal tumors, identified as GD_2 (53). A melanoma-associated antibody (AH) detected in the serum of patients was also shown to be specific for GD_2 (54). It is assumed that GM_2 and GD_2 are relatively minor or cryptic components of normal cells. These components could be highly exposed and accumulate when higher gangliosides (GM_1, GD_{1a}, GD_{1b}, GT_{1b}) are deleted in tumor cells.

GLOBO-SERIES ANTIGENS IN HUMAN CANCER

Gb_3 as Burkitt lymphoma antigen The antigen specifically expressed on Burkitt tumor cells irrespective of their possessing the Epstein-Barr virus (EBV) genome has been defined by a rat monoclonal IgM, 38–13 (55). The antigen was identified as globotriaosylceramide (Gb_3) [Galα1$\rightarrow$4Galβ1$\rightarrow$4Glcβ1$\rightarrow$1Cer (56)], which had previously been identified as the blood group P^k antigen (57).

Annual Reviews

On chemical analysis, only Burkitt lymphoma cell lines had a large quantity of this glycolipid (200–800 μg/10 mg protein). Normal human erythrocytes contain a moderate quantity of Gb_3, but they do not react with this monoclonal antibody (55) nor to anti-P^k antibodies (57). In contrast, blood group P^k erythrocytes, which contain much more Gb_3 than is found in normal erythrocytes (57), react well with anti-P^k antibodies. The small quantity of Gb_3 in various normal cells may be cryptic.

Forssman glycolipid ($IV^3\alpha$-GalNAc Gb_4) Humans have been regarded as a Forssman-negative species (58). Although Forssman glycolipid antigen can be detected in small quantity in tissues of 20–30% of the population it was assumed to be lacking in the remaining 70–80% (59). Isoantigenic expression of Forssman antigen in human tissue is further supported by the corresponding presence of anti-Forssman antibodies in these groups (60). In human gastrointestinal cancer derived from Forssman-negative tissue, Forssman antigen was present (59). In normal human lung tissue, an extremely low concentration (0.3 μg/10 g of tissue) of Forssman glycolipid was present, which is lower than the "detectable level" in the usual chemical analysis. Various cases of squamous carcinoma and adenocarcinoma of lung as well as fetal lung contained 60–90 times this concentration of Forssman glycolipids (61). The enzyme activity for synthesis of Forssman glycolipid was greatly increased in the majority of squamous carcinomas and in some adenocarcinomas (62). Forssman antigen has been detected in various human lung, breast, and gastric cancer cell lines by indirect immunofluorescence (63) and by immunostaining of thin-layer chromatography of total glycolipids (64). Forssman antigen with unusual structures, such as ceramide trisaccharide (113) and a compound higher than a ceramide pentasaccharide (64), has been detected in some human malignancies.

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LACTO-SERIES GLYCOLIPIDS AS HUMAN TUMOR-ASSOCIATED ANTIGENS

Sialosyl-Le^a antigen A number of monoclonal antibodies directed to colorectoadenocarcinomas have been prepared by Koprowski and associates (65). Among such antibodies, one, N–19–9, was found to be specific not only for colorectoadenocarcinoma but also for gastric and pancreatic cancer. The binding of the antibody to carcinoma tissue extract was inhibited by serum from patients with adenocarcinoma of colon but not by serum from patients with other bowel diseases or from healthy volunteers (66). The specific antigen defined by this monoclonal antibody was found in the ganglioside fraction of tumors and was identified as sialosyl-Le^a by Magnani et al (67) and Falk et al (68) (see Table 3). The antigen was also found in meconium, a substance that consists essentially of fetal intestinal epithelial membranes. However, on immunohistologic examination, the antibody stained only limited loci of glan-

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Table 2 Precursor glycolipids accumulating in some human cancers and recognized by monoclonal "tumor-specific" antibodies

Glycolipid	Structure	Observation
1. Globotriaosylceramide (Gb_3) in Burkitt lymphoma (56)	Galα1→4Galβ1→ 4Glcβ1→1Cer	a. Rat hybridoma IgM antibody (38–13) was selected for specific reactivity to Burkitt lymphoma. b. The antibody defined globo-triaosylceramide (P^k antigen) c. A small amount of Gb_3 in erythrocytes and non–Burkitt lymphoma cells did not show the reactivity with 38–13 antibody
2. GD_3 ganglioside in human melanoma (46,47)	NeuAcα2→8NeuAcα2→ 3Galβ1→4Glcβ1→1Cer (GD_3)	a. Mouse hybridoma antibodies (R24, 4.2) selected by the specific reactivity with human melanoma were identified as being directed to GD_3 having long-chain fatty acid (47). b. A small amount of GD_3 in normal kidney and colon did not show the reactivity with 4.2 antibody (45).
3. GM_2 in human breast cancer, brain tumor, melanoma and GD_2 in human melanoma and other neuroectodermal tumors (51–54)	NeuAcα2→3[GalNAcβ1→4] Galβ1 →4Glcβ1→1Cer (GM_2) NeuAcα2→8NeuAcα2→3[GalNAcβ1→ 4]Galβ1→4Glcβ1→1Cer (GD_2)	a. Antigens defined by human monoclonal antibodies that show specificities to various human cancers were identified as GM_2 and GD_2

Annual Reviews

dular epithelia of normal pancreatic and gastric mucosae (69). In serum and secretions, the antigen is present on glycoproteins (101).

X- and poly-X haptens and monoclonal antibodies directed to them showing an apparent tumor specificity The monoclonal antibodies directed to gastric cancer (WGHS 29–1), colonic adenocarcinoma (ZWG13, ZWG14, ZWG111) (70), a series of antibodies directed to small cell adenocarcinoma and squamous carcinoma of lung (55A5, 534F8, 535F12) (71,72), and various antibodies directed to the promyelocytic leukemia cell line HL–60 and termed My–1 (73), VEP–8 and 9 (74), and 1G10 (75), have been identified as being directed to the X-determinant (Galβ1→4[Fucα1→3]GlcNAc). Antibodies to the embryonic antigen SSEA–1—an antigen maximally expressed from the morulae to early blastocyte stage of mice (76)—have also been identified as directed to the X-determinant (77,78). All these antibodies detect a series of glycolipids in normal granulocytes (74), erythrocytes (79), normal colonic mucosa, and normal liver (80). These glycolipids are characterized by an unbranched type-2 chain with fucosylα1→3 linkage at the penultimate GlcNAc as shown in Y_2-, Z_1-, Z_2-glycolipid (79,80). In contrast, cancer tissue, particularly adenocarcinoma, showed a large accumulation of lactofucopentaosyl(III)ceramide ($III^3FucnLc_4$) (80,81), difucosyllacto*nor*hexaosylceramide (III^3 $V^3Fuc_2nLc_6$) (80,82), and trifucosyllacto*nor*octaosylaceramide (III^3 V^3 VII^3 Fuc_3nLc_8) (80,82), which are virtually absent in normal liver (Table 3, item 2) (82). Although the immunostaining pattern of glycolipids separated by TLC with anti-X antibodies is similar between normal and cancer tissues, the three components with novel structure described above are highly characteristic for adenocarinoma. Recently, two monoclonal antibodies (FH4 and ACFH12) showing a preferential reactivity with difucosyl and trifucosyl type-2 chain were established (83). These may distinguish those polyfucosylated glycolipids accumulating in cancer from those monofucosyl compounds present in normal tissue.

N-Acetylneuraminosylα2→6 ganglioside defined by monoclonal antibody IB–9 A minor ganglioside containing the sialosylα2→6 linkage has been found in adult human erythrocytes and other tissues (84). It is the major component in human meconium (85). Some cases of human colonic and liver adenocarcinomas have been characterized by a large quantity of ganglioside with the structure as shown in Table 3. Of particular interest is the accumulation of sialosyl2→6 lacto*nor*hexaosylceramide with a fucosyl α1→3 substitution at the internal GlcNAc residue. This ganglioside was absent in normal colonic mucosa and normal liver (86) and was proposed to be a cancer-associated ganglioside antigen.

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Table 3 Complex glycolipids accumulating in human cancer and defined by monoclonal antibodies

Glycolipid	Structure	Background
1. Sialosyl-Le[a] antigen defined by the antibody N–19–9 (66–68)	NeuAcα2→3Galβ1→3GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 4 (on GlcNAc) ↑ Fucα1 (brace) R R can be linked to proteins and other carrier lipids.	a. The mouse IgG_{2b} antibody (N–19–9) was selected by the specific reactivity with human colorectoadenocarcinoma. The antigen is identified as shown. The antigen in serum of cancer patient is present as the form of glycoprotein (101). b. The antibody binding to colon carcinoma cells was inhibited by serum from patients with cancers, and the degree of the binding inhibition is useful for diagnosis of colorectocarcinoma.
2. Poly-X antigen in various human adenocarcinoma (80,82) and defined by monoclonal antibody "FH4" (83)	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 (on each GlcNAc) ↑ Fucα1 Fucα1 $III^3V^3Fuc_2nLc_6$ Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 3 (on each GlcNAc) ↑ Fucα1 Fucα1 Fucα1 $III^3V^3VII^3Fuc_3nLc_8$	a. Detected originally by anti-X monoclonal antibody; accumulating in colonic and liver cancer; absent in normal colonic mucosa, normal liver, and normal granulocytes b. FH_4 antibody directed to di- or trifucosylated structure and was not reactive to a monofucosyl-X hapten. The antibody showed a restricted but more specific reactivity to human adenocarcinoma than antibodies to monofucosyl-X hapten.
3. A novel fucoganglioside of human colonic cancer defined by monoclonal antibody (IB9) (86)	Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer 6 (on terminal Gal) 3 (on inner GlcNAc) ↑ ↑ NeuAcα2 Fucα1 $III^3FucVI^6NeuAcnLc6$	a. Detected by IB9 antibody directed to NeuAcα2→6Gal residue; present in colonic adenocarcinomas and absent in normal liver and normal colonic mucosa

Annual Reviews

Gangliosides containing N-glycolylneuraminic acid, the Hanganutziu-Deicher type heterophile antigen (HD antigen) in human cancer The heterophile antibodies occurring in patients who received heterologous antiserum are called Hanganutziu-Deicher antibodies (for a review, see 87). The antigen (abbreviated HD antigen) was identified as gangliosides containing the N-glycolylneuraminosylα2→3galactosyl residue (cited in 88,89). Humans are a unique species in that entire tissues, organs, and blood cells do not contain N-glycolylneuraminic acid. Interestingly, two independent studies indicated that HD antigen was detected in various types of human cancer using heterophile HD antibodies and the purified antibodies to N-glycolylhematoside (88,89). These findings suggested that some human cancer contained N-glycolylneuraminic acid bound to lipids or proteins.

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GLYCOPROTEIN TUMOR-ASSOCIATED ANTIGENS

The core structure of aspargine-linked carbohydrate chain changes consistently in many tumor cells (14–17), but this usually does not affect the antigenic properties of glycoproteins. In contrast, changes in the peripheral sequences of either aspargine-N-linked or serine/threonine-O-linked chains can contribute to antigenic changes in tumor cells. While these structures are often shared with lacto-series glycolipids, two antigens characteristic of the O-linked chain in glycoproteins have been reported.

T (Thomsen-Friedenreich) Antigen

This antigen was originally described as the receptor of "panagglutinable" erythrocytes that is exposed by contact with bacterial enzyme (Thomsen-Friedenreich's phenomenon). The panagglutination can be caused by the common antibodies (anti-T) present in all adult sera (for a review, see 90). Uhlenbruck et al observed that sialidase treatment of human erythrocytes exposed T antigens that could be detected by natural anti-T antibodies or with peanut (*Arachis hypogaea*) lectin (91); the antigens are assumed to have the structure Galβ1→3GalNAcα1→O-Ser/or Thr (92). Springer and associates claim that the antigen activity was greatly enhanced in over 90% of breast, lung, and pancreatic cancer, and that most cancer patients display an increased humoral or cellular immune response to the T antigen (for a review, see 93). In a mouse lymphoma model, the expression of T antigen was much greater in high- than in low-metastatic cell lines (94). T antigen is prominently expressed in malignant urinary bladder carcinoma, and its degree of expansion can assist prognosis (95). Peanut lectin receptors on T lymphocytes are essentially differentiation-dependent, being expressed highly in immature cells (123). However, the lectin cross-reacts with various Gal structures other than those on the T antigen. Recently the hybridoma monoclonal antibody directed to the

Annual Reviews

sequence Galβ1→3GalNAcα1→O-Ser/Thr has been established, which does not recognize the Galβ1→3GalNAcβ1→R structure found in the glycolipid Gg_4 (96). This reagent will be useful in further evaluation of the status of the T antigen in a variety of normal and tumor cells at different stages of differentiation.

A Human Cancer Antigen (Ca) Defined by a Monoclonal IgM Antibody (Ca 1)

A monoclonal antibody that distinguishes between various malignant and nonmalignant cells was selected by the reactivity of a panel of hybrid cells produced by fusion of diploid fibroblasts with malignant cells originating from cervical carcinoma. The antibody, Ca 1, discriminates between the hybrids in which malignancy was suppressed and the malignant segregant. The antigen defined by this antibody was claimed to have high molecular weights (390,000 and 350,000), and the reactivity was abolished by sialidase as well as by endo-β-galactosidase (97). In subsequent studies, the antigen was purified from HEp2 carcinoma cells by a series of steps including affinity chromatography on a column of Sepharose 4B coupled with Ca 1. The isolated antigen was found to be a typical mucine-type glycoprotein having a large quantity of short-chain sialosyloligosaccharides. The carbohydrate chain showed no novel structure although the antigenic activity was sensitive to sialidase treatment (98). It is suggested that the densely spaced sialosyloligosaccharides linked to a mucine-type glycoprotein could be recognized by a Ca 1 antibody. After purification, the antigen is no longer susceptible to endo-β-galactosidase (98).

Shared Antigens Between Glycoproteins and Glycolipids

Lacto-series glycolipid structures have also been found in the peripheral region of N-linked or O-linked side chains of glycoproteins. This is typically demonstrated by the presence of lactosaminoglycan bearing blood group ABHIi determinant in both glycolipids and band-3 glycoprotein of erythrocytes (99). Although antibodies to GM_1 or to Gb_4 react with glycoproteins of NIL and 3T3 cells (100), no strong chemical evidence supports the presence of globo- and ganglio-series structures in side chains of glycoproteins.

Since blood group ABH, Lewis, and Ii antigens belong essentially to lacto-series structures, the major antigens shared between glycolipid and glycoprotein are blood group antigens. Thus the major tumor-associated antigens shared between glycolipids and glycoproteins are those related to a modified blood group antigen. Sialosyl-Le^a antigen, a modified blood group antigen originally identified as monosialoganglioside (67,68), has been found in sera of patients with various types of colorectocarcinoma as a glycoprotein with the O-linked carbohydrate chain (101). The HD heterophile antigen that appears in some human cancer (98,99) should also be shared between glycolipid and glyco-

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protein, since the N-glycolylneuraminosyl α2→3 galactosyl residue is usually found in glycolipids and glycoproteins.

THE MODIFICATION OF BLOOD GROUP ANTIGENS IN HUMAN CANCER

Blood group antigens, the major alloantigens in humans, are present on blood cells and various epithelial cells. Since the majority of human cancers are derived from epithelial cells, the changes in blood group antigens are an important topic in human tumor immunology.

Deletion of A and B Determinants with Precursor Accumulation

The most frequent and remarkable change in blood group determinants in human cancer is the deletion of the A and B antigens (for a review, see 102). The change can also be found in premalignant dysplasia with disorganization of precursor structure (103,104).

Incomplete synthesis of type-2 chain blood group ABH antigen may result in accumulation of Ii antigen (105), although Ii reactivity of mucosae is also dependent on the secretor/or nonsecretor status of the individual and to the presence of malignancy (104). Normal oral mucosa is characterized by a well-organized carbohydrate architecture which becomes disarranged in premalignant dysplasia. There is a loss of A antigens associated with accumulation and dislocation of type-2 chain H (106).

Incomplete synthesis of blood group ABH antigen may also result in the accumulation of type-1 chain precursor that is unrelated to Ii. In a case of broncogenic lung cancer with an incidental gammopathy (107), the serum of the patient was found to contain a warm-reactive monoclonal IgM that was shown to react with the lacto-N-tetraosyl structure. The lung cancer presumably regressed because the patient had a concurrent gammopathy that was directed to the type-1 chain precursor that presumably accumulated in the tumor. Recently a hybridoma monoclonal antibody directed to human teratocarcinoma cells was identified as being directed to lacto-N-tetraosyl (type-1 chain) (108). This reagent will be useful in quantitation of type-1 chain structure in human malignancy.

Incompatible Blood Group Antigens in Human Tumors

A-LIKE OR B-LIKE ANTIGEN The presence of an A-like antigen in tumors of patients with blood group O and B, was initially suggested 50 years ago (for review, see 102). More recent studies (109–113) indicate that such antigen includes various A-like structures that can be distinguished from Forssman

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antigen (112,113). A probable presence of an A-like difucosyl glycolipid in a tumor of a host with blood type B was suggested by mass spectrometry (111). Glycolipids with a clear A activity were demonstrated in a few cases of cancer from blood group O individuals (112,113). The ceramide hexasaccharide fraction isolated from the hepatocarcinoma had a weak A activity but had no Forssman activity, showed a single spot on TLC, and was degraded by hog liver α-N-acetylgalactosominidase. Direct probe mass spectrometry indicated the presence of a sequence GalNAcα→Hex→HexN→R but the absence of the usual A-determinant with a fucosyl residue (113) (Table 1, item 3). The tissue of this tumor also contained a novel Forssman antigen with a ceramide trisaccharide Forssman (Table 1, item 4). Human adenocarcinoma may produce an unusual α-GalNAc transferase with less restricted substrate specificity so that GalNAc residue could be added to the unusual structure. Sera of two siblings in a family at high-risk for colonic cancer had high-titer antibodies directed to cell-surface antigen of colonic tumor cell lines. Inhibition studies indicated that the antigen could be A- or B-like determinant with type-1 chain but not the Ii precursor type (114).

P, P_1, P^k ANTIGEN Another striking example of the presence of an incompatible blood group antigen in a cancer comes from the work of Levine and associates (115,116). The gastric cancer of a patient, Mrs. J., with blood group genotype pp seemed to have regressed completely after the patient received a transfusion of 25 ml of normal P erythrocytes. A severe reaction developed and the anti-PP_1P titer increased from 8 to 512. Only a subtotal gastrectomy was performed but the patient survived for 22 years without a sign of cancer recurrence. Recent analysis of her tumor glycolipid and glycoprotein revealed that the major glycolipid had the structure shown in Table 1 (item 5)—i.e. β-GalNAc attached to nLc_4 paragloboside, whose terminal structure was identical to that of the P-antigen (globoside). A neutral glycolipid with P_1 activity was also demonstrated (117) (Table 1, item 5). Thus the glycolipid alteration in this tumor having a rare pp genotype may involve neosynthesis of incompatible blood group P_1 and enhanced synthesis of P-like antigen.

FACTORS AFFECTING ANTIGENICITY AND IMMUNOGENICITY OF TUMOR-ASSOCIATED CARBOHYDRATES

The antigenicity and immunogenicity of carbohydrate antigens at the cell surface are not only defined by the primary chemical structures of the carbohydrate antigen but are also influenced by the organization of carbohydrates at the surface of the membrane. Even though the primary structures of the car-

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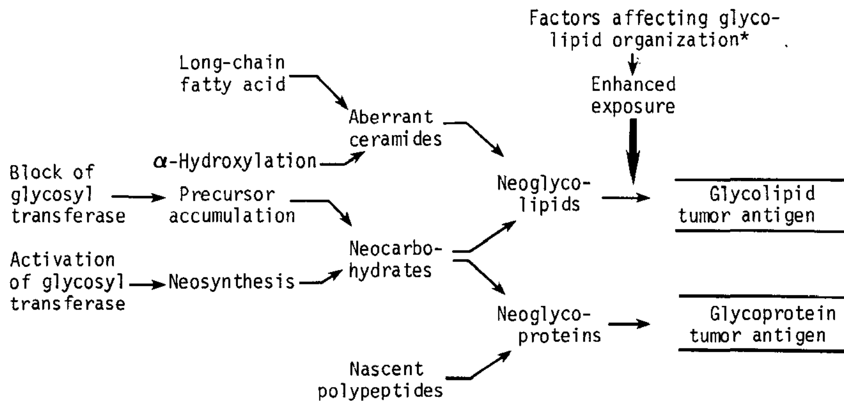

Figure 1 Mechanisms for expression of tumor-associated carbohydrate antigens. Neocarbohydrates are present or accumulate in tumor cells as a result of a blocked synthesis or neosynthesis. The neocarbohydrates may be linked to ceramides with unusual composition or to a side chain of glycoproteins, thus forming neoglycolipids or neoglycoproteins. Various factors as discussed in the text affect membrane organization, which ensures increased exposure of neoglycolipids; such structures with unique organization are recognized as tumor-associated antigens. Factors affecting expression of glycoprotein antigens are unknown.

bohydrate chains are not novel, they could be uniquely immunogenic and recognizable as a discrete antigen if the carbohydrate chain is organized in a distinctive way at the cell surface. Dominant factors affecting expression of the antigens are the density and the crypticity of carbohydrate chains at the cell surface in addition to the uniqueness of the carbohydrate structure.

Density of Carbohydrate Chain; High- and Low-Density Organization

Carbohydrate structures on the membrane at high density may behave qualitatively differently from the same carbohydrate chain distributed at low density, both in reacting with a defined monoclonal antibody and in initiating an immune response. The high-density carbohydrate chain can be found at a clustered glycosylation site in a specific domain of a polypeptide. The Ca 1 antibody, for example (97), may recognize a cluster of sialosyloligosaccharide chain at certain regions of the Ca polypeptide (98). A similar high-density cluster of glycolipids can be created by aggregation of glycolipids at certain regions of the membrane bilayer. There is a clear threshold value of $III^3FucnLc_4$ concentration in liposome lysis by anti-SSEA-1 antibody. No lysis occurred at lower than 15 nmoles of the glycolipid antigen (77). Similarly, melanoma-specific monoclonal antibody 4.2 specifically recognized GD_3 at high density (45,46), and the Burkitt lymphoma-specific monoclonal antibody 38–13 specifically reacts with Gb_3 at high density (56) at the cell surface. These monoclonal antibodies are apparently produced because their antigens are orga-

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nized in high density at the cell surface. These carbohydrate chains are distributed in low density in various normal cells and tissues and hence may not be immunogenic, or may not react well with antibodies at the cell surface.

Crypticity of Glycolipid Antigens: Effects of Coexisting Glycolipid, Proteins, and Ceramide Composition

Many glycolipid tumor antigens are characterized by their high degree of exposure at the cell surface (5,9,10). In normal cells, many glycolipids with shorter-chain carbohydrates are masked by glycolipids with longer carbohydrate chains. Since glycolipids with longer carbohydrate chains are deleted in many transformed cells (1–3), the shorter-chain glycolipids are exposed, as typically seen with GD_2, GD_3 in human melanoma (45,54), and Gg_3 in mouse lymphoma and sarcoma (4,13). The reactivity of Gg_3 to galactose oxidase or to antibody at the surface of lymphoma L–5178 can be increased ten times by treatment with sialidase. Since no sialylated form of Gg_3 could be detected and the chemical quantity of Gg_3 was not increased by sialidase treatment, it is assumed that cryptic Gg_3 became exposed by desialylation of a second glycoconjugate (118). The reactivity of Gg_3 to its antibody on these cells has also been correlated with the presence of longer-chain glycolipid GM_{1b}. The low Gg_3 expressor had more GM_{1b} than higher expressor, and vice versa (119). It is proposed that the expression of tumor antigen Gg_3 is regulated by coexisting GM_{1b} which is sialidase sensitive (119). Glycolipids in membranes could also be masked by proteins in adjacent domains (5,120). A removal of such proteins by proteolysis increases exposure of glycolipids (5,9,120,121).

Ceramide composition also affects the crypticity and antigenicity of glycolipid in membranes. Membranes containing ceramides having longer-chain fatty acids show greater stability than those with ceramides having shorter-chain fatty acids (122). The antigenicity of a tumor-associated Gg_3 antigen in a mouse lymphoma was much greater in clones that had a greater concentration of Gg_3 having α-hydroxy palimitic acid. The low-expressor variant was characterized by the absence of α-hydroxy fatty acid and a high content of shorter-chain fatty acids (119). Lactosylceramide having longer-chain fatty acid (C22–24) showed a greater reactivity with its monoclonal antibody than lactosylceramide having shorter fatty acid (124).

Uniqueness of Carbohydrate Structures Present in Tumor Cells

The presence of a unique carbohydrate chain has long been sought as the candidate for tumor-associated antigen; however, evidence that such an antigen is based on a specific carbohydrate structure for tumor cells is highly limited. A-like antigen in tumors of blood group B or O individuals (109–113), Forssman antigen in tumors derived from Forssman-negative individuals (60), and P_1 antigen in the tumors of the p individual (117) may represent such examples.

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Sialosyl-Le[a] (68,69), polyfucosylated type-2 chain (poly X) (80,82), and a novel sialosyl 2→6fucoganglioside may also be unique structures for tumor cells, although a small quantity of these antigens could also be present in highly limited loci in normal tissues. If their organization in membranes is appropriate, these aberrant structures may act as highly efficient tumor antigens.

CONCLUSIONS AND PERSPECTIVES

Essentially all tumor cells are characterized by changes in carbohydrate composition and structure from their progenitors, irrespective of the causal agent and mechanism of oncogenic transformation.

A systematic chemical and immunochemical analysis of glycolipids has revealed that some tumors accumulate glycolipids that are either absent or are present only in small quantities in normal tissues. Gg_3 in mouse lymphoma and A-like antigen in cancers of blood group O or B host, and Forssman antigen in Forssman-negative (fsfs) individuals are such examples (summarized in Table 1).

In recent studies with monoclonal antibodies, an additional version of carbohydrate tumor antigens has emerged. Monoclonal antibodies have been selected by their specific reactivities to tumor cells and by the absence of reactivity to unrelated tumor cells and normal cells. These antibodies have often been found to be directed to glycolipids or to the carbohydrate moieties of glycoproteins (summarized in Tables 2 and 3). While some antibodies were directed to a novel structure such as sialosyl-Le[a] polyfucosyl type-2 chain (poly X), others were directed to the precursors of complex glycolipids, such as GD_2 and GD_3 in melanoma, and Gb_3 in Burkitt lymphoma.

In addition to the aberrant composition and structure of carbohydrates, factors that influence antigenicity and immunogenicity are the density and crypticity of carbohydrate antigens. Both the density and the crypticity of carbohydrate antigens in glycoproteins can be controlled by the primary structure of the polypeptide chain, whereas those in glycolipids are controlled by coexisting membrane components and the ceramide composition.

Similar or identical tumor-associated carbohydrate profiles can be detected at the cell surfaces during certain stages of development and differentiation; nevertheless, the profile is useful in detection of tumor cells and may eventually be utilized in the treatment of human cancer. Successful immunotherapy of experimental tumors (lymphoma L–5178 in DBA/2 mice) utilizing monoclonal anti-glycolipid IgG_3 antibody (13) and two clinical cases (107,115) (see also the section on Modified Blood Group Antigens) led to an optimistic view. In naturally occurring human cancer, however, heterogeneity in glycolipid expression and organization may be the major problem preventing a successful utilization of glycolipid markers for treatment. Multiple glycolipid or other

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markers present within heterogeneous populations of tumor cells could be useful targets for a mixture of monoclonal antibodies with different specificities.

The functional significance of the changes in carbohydrate associated with oncogenesis is not within the scope of this review; however, the aberrant structure and organization of cell-surface carbohydrates could reflect aberrant cell-cell recognition and cell adhesion. Carbohydrates may also regulate the antigenicity of membrane proteins (39), they may modulate the function of receptors (35) and other membrane proteins (36,37). Thus, expression of tumor-associated carbohydrate antigens, which are recognized by immunological methods, may be essential for tumor cells to conserve their transformed phenotype. A genetic or epigenetic mechanism that controls transcription of glycosyltransferase and that regulates organization of glycosyltransferase will undoubtedly be an area of major focus for the study of tumor-associated carbohydrate antigens in the immediate future.

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Literature Cited

1. Hakomori, S. 1973. Glycolipids of tumor cell membrane. *Adv. Cancer Res.* 18:265–315
2. Brady, R.O., Fishman, P. 1974. Biosynthesis of glycolipids in virus-transformed cells. *Biochim. Biophys. Acta* 335:121–48
3. Richardson, C.L., Baker, S.R., Morré, D.J., Keenan, T.W. 1975. Glycosphingolipid synthesis and tumorigenesis. A role for the golgi apparatus in the origin of specific receptor molecules of the mammalian cells surface. *Biochim. Biophys. Acta* 417:175–86
4. Rosenfelder, G., Young, W.W. Jr., Hakomori, S. 1977. Association of the glycolipid pattern with antigenic alteration in mouse fibroblasts transformed by murine sarcoma virus. *Cancer Res.* 37:1333–39
5. Gahmberg, C.G., Hakomori, S. 1975. Surface carbohydrates of hamster fibroblasts. I. Chemical characterization of surface-labeled glycosphingolipids and a specific ceramide tetrasaccharide for transformants. *J. Biol. Chem.* 250:2438–46
6. Lokney, M.W., Soltysiak, R.M., Sweeley, C.C. 1982. Glycolipid N-acetylgalactosaminyltransferase activity in normal and Kirsten murine sarcoma virus transformed Balb/c 3T3 cells. New vistas in glycolipid research. *Adv. Exp. Med. Biol. Monog.* 152:139–47
7. Holmes, E.H., Hakomori, S. 1982. Isolation and characterization of a new fucoganglioside accumulated in precancerous rat liver and in rat hepatoma induced by N-2-acetylaminofluorene. *J. Biol. Chem.* 257:7698–7703
8. Holmes, E.H., Hakomori, S. 1983. Enzymatic basis for changes in fucoganglioside during chemical carcinogenesis: Induction of a specific α-fucosyltransferase in precancerous rat liver and hepatoma. *J. Biol. Chem.* 258:3706–13
9. Hakomori, S., Teather, C., Andrews, H.D. 1968. Organizational difference of cell surface of hematoside in normal and virally transformed cells. *Biochem. Biophys. Res. Commun.* 33:563–68
10. Tsuchiya, S., Hakomori, S. 1983. Cell surface glycolipids of transformed NIH 3T3 cells transfected with DNAs of human

Annual Reviews

bladder and lung carcinomas. *EMBO J.* 2:2323-26
11. Hakomori, S., Kannagi, R. 1983. Glycosphingolipids as tumor-associated and differentiation markers. *J. Natl. Cancer Inst.* 71:231–51
12. Sundsmo, J., Hakomori, S. 1976. Lacto-N-neotetraosylceramide ("Paragloboside") as a possible tumor-associated surface antigen of hamster NILpy tumor. *Biochem. Biophys. Res. Commun.* 68:799–806
13. Young, W.W. Jr., Hakomori, S. 1981. Therapy of mouse lymphoma with monoclonal antibodies to glycolipid: Selection of low antigenic variants *in vivo*. *Science* 211:487–89
14. Buck, C.A., Glick, M.C., Warren, L. 1970. A comparative study of glycoprotein from the surface of control and Rous sarcoma virus transformed hamster cells. *Biochemistry* 9:4567–76
15. Warren, L., Buck, C.A., Tuszyndki, G.P. 1978. Glycopeptide changes and malignant transformation. A possible role for carbohydrate in malignant behavior. *Biochim. Biophys. Acta* 516:97–123
16. Ogata, S., Muramatsu, T., Kobata, A. 1976. New structural characteristic of the large glycopeptides from transformed cells. *Nature* 259:580–82
17. Takasaki, S., Ikehira, H., Kobata, A. 1980. Increases of asparagine-linked oligosaccharides with branched outer chains caused by cell transformation. *Biochem. Biophys. Res. Commun.* 92(3):735–42
18. Yamashita, K., Hitoi, A., Taniguchi, N., Yokosawa, N., Tsukada, Y., Kobata, A., 1983. Comparative study of the sugar chains of γ-glutamyltransferases purified from rat liver and rat AH-66 hepatoma cells. *Cancer Res.* 43:5059–63
19. Bhavanandan, V.P., Kemper, J.G., Bystyn, J.C. 1980. Purification and partial characterization of a murine melanoma-associated antigen. *J. Biol. Chem.* 255:5145–53
20. Chandrasekaran, E.V., Davidson, E.A. 1979. Sialoglycoproteins of human mammary cells: Partial characterization of sialoglycopeptides. *Biochemistry* 18:5615–20
21. Funakoshi, I., Yamashina, I. 1982. Structure of O-glycosidically linked sugar units from plasma membranes of an ascites hepatoma, AH 66. *J. Biol. Chem.* 257:3782–87
22. Yamashita, K., Tachibana, Y., Takemchi, T., Kobata, A. 1981. Structural study of the sugar chains α-amylases produced ectopically in tumors. *J. Biochem.* 90:1281–89
23. Ivatt, R.J. 1981. Regulation of glycoprotein biosynthesis by formation of specific glycosyltransferase complexes. *Proc. Natl. Acad. Sci. USA* 78:4021–25
24. Hakomori, S., Wyke, J.A., Vogt, P.K. 1977. Glycolipids of chick embryo fibroblasts infected with temperature-sensitive mutants of avian sarcoma viruses. *Virology* 76:485–93
25. Gahmberg, C.G., Kiehn, D., Hakomori, S. 1974. Changes in a surface-labelled galactoprotein and in glycolipid concentrations in cells transformed by a temperaure-sensitive polyoma virus mutant. *Nature* 248:413–15
26. Warren, L., Critchley, D., Macpherson, I. 1972. Surface glycoproteins and glycolipids of chicken embryo cells transformed by a temperature-sensitive mutant of Rous sarcoma virus. *Nature* 235:275–77
27. Glick, M.C., Santer, U.V., Gilbert, F. 1983. Glycopeptide change after transfection of NIH 3T3 with human tumor DNA. *Fed. Proc.* 42:2189 (Abstr. 2520)
28. Hakomori, S., Fukuda, M., Nudelman, E. 1982. Role of cell surface carbohydrates in differentiation: Behavior of lactosaminoglycans in glycolipids and glycoproteins. *Teratocarcinoma and EmbryonicCell Surface (Proc. 1st Hiei Symp Teratocarcinoma)*, ed. T. Muramatsu, Y. Ikawa., pp. 179–200. Heidelberg/New York: Springer
29. Feizi, T. 1983. Carbohydrate differentiation antigens. *Biochem. Soc. Transact.* 2:263–66
30. Emmelot, P. 1973. Biochemical properties of normal and neoplastic cell surfaces, a review. *Eur. J. Cancer.* 9:319–33
31. Loewenstein, W.R. 1979. Junctional intercellular communication and control of growth. *Biochim. Biophys. Acta* 560:1–65
32. Sharom, F.J., Grant, C.W.M. 1978. A model for ganglioside behavior in cell membranes. *Biochim. Biophys. Acta* 507:280–93
33. Lee, P.M., Ketis, N.V., Barber, K.R., Grant, C.W.M. 1980. Ganglioside headgroup dynamics. *Biochim. Biophys. Acta* 601:302–14
34. Maggio, B., Cumar, F.A., Capputo, R. 1981. Molecular behaviors of glycosphingolipids in interfaces. Possible participation in some properties of nerve membranes. *Biochim. Biophys. Acta* 650:68–87
35. Bremer, E.G., Hakomori, S. 1982. GM_3 Ganglioside induces hamster fibroblast

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Annual Reviews

growth inhibition in chemically-defined medium: Ganglioside may regulate growth receptor function. *Biochem. Biophys. Res. Commun.* 106(3):711–18

36. Caputto, R., Maccioni, A.H.R., Caputto, H.L. 1977. Activation of deoxycholate solubilized adenosine triphosphatase by ganglioside and asialoganglioside preparations. *Biochem. Biophys. Res. Commun.* 74:1046–52
37. Partington, C.R., Daly, J.W. 1979. Effect of gangliosides on adenylate cyclase activity in rat cerebral cortical membranes. *Mol. Pharmacol.* 15:484–91
38. Olden, K., Parent, J.B., White, S.L. 1982. Carbohydrate moities of glycoproteins, a re-evaluation of their function. *Biochim. Biophys. Acta* 650:209–32
39. Sadler, J.E., Paulson, J.C., Hill, R.L. 1979. The role of sialic acid in the expression of human MN blood group antigens. *J. Biol. Chem.* 254:2112–19
40. Watanabe, K., Hakomori, S., Powell, M.E., Yokota, M. 1980. The amphipathic membrane proteins associated with gangliosides: The Paul-Bunnell antigen is one of the gangliophilic proteins. *Biochem. Biophys. Res. Commun.* 92:638–48
41. Young, W.W. Jr., MacDonald, E.M.S., Nowinski, R.C., Hakomori, S. 1979. Production of monoclonal antibodies specific for distinct portions of the glycolipid asialo GM_2 (gangliotriaosylceramide). *J. Exp. Med.* 150:1008–19
42. Ito, M., Suzuki, E., Naiki, M., Sendo, F., Arai, S. 1983. Carbohydrates as tumor-associated antigens. *J. Natl. Cancer Inst.* In press
43. Ansel, S., Huet, C. 1980. Specific Glycolipid Antigen in SV40-transformed cell membranes. *Int. J. Cancer* 25:797–803
44. Dippold, W.G., Lloyd, K.O., Li, L.T., Ikeda, H., Oettgen, H.F., Old, L.J. 1980. Cell surface antigens of human malignant melanoma: Definition of six antigenic systems with mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* 77:6114–18
45. Yeh, M.-Y., Hellstrom, I., Abe, K., Hakomori, S., Hellstrom, K.E. 1982. A cell-surface antigen which is present in the ganglioside fraction and shared by human melanomas. *Int. J. Cancer* 29:269–75
46. Pukel, C.S., Lloyd, K.O., Trabassos, L.R., Dippold, W.G., Oettgen, H.F., Old, L.J. 1982. GD_3 a prominent ganglioside of human melanoma: Detection and characterization by mouse monoclonal antibody. *J. Exp. Med.* 155:1133–47
47. Nudelman, E., Hakomori, S., Kannagi, R., Levery, S., Yeh, M.-Y., Hellstrom, K.E., Hellstrom, I. 1982. Characterization of human melanoma-associated ganglioside antigen defined by a monoclonal antibody, 4.2. *J. Biol. Chem.* 257:12752–56
48. Holm, M., Mansson, J.-E., Vanier, M.-T., Svennerholm, L. 1972. Gangliosides of human, bovine and rabbit retina. *Biochim. Biophys. Acta* 280:356–64
49. Puro, K. 1969. Carbohydrate components of bovine-kidney gangliosides. *Biochim. Biophys. Acta* 189:401–13
50. Portoukalian, J., Zwingelstein, G., Dore, J.-F. 1979. Lipid composition of human malignant melanoma tumors at various levels of malignant growth. *Eur. J. Biochem.* 94:19–23
51. Irie, R.F., Sze, L.L., Saxton, R.E. 1982. Human antibody to OFA-I, a tumor antigen, produced *in vitro* by Epstein-Barr virus-transformed human β-lymphoid cell lines. *Proc. Natl. Acad. Sci. USA* 79:5666–70
52. Tai, T., Paulson, J.C., Cahan, L.D., Irie, R.F. 1983. Ganglioside GM_2 as a human tumor antigen (OFA–I–1). *Proc. Natl. Acad. Sci. USA* 80:5392–96
53. Cahan, L.D., Irie, R.I., Singh, R., Cassidenti, A., Paulsen, J.C. 1982. Identification of human neuroectodermal tumor antigen (IA–I–2) as ganglioside GD_2. *Proc. Natl. Acad. Sci. USA* 79:7629–33
54. Watanabe, T., Pukel, C.S., Takeyama, H., Lloyd, K.O., Shiku, H., Li, L.T.C., Trabassos, L.R., Oettgen, H.F., Old, L.J. 1982. Human melanoma antigen AH is an autoantigen ganglioside related to GD_2. *J. Exp. Med.* 156:1884–89
55. Wiels, J., Fellous, M., Tursz, T. 1981. Monoclonal antibody against a Burkitt lymphoma associated antigen. *Proc. Natl. Acad. Sci. USA* 78:6485–88
56. Nudelman, E., Kannagi, R., Hakomori, S., Parsons, M., Lipinski, M., Wiels, J., Fellous, M., Tursz, T. 1983. A glycolipid antigen associated with Burkitt lymphoma defined by a monoclonal antibody. *Science* 220:509–11
57. Marcus, D.M., Kundu, S.K., Suzuki, A. 1981. The P blood group system: Recent progress in immunochemistry and genetics. *Semin. Hematol.* 18:63–71
58. Buchbinder, L. 1935. Heterophile phenomena in immunology. *Arch. Pathol* 19:841–80
59. Hakomori, S., Wang, S.-H., Young, W.W. Jr. 1977. Isoantigenic expression of Forssman glycolipid in human gastric and colonic mucosa: its possible identity with "A-like antigen" in human cancer. *Proc. Natl. Acad. Sci. USA* 74:3023–27
60. Young, W.W. Jr., Hakomori, S., Levine,

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Annu. Rev. Immunol. 1984.2:103-126. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

P. 1979. Characterization of anti-Forssman (anti-Fs) antibodies in human sera: their specificity and possible changes in patients with cancer. *J. Immunol.* 123:92–96

61. Yoda, Y., Ishibashi, T., Makita, A. 1980. Isolation, characterization and biosynthesis of Forssman antigen in human lung and lung carcinoma. *J. Biochem.* 88:1887–90
62. Taniguchi, N., Yokosawa, N., Narita, M., Mitsuyama, T., Makita, A. 1981. Expression of Forssman antigen synthesis and degradation in human lung cancer. *J. Natl. Cancer Inst.* 67:577–83
63. Mori, T., Sudo, T., Kano, K. 1983. Expression of heterophile Forssman antigens in cultured malignant cell lines. *J. Natl. Cancer Inst.* 70:811–14
64. Mori, E., Mori, T., Sanai, Y., Nagai, Y. 1982. Radioimmuno-thin-layer chromatographic detection of Forssman antigen in human carcinoma cell lines. *Biochem. Biophys. Res. Commun.* 108: 926–32
65. Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D., Fuhrer, P. 1979. Colorectal carcinoma antigens detected by hybridoma antibodies. *Soma. Cell Genet.* 5:957–72
66. Koprowski, H., Herlyn, M., Steplewski, Z., Sears, H.F. 1981. Specific antigen in serum of patients with colon carcinoma. *Science* 212:53–55
67. Magnani, J.L., Nilsson, B., Brockhaus, M., Zopf, D., Steplewski, Z., Koprowski, H., Ginsburg, V. 1982. A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentaose II. *J. Biol. Chem.* 257:14365–69
68. Falk, K.-E., Karlsson, K.-A., Larson, G., Thurin, J., Blaszczyk, M., Steplewski, Z., Koprowski, H. 1983. Mass spectrometry of a human tumor glycolipid antigen being defined by mouse monoclonal antibody NS-19-9. *Biochem. Biophys. Res. Commun.* 100:383–91
69. Atkinson, B.E., Ernst, C.S., Herlyn, M., Steplewski, Z., Sear, H.F., Koprowski, H. 1982. Gastrointestinal cancer-associated antigen in immunoperoxidase assay. *Cancer Res.* 42:4820–23
70. Brockhaus, M., Magnani, J.L., Herlyn, M., Blaszczyk, M., Steplewski, Z., Koprowski, H., Ginsburg, V. 1982. Monoclonal antibodies directed against the sugar sequence of lacto-N-fucopentaose III are obtained from mice immunized with human tumors. *Arch. Biochem. Biophys.* 217:647–51
71. Cuttitta, F., Rosen, S., Gazdar, A.F., Minna, J.D. 1981. Monoclonal antibodies that demonstrate specificity for several types of human lung cancer. *Proc. Natl. Acad. Sci. USA* 78:4591–95
72. Huang, L.C., Brockhaus, M., Magnani, J.L., Cuttitta, S.R., Rosen, S., Minna, J.D., Ginsburg, V. 1983. Many monoclonal antibodies with an apparent specificity for certain lung cancers are directed against a sugar sequence found in lacto-N-fucopentaose III. *Arch. Biochem. Biophys.* 220:318–20
73. Huang, L.C., Civin, C.I., Magnani, J.L., Shaper, J.H., Ginsburg, V. 1983. My-1, the human myeloid-specific antigen detected by mouse monoclonal antibodies, is a sugar sequence found in lacto-N-fucopentaose III. *Blood* 61:1020–23
74. Gooi, H., Thorpe, S.J., Hounsell, E.F., Rumpold, H., Kraft, D., Forster, O., Feizi, T. 1983. Marker of peripheral blood granulocytes and monocytes of man recognized by two monoclonal antibodies VEP8 and VEP9 involves the trisaccharide 3-fucosyl-N-acetyllactosamine. *Eur. J. Immunol.* 13:306–12
75. Urdal, D.L., Bretnall, T.A., Bernstein, I.D., Hakomori, S. 1983. The granulocyte reactive monoclonal antibody, IG10, identifies the Le^x carbohydrate determinant depressed in HL-60 cells in both glycolipid and glycoprotein molecules. *Blood.* 62 1022-1026
76. Solter, D., Knowles, B.B. 1978. Monoclonal antibody defining a stage-specific embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci. USA* 75:5565–69
77. Hakomori, S., Nudelman, E., Levery, S., Solter, D., Knowles, B.B. 1981. The hapten structure of a developmentally regulated glycolipid antigen (SSEA-1) isolated from human erythrocytes and adenocarcinoma: a preliminary note. *Biochem. Biophys. Res. Commun.* 100:1578–86
78. Gooi, H.C., Feizi, T., Kapadia, A., Knowles, B.B., Solter, D., Evans, J.M. 1981. Stage-specific embryonic antigen involves $\alpha 1 \rightarrow 3$-fucosylated type 2 blood group chains. *Nature* 292:156–58
79. Kannagi, R., Nudelman, E., Levery, S.B., Hakomori, S. 1982. A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. *J. Biol. Chem.* 257:14865–74
80. Hakomori, S., Nudelman, E., Kannagi, R., Levery, S.B. 1982. The common structure in fucosyllactosaminolipids accumulating in human adenocarcinomas, and its absence in normal tissue. *Biochem. Biophys. Res. Commun.* 109:36–44
81. Yang, H.-J., Hakomori, S. 1971. A

Annual Reviews

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sphingolipid having a novel type of ceramide and lacto-N-pentaose III. *J. Biol. Chem.* 246:1192–1200
82. Hakomori, S., Nudelman, E., Levery, S.B., Kannagi, R. 1984. Novel fucolipids accumulation in human adenocarcinoma. I. Glycolipids with di- or trifucosylated type 2 chain. *J. Biol. Chem.* In press
83. Fukushi, Y., Hakomori, S., Nudelman, E., Cochran, N. 1984. Novel fucolipids accumulating in human adenocarcinoma. II. Selective isolation of hybridoma antibodies that differentially recognize mono-, di-, trifucosylated type 2 chain. *J. Biol. Chem.* Submitted
84. Watanabe, K., Powell, M.E., Hakomori, S. 1979. Isolation and characterization of gangliosides with a new sialosyl linkage and core structures. II. Gangliosides of human erythrocyte membranes. *J. Biol. Chem.* 254:8223–29
85. Nilsson, O., Mansson, J.E., Tibblin, E., Svennerholm, L. 1981. Gangliosides of tumor meconium, possible detection of fetal antigen. *FEBS Lett.* 133:197–200
86. Hakomori, S., Nudelman, E., Levery, S.B., Patterson, C.M. 1983. Human cancer associated gangliosides defined by a monoclonal antibody (IB9) directed to sialosylα2→6galactosyl residue: A preliminary note. *Biochem. Biophys. Res. Commun.* 113:791–98
87. Kano, K., Milgrom, F. 1977. Heterophile antigens and antibodies in medicine. *Curr. Top. Microbiol. Immunol.* 77:43–69.
88. Nishimaki, T., Kano, K., Milgrom, F. 1979. Hanganutziu-Deicher antigen and antibody in pathologic sera and tissues. *J. Immunol.* 122:2314–18
89. Ikuta, K., Nishi, Y., Shimizu, Y., Higashi, H., Kitamoto, N., Kato, S., Fujita, M., Nakano, Y., Taghuchi, T., Naiki, M. 1982. Hanganutziu-Deicher type-heterophile antigen-positive cells in human cancer tissues demonstrated by membrane immunofluorescence. *Biken J.* 25:47–50
90. Prokop, O., Uhlenbruck, G. 1969. *The Thomsen Phenomenon in Human Blood and Serum Groups.* London: MacLaren and Sons, pp. 102–10
91. Uhlenbruck, G., Pardoe, G.I., Bird, G.W.G. 1969. On the specificity of lectins with a broad agglutination spectrum. II. Studies on the nature of T-antigen and the specific receptors for the lectin of *Arachis hypogoea* (ground nut). *Z. Immunitätsforsch. Allg. Klin. Immunol.* 38:423–33
92. Springer, G.F., Desai, P.R., Banatwala, I. 1975. Blood group MN antigen as precursor in normal and malignant human breast glandular tissue. *J. Natl. Cancer Inst.* 54:335
93. Springer, G.F., Desai, P.R., Fry, W.A., Goodale, R.L., Shearen, J.G., Scanlon, E.F. 1983. T antigen, a tumor marker against which breast, lung and pancreas carcinoma patients mount immune responses. *Cancer Detect. Preven.* 6:111–18
94. Springer, G.F., Cheingsong-Popov, R., Schirrmacher, V., Desai, P.R, Tegtemeyer, H. 1983. Proposed molecular basis of murine tumor cell-hepatocyte interaction. *J. Biol. Chem.* 258:5702–6
95. Summer, J.L., Coon, J.S., Ward, R.M., Falor, W.H., Miller, A.W., Weinstein, R.S. 1983. Prognosis in carcinoma of the urinary bladder based upon tissue blood group ABH and Thomsen-Freidenreich antigen status and karyotype of the initial tumor. *Cancer Res.* 43:934–39
96. Rahman, A.F.B., Longeneker, B.M. 1982. A monoclonal antibody specific for the Thomsen-Friedenreich cryptic T-antigen. *J. Immunol.* 129:2021–24
97. Ashall, F., Bramwell, M.E., Harris, H. 1982. A new marker for human cancer cells. 1. The Ca antigen and the Ca 1 antibody. *Lancet* 2:1–6
98. Bramwell, M.E., Bhavanandan, V.P., Wiseman, G., Harris, H. 1983. Structure and function of CA antigen. *Brit. J. Cancer* 48:177–83
99. Fukuda, M. N., Fukuda, M., Hakomori, S. 1979. Cell surface modification by endo-β-galactosidase. Change of blood group activities and release of oligosaccharides for glycoproteins and sphingolipids of human erythrocytes. *J. Biol. Chem.* 254:5458–65
100. Tonegawa, Y., Hakomori, S. 1977. "Ganglioprotein and globoprotein": The glycoproteins reacting with anti-ganglioside and anti-globoside antibodies and the glycoprotein change associated with transformation. *Biochem. Biophys. Res. Commun.* 76:9–17
101. Magnani, J., Steplewski, Z., Koprowski, H., Ginsburg, V. 1983. Identification of the gastrointestinal and pancreatic cancer-associated antigen detected by monoclonal antibody 19-9 in the sera of patients as mucin. *Cancer Res.* 43:5489-92
102. Hakomori, S., Young, W.W. Jr. 1978. Tumor associated glycolipid antigens and modified blood group antigens. *Scand. J. Immunol.* 7:97–117
103. Dabelsteen, E., Fejerskov, O. 1974. Distribution of blood group antigen A in human oral epithelium. *Scand. J. Dent. Res.* 82:206–11
104. Kapadia, A., Feizi, T., Jewell, D., Keeling, J., Slavin, G. 1981. Immunocyto-

Annual Reviews

chemical studies of blood group A,H,I, and i antigens in gastric mucosae of infants with normal gastric histology and of patients with gastric carcinoma and chronic benign peptic ulceration. *J. Clin. Pathol.* 34:320–37

105. Feizi, T., Turberville, C., Westwood, J.H. 1975. Blood-group precursors and cancer-related antigens. *Lancet* 2:391–93
106. Dabelsteen, E., Vedtofte, P., Hakomori, S., Young, W.W. Jr. 1983. Accumulation of blood group antigen precursors in oral premalignant lesions. *Cancer Res.* 43:1451–54
107. Kabat, E.A., Liao, J., Shyong, J., Osserman, E.F. 1982. A monoclonal IgM macroglobulin with specificity for lacto-N-tetraose in a patient with bronchogenic carcinoma. *J. Immunol.* 128:540–44
108. Gooi, H.C., Williams, L.K., Uemura, K., Hounsell, E.F., McIlhinney, R.A.J., Feizi, T. 1983. A marker of human foetal endoderm defined by a monoclonal antibody involves type 1 blood group chains. *Mol. Immunol.* 20:607–13
109. Hakomori, S., Koscielak, J., Bloch, K.V., Jeanloz, R.W. 1967. Studies on the immunological relation between the tumor glycolipids and blood group substances. *J. Immunol.* 98:31–38
110. Häkkinen, I. 1970. A-like blood group antigen in gastric cancer cells of patients in blood groups O and B. *J. Natl. Cancer Inst.* 44:1183–93
111. Breimer, M.E. 1980. Adaptation of mass spectrometry for the analysis of tumor antigens as applied to blood group glycolipids of a human gastric carcinoma. *Cancer Res.* 40:897–908
112. Hattori, H., Uemura, K., Taketomi, T. 1981. Glycolipids of gastric cancer. The presence of blood group A-active glycolipids in cancer tissues from blood group O patients. *Biochim. Biophys. Acta* 666:361–69
113. Yokota, M., Warner, G., Hakomori, S. 1981. Blood group A-like glycolipid and a novel Forssman antigen in the hepatocarcinoma of a blood group O individual. *Cancer Res.* 41:4185–90
114. Knuth, A., Lloyd, K.O., Lipkin, M., Oettgen, H.F., Old, L.J. 1983. Natural antibodies in human sera directed against blood-group-related determinants expressed on colon cancer cells. *Int. J. Cancer* 32:199–204
115. Levine, P., Bobbit, O.B., Waller, R.K., Kuhmichel, A. 1951. Isoimmunization by a new blood factor in tumor cells. *Proc. Soc. Exp. Biol. Med.* 777:403–5
116. Levine, P. 1978. Blood group and tissue genetic markers in familial adenocarcinoma: Potential specific immunotherapy. *Semin. Oncol.* 5:28–34
117. Kannagi, R., Levine, P., Watanabe, K., Hakomori, S. 1982. Glycolipid and glycoprotein profiles and characterization of the major glycolipid antigen in gastric cancer of the 1951 patient of blood group genotype pp (Mrs. D.J.). *Cancer Res.* 42:5249–54
118. Urdal, D.L., Hakomori, S. 1983. Characterization of tumor-associated ganglio-N-triaosylceramide in mouse lymphoma and the dependency of its exposure and antigenicity on the sialosyl residue of a second glycoconjugate. *J. Biol. Chem.* 258:6869–74
119. Kannagi, R., Stroup, R., Cochran, N.A., Urdal, D.L., Young, W.W. Jr., Hakomori, S. 1983. Glycolipid tumor antigen in cultured murine lymphoma cells and factors affecting its expression at the cell surface. *Cancer Res.* 43:4997–5005
120. Stein, K.N., Schwarting, G.A., Marcus, D.M. 1978. Glycolipid markers of murine lymphocyte subpopulations. *J. Immunol.* 120:676–79
121. Hakomori, S. 1969. Differential reactivities of fetal and adult human erythrocytes to antisera directed against glycolipids of human erythrocytes. *Vox Sang.* 16:478–84
122. Oldani, D., Hauser, H., Nichols, B.W., Philips, M.C. 1975. Monolayer characteristics of some glycolipids at the air-water interface. *Biochim. Biophys. Acta* 382:1–9
123. Reisner, Y., Linker-Israeli, M., Sharon, N. 1976. Separation of mouse thymocytes into two subpopulations by the use of peanut agglutinin. *Cell. Immunol.* 25:129–34
124. Symington, F.W., Burnstein, I.D., Hakomori, S. 1984. Monoclonal antibody specific for lactosylceramide (LacCer). *J. Biol. Chem.* Submitted
125. IUPAC–IUB Commission on Biochemical Nomenclature. 1977. *Lipids* 12:455–63
126. Svennerholm, L. 1964. The gangliosides. *J. Lipid Res.* 5:145–55

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:103-126. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:103-126. Downloaded from arjournals.annualreviews.org
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SUPPRESSOR CELLS AND IMMUNOREGULATION

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INTRODUCTION

Several mechanisms result in suppression of the immune response. For example, either T cells (1-3), B cells (4), or macrophages (5-7) can mediate antigen nonspecific suppression. However, discussion of these phenomena is beyond the scope of the present review. In this chapter we consider only T-cell suppressor systems specific for the antigen that stimulated their development.

In addition to helper T lymphocytes, which permit development of immune responses to T-dependent antigens, there is a distinct subclass of T cells that suppress specific immune responses. The existence of antigen-specific suppressor T cells following antigen administration was originally demonstrated by Gershon & Kondo (8) and has been studied subsequently in many laboratories. Suppressor T cells are responsible for many phenomena associated with immunological tolerance. In addition, suppressor T cells are an essential component of a homeostatic mechanism that keeps the course and size of specific immune responses under control.

FEATURES SHARED AMONG DIFFERENT SUPPRESSOR T-CELL SYSTEMS

Agreement on a comprehensive antigen-specific suppressor T-cell schema that integrates all available data has been hampered by the fact that most laboratories have chosen to analyze suppressor T-cell activity in different model systems. The ability to observe immune suppression in so many experimental systems underscores the importance of suppressor T cells. Suppressor T cells can modulate a variety of immune responses, involving both humoral (9-13) and cellular immunity. The latter includes modulation of helper-cell (14,15), delayed-type hypersensitivity (16,17), contact sensitivity (18,19), cytolytic T-

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Annual Reviews

cell (20,21), and proliferating T-cell responses (22,23). Suppressor T cells can also be demonstrated in tumor immunity (24,25) and can be observed in several disease processes (26-28). Furthermore, antigen-specific suppressor T cells have been identified in several animal species, including humans (29-31). Since murine suppressor T cells have been most extensively studied, we confine our discussion to this species.

Several features are common to most suppressor T-cell systems. In this section we emphasize the features shared by most of the well-characterized model systems (Table 1). First, several suppressor T-cell subsets interact sequentially. In general, these consist of at least two and in some cases three distinct suppressor T-cell subpopulations (9,30,32-34). The earliest-acting suppressor T cells have been categorized as inducer cells or Ts_1 by most laboratories (17,23,35-37). These are Lyt-1^+,2^- cells and may be involved in the generation of another suppressor T-cell subset. In contrast, the effector suppressor T cells generally have an Lyt-1^-,2^+ phenotype and operate late in the course of the immune response (15,18,23,33,34,38-40). In some systems an additional T-cell population is active in the pathway between the inducer and effector suppressor cells (15,29,41,42).

Another important feature of suppressor T cells is their ability to bind antigen in the absence of accessory cells or specific H-2 gene products. In many systems, suppressor T cells can be depleted following passage over antigen complexed to polystyrene surfaces (17,43-45). Enriched populations of antigen-specific suppressor T cells can then be recovered from the plastic surface following a temperature shift. In contrast, helper, proliferating, and killer T cells fail to bind antigen unless it is associated with appropriate H-2 gene products. This ability of suppressor T cells to bind free antigen suggested initially that they might utilize immunoglobulin receptors. However, this hypothesis has clearly been disproven by convincing serological (lack of heavy- and light-chain constant-region determinants) and molecular genetic (lack of VDJ rearrangements) evidence (46-49). Nonetheless, several groups have demonstrated that some reagents specific for V_H and/or idiotypic determinants on immunoglobulin molecules also bind suppressor T cells or their factors in

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Table 1 Features shared among several suppressor T-cell systems

1. Utilize multiple distinct T-cell subsets
2. Suppressor T cells bind free antigen
3. Antigen receptors share some serological determinants with major idiotypes
4. I-region gene products are involved in suppressor-cell interactions
5. Soluble suppressor factors exhibit biological activity
6. Distinctive mechanisms of antigen presentation to suppressor T cells
7. Final suppressor mechanism is antigen-dependent but nonspecific

Annual Reviews

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selected systems (15,17,39,43,49-53). These idiotype-like determinants may play a critical role in the network-like interactions among suppressor T-cell subsets. The significance of the sharing of immunoglobulin idiotypic specificity by T cells is discussed in a later section.

A general feature of the suppressor T-cell system is the involvement of major histocompatibility complex (MHC) genes in the induction and expression of suppressor T-cell activity. This was originally demonstrated with antigens such as the linear synthetic polymer poly-(L-glutamic acid, L-alanine, L-tyrosine), abbreviated GAT (54,55). The ability to mount an immune response to GAT is controlled by I-A region-encoded immune-response genes (56). $H\text{-}2^q$ and $H\text{-}2^s$ nonresponder mice to GAT generally developed active suppressor T cells following immunization with this polypeptide (57). The genetics of suppression were first evaluated using the copolymer of L-glutamic acid, L-tyrosine (GT), which is not immunogenic in inbred strains of mice (58,59). The data demonstrated that genes encoded in the I-A and I-C subregions of the H-2 complex were involved in the process of suppressor T-cell generation against GT (58). Subsequent studies in these and other systems demonstrated that a third subregion (I-J) of the H-2 complex was also associated with suppressor T-cell activity and restriction specificity (60-63). The involvement of I-A, I-J, and/or I-C genes in immune suppression has been confirmed in numerous systems (22,23,38,60,64-66), although few model systems allow comparison among all three genes or gene products simultaneously. These various I-region gene products appear to be expressed on suppressor T cells and/or their factors in selected systems. In addition, the interactions of various suppressor T-cell subsets are also restricted by these genes.

Suppressor T cells produce soluble factors that mediate suppressive activity. Each suppressor T-cell subset produces its own type of suppressive factor. Thus, inducer T cells make inducer suppressor factor and effector suppressor T lymphocytes produce molecules that mediate effector-cell function. These factors provide the induction, activation, and/or effector signals required for communication among the various suppressor T-cell populations. Soluble factors permit interactions to proceed at distances from the T cell that produced the factor and thereby obviate the need for direct cell-cell contact among the various suppressor T-cell subsets.

It had long been recognized that in vivo–filtered antigen or deaggregated (by ultracentrifugation) antigen selectively induces tolerance (67-69). This selective induction was attributed to the inability of antigen-presenting cells to process these forms of antigen. Subsequently, most of these tolerance models were shown to involve active mechanisms of T cell–mediated immunosuppression (67,69). In addition, other experimental methods designed to eliminate the antigen-presenting cells required for helper T-cell induction were shown to favor the production of suppressor T lymphocytes. Thus, treatment with

Annual Reviews

ultraviolet light, which eliminates the I-A bearing antigen-presenting cells, resulted in the selective induction of specific suppressor T lymphocytes (70-72). However, direct analysis of suppressor T-cell induction has demonstrated a unique requirement for an accessory population that bears I region–encoded determinants and that is involved in antigen presentation (73-77). One interesting feature of the antigen-presenting cells for suppressor T-cell induction is their remarkable efficiency. Thus, as few as 10^2–10^3 antigen-coupled adherent cells could induce T cell-mediated immune suppression in vivo (74,76,78,79). Recent studies (detailed below) further characterized the critical role of antigen-presenting cells in suppressor T-cell induction. In brief, it appears that a subclass of antigen-presenting cells is responsible for the induction of the genetic restrictions of suppressor T cells and for guiding the communications between suppressor T cells and factors (74,77,80,81).

The issue of the specificity of effector suppressor T cells must be clarified before we can understand the final mechanism of T-cell suppression. If, as some investigators (38,82) have claimed, suppressor T cells selectively interact with the antigen receptors on helper T lymphocytes, we would expect to observe strict antigen or idiotype specificity. However, there are numerous indications that the final stages of the suppressor T-cell pathway involve nonspecific mediators (66,83-87). Although one can interpret the combined data to suggest the existence of multiple effector-phase suppression mechanisms (88), careful analysis of the data imply that the terminal suppressive mechanism is antigen dependent but antigen nonspecific in many cases. This combination provides an efficient mechanism for suppressing various T-cell subsets and simultaneously controlling the release of potent biological mediators.

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SUPPRESSOR T-CELL SUBSETS

In the remainder of this review we discuss data primarily from our laboratories describing the cascade of cellular events that results in immune suppression by suppressor T cells. The first system analyzed by us involved the regulation of plaque-forming cell (PFC) responses to GAT. The GAT system offered unique advantages for such studies because administration of this molecule to responder mice stimulated helper T-cell activity and antibody formation, while GAT given to nonresponder mice not only failed to elicit demonstrable immunity but also stimulated the development of GAT-specific suppressor T cells in these strains. GAT-specific suppressor T cells were detected by their ability to inhibit the normal GAT response of nonresponder strains to the immunogenic complex of GAT with methylated bovine serum albumin (GAT-MBSA) (55). These features of immune response (Ir) gene systems—i.e. the difference in regulatory T-cell responses of responder versus nonresponder mice and the opportunity to study nearly pure suppressor T-cell responses in nonresponder

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mice—have proven most useful for the study of immune regulation. Other well-characterized suppressor T-cell systems also utilized antigens the response to which is under H-2-linked Ir gene control (23,89). Splenic cell extracts from GAT nonresponder mice yield suppressor T-cell factors that function in vivo and in vitro (90). These factors are derived from suppressor T-cell subsets that correspond to the inducer or Ts_1 population. Investigation of how these factors mediate T cell–suppressive activity indicated that injection of GAT-suppressor factor into normal nonresponder mice elicited the generation of a second population of GAT-specific suppressor T cells (91). These results indicated that one important function of suppressor factor was the recruitment of a second population of suppressor cells from unprimed T cells. From the above results, it was apparent that the GAT suppressor T-cell circuit involved a pathway comprising at least two distinct T cells and one suppressor factor.

As the above studies progressed it also became apparent that B-cell idiotype networks were also involved in some suppressor T-cell systems (92-96). Therefore we initiated two independent approaches to evaluate the role of major idiotypes in suppressor T-cell cascades. First we analyzed the common idiotypes of the GAT and GT systems (97,98) and detected idiotypic determinants on suppressor T-cell factors (50). Second we investigated suppressor T cells in systems where the major idiotypes had been well characterized. The 4-hydroxy-3-nitrophenyl acetyl (NP) and azobenzenearsonate (ABA) haptens were chosen for the analysis of suppressor T-cell activity since the antibody responses and idiotypes to these haptens had been thoroughly studied (96,99,100). Furthermore, the requirement for recognition of idiotype-related determinants for T-cell interactions was investigated in systems in which both T- and B-cell responses could be assayed. Suppression of hapten-specific T-cell responses was evaluated by measurement of contact sensitivity (CS) (34,101), delayed-type hypersensitivity (DTH) (16,17), or cytolytic T-cell (21) responses following priming with either a chemically active form of the hapten or hapten coupled to proteins or cells. Following priming, animals were challenged for DTH or CS reactivity with the chemically active form of the hapten or hapten-coupled spleen cells. Modulation of B-cell responses were determined in the NP system by assay of plaque-forming cell responses (PFC) following in vivo priming or in vitro challenge of NP-primed spleen cells (102,103). In the latter system the ability of suppressor T cell populations to affect both the magnitude of the response and the percent of NP^b idiotype bearing B cells was monitored. In each of these systems it was demonstrated that the regulation of immune responses depended upon idiotype recognition by T-cell subsets.

In the NP, ABA, and other suppressor T-cell systems at least three distinct suppressor T-cell subsets have been identified (29,33,104,105). These suppressor T lymphocytes function in a tandem sequence often referred to as the suppressor T-cell cascade. The suppressor T cell populations share several

Annual Reviews

properties, including the ability to bind free ligand, the ability to produce soluble suppressor factors, and the dependence on accesory cells for induction and/or expression of functional activity. The major differences among these populations concern their Lyt phenotype, kinetics, functional properties, and binding specificity. Some suppressor T cells bind antigen via idiotype-like receptors while other suppressor T cells use reciprocal anti-idiotypic receptors that can serve as an internal image of antigen (80). The latter suppressor T cells presumably serve to bridge the other elements of the cascade. The availability of hybridoma or cloned suppressor T-cell lines has permitted careful analysis of the properties of each suppressor T-cell subset. Such cell lines constitutively produce soluble factors possessing the biological activity of the intact cells. Since each of these suppressor-cell subsets has been identified in the NP system and since each has been used for the preparation of cloned suppressor T-cell hybridomas, much of our discussion focuses on the comparisons among suppressor cell subsets in this system.

The three T-cell elements involved in the suppressor pathway have been given various names in different systems. Our laboratories refer to these cell populations as Ts_1, Ts_2, and Ts_3. Ts_1 cells are also referred to as inducer suppressor cells, analogs of the Ts_2 have been referred to as transducer cells, while Ts_3 cells have been termed T auxiliary cells and effector suppressor cells by other laboratories. Studies from our laboratories indicated that in the NP and ABA systems the Ts_2 population has anti-idiotypic receptors (41,42), while the Ts_1 and Ts_3 subsets have antigen binding receptors that possess idiotype-related determinants (17,49,106).

Ts_1 INDUCTION

The initial reports of Battisto & Bloom (19) in 1966 showed that the intravenous injection of antigen-conjugated cell membranes into syngeneic mice induced antigen-specific nonresponsiveness. Subsequently, such nonresponsiveness was attributed to the induction of suppressor T cells (107). To define the nature of the cells responsible for Ts_1 induction, Sherr et al (76) injected mice with graded numbers of antigen-coupled syngeneic splenic adherent (2 hr) or nonadherent cells. After 5–7 days, splenocytes from the Ts_1 donors were adoptively transferred to syngeneic recipients at the time of antigen priming. As few as 10^2–10^3 antigen-coupled Ia-bearing adherent cells were sufficient to induce significant levels of antigen-specific suppression. In contrast, at least 10^6 antigen-coupled nonadherent cells were needed to induce similar levels of suppression (76). Thus, antigen-coupled splenic cells are at least 1000-fold more efficient at inducing Ts_1 activity than antigen-coupled nonadherent cells.

The phenotypic characterization of the cells capable of inducing Ts_1 was further explored in the NP system (79). The adherent population responsible

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Annual Reviews

for Ts_1 induction carried I-A determinants but lacked conventional T-cell markers, including Thy-1 and Lyt-1. The antigen-presenting cells that induced Ts_1 were resistant to 500R and treatment with cyclophosphamide (79). The cells were contained within the 24-hr adherent, phagocytic, FcR^+ fraction. In contrast, the low-density, FcR^- nonphagocytic, nonspecific esterase-negative, dendritic fraction was unable to induce Ts_1 activity (79). Thus, the antigen-presenting cells required for Ts_1 induction have the properties of macrophages (Table 2).

The induction of Ts_1 cells also required presentation of antigen in the context of H-2 coded determinants. Thus, I-J homology was required between the antigen-presenting cell population and the Ts_1 donor (79). This I-J restriction on the induction of Ts_1 cells implies that the macrophage-like cells involved in Ts_1 induction also possess I-J determinants.

Additional methods of Ts_1 induction may also exist. Thus intravenous administration of high doses of hapten conjugated to the polysaccharide antigen pullulan can also induce NP-specific cells indistinguishable from the Ts_1 cells characterized above (108). Further experiments are required to evaluate the role of antigen-presenting cells under the latter set of experimental conditions.

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Ts_1 CELLS AND FACTORS

The ability of NP-coupled syngeneic spleen cells to induce antigen-specific T suppressor cells was first demonstrated by Weinberger et al (17). It was shown that spleens of mice injected seven days previously with NP-coupled syngeneic spleen cells contain an NP-binding T-cell population capable of suppressing DTH-mediated footpad swelling responses to NP, only when these cells were added in the induction phase (i.e. during priming) of the response. This T-cell population, initially termed Ts^i, adhered to NP-BSA coated petri dishes. Furthermore, treatment of T cells from NP spleen–treated C57BL/6 (Igh^b) mice with guinea-pig anti-NP^b idiotypic antiserum plus complement abrogated the ability to transfer NP-specific suppression to normal syngeneic recipients (17,34).

Table 2 Comparisons among the accessory cells involved in suppressor T-cell generation

Parameter	Ts_1 Induction	Ts_3 Induction
adherence	24 hr adherent	24 hr adherent
FcR	FcR^+	FcR^+
phagocytosis	+	+
I-J	+	+
I-A	+	–
cyclophosphamide	resistant	sensitive
500R	resistant	not tested

Annual Reviews

Such readily detectable NP^b-related idiotypic determinants were also found on Ts_1 cells derived from SJL mice (17). Since SJL mice express little serum NP^b idiotype following immunization with NP-coupled proteins (99), it appeared that the regulation of T-cell idiotypic determinants was independent of idiotype expression on immunoglobulin molecules. The results also suggested that the T cell– and B cell–derived idiotypic receptors were distinct (12).

In the NP and ABA systems, Ts_1 cells have been successfully hybridized with the BW5147 thymoma to generate cloned hybridoma lines that have the characteristic properties of hapten-specific Ts_1 (49,109). Screening for NP-specific Ts_1 hybridomas was partially based on the ability of allele-specific anti-I-J alloantisera and heterologous anti-NP^b idiotypic antisera to lyse the hybridoma cells (49). In addition, functional assays were performed on the culture supernatants to establish the biological activity of the hybridoma cell–derived factors (110).

The Ts_1 hybridomas constitutively released suppressor factors (TsF_1) into the culture supernatant (109,110). These TsF_1 functionally substituted for intact Ts_1 cells. Thus, i.v. administration of TsF_1 during the induction phase of the immune response mediated antigen-specific suppression. Suppressor factors derived from three NP-specific and one ABA-specific T-cell hybridomas were characterized. These TsF_1 specifically inhibited T cell–mediated CS and DTH responses. The factors specifically adsorbed to columns containing allele-specific anti-I-J alloantisera, heterologous anti-idiotypic antisera, or appropriate hapten-conjugated proteins (109,110). In contrast, hapten-specific TsF_1 did not adhere to immunoadsorbent columns of antibodies to immunoglobulin constant-region determinants, irrelevant anti-I-J or anti-idiotype antisera, or TNP-conjugated proteins. Thus, TsF_1 appeared to have the properties of a soluble form of Ts_1 cell receptor. Further support for this hypothesis was derived from the fact that cell-free membrane preparations from NP-specific Ts_1 hybridomas also display suppressive activity (111).

The TsF_1 cells and their factors are also referred to as inducer factors, since they (*a*) function as afferent suppressor cells requiring several days for suppression to become effective and (*b*) can induce a second population of suppressor cells (Ts_2) after injection into normal recipients. Hybridoma-derived NP- or ABA-specific TsF_1 induce Ts_2 cells in the absence of an exogenous source of antigen (109,110). In vivo the induction process requires 4–6 days, which accounts for the fact that Ts_1 and TsF_1 must be administered early in immunization to observe suppressive activity. TsF_1 specifically inhibits T-cell responses in appropriate strains of mice. The Ts_1-derived hybridoma suppressor factors can directly suppress H-2-incompatible strains of mice provided they are Igh-V homologous with the strain producing the factor. Thus, there is an apparent Igh-V restriction in the activity of these factors. This is a pseudorestriction because these factors can generate second-order suppressor

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cells (Ts_2) in Igh-incompatible mice (110). However, the latter Ts_2 cells only function when adoptively transferred into recipients Igh compatible with the strain producing the suppressor factor.

In a related system, splenic Ts_1 cells from mice treated with NP-coupled syngeneic cells were shown to specifically suppress the in vivo plaque-forming cell (PFC) response to T-dependent or T-independent forms of NP conjugates when transferred to normal, syngeneic recipients (110). In this system, the percent of the NP-specific PFC that expressed NP^b determinants was determined by addition of microliter quantities of NP^b idiotype-specific antiserum to the plaquing mixture. While the idiotype content of the PFC response from control mice ranged from 30 to 50% of the total NP-specific PFC response, the idiotype level in recipients of suppressor T cells was 0%. The complete suppression of NP^b idiotype–bearing B-cell clones correlated well with a 30–50% suppression of the magnitude of the response and a preferential suppression of the high-affinity PFC (12). Thus, suppression induced with NP-modified spleen cells preferentially affects the high-affinity NP^b idiotype–bearing B cells as well as the T cell–mediated CS response.

One of the NP-specific Ts_1-derived hybridoma factors was also assayed for its ability to suppress in vitro PFC responses (112). The same TsF_1-containing supernatants that were capable of suppressing T cell–mediated immune responses also suppressed PFC responses when added in the induction phase. Again the TsF_1 functioned by inducing Ts_2 cells. Furthermore, the factor that suppressed B-cell responses bound NP and reacted with anti-I-J and anti-NP^b antisera. Thus, it appears that the same TsF_1 factor that induces suppression of T cell–dependent CS responses also induces suppression of B-cell responses (112).

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Ts_2 CELLS AND FACTORS

In order to determine how induction-phase Ts_1 suppressor cells or factors can recruit other T-cell subsets, supernatants from the NP- or ABA-specific Ts_1 hybridomas described above were used to induce second-order (Ts_2) cells in normal syngeneic recipients. Four to seven days later spleen cells from these recipients contained antigen-specific suppressive activity (109,110). This latent period presumably represents the temporal requirement for a Ts_1-derived factor (TsF_1) to induce Ts_2 suppressor cells. Unlike Ts_1 cells with which they were induced, these second-order suppressor T cells (Ts_2) were capable of suppressing responses when added in the effector phase of the DTH, CS, or PFC response (109,110). Effector-phase Ts_2 suppressor cells can also be generated by in vitro incubation of NP-specific Ts_1 hybridoma supernatants with normal, syngeneic spleen cells (112).

The induction of Ts_2 cells requires presentation of TsF_1 by a specialized population of factor-presenting cells (80). The I-J phenotype of this factor-

Annual Reviews

presenting cell population controls the functional H-2 (I-J) restriction of the Ts_2 cells. When parental accessory cells were pulsed with TsF_1 and used to generate Ts_2 cells in F_1 recipients, the Ts_2 cells were only restricted to the I-J type of the parental accessory-cell population (80). The splenic cells responsible for presenting TsF_1 appear to be macrophages; they adhere to plastic and lack the Thy-1 marker (80).

Ts_2 cells are also observed to develop in the spleen 6–7 days following i.v. administration of NP-modified syngeneic cells (34,42). Although this protocol produces both Ts_1 and Ts_2 cells, the Ts_2 cells differ radically from Ts_1 in their properties and functions. In the NP and ABA systems, Ts_2 cells are anti-idiotypic and can be shown to bind to NP^b-related idiotype or ABA-associated CRI idiotype–coated plates, respectively (41,42). The anti-idiotypic receptor on Ts_2 cells may also be viewed as representing an internal image of antigen, thereby serving as a bridge to interact with the antigen-specific idiotypic Ts_1 and Ts_3 cells (80). Ts_2 cells react with anti-Lyt-2 and allele-specific anti-I-J alloantisera (34,106). Another very important difference between Ts_1 and Ts_2 cells concerns the genetic restrictions that govern their interaction with target cells. Contrary to results obtained with Ts_1 cells, the expression of Ts_2-cell activity is restricted by genes in both the Igh and I-J complexes (34,106). Thus, Ts_2 cells or their factors will not function unless adoptively transferred into recipients homologous at both the H-2 (I-region) and Igh gene complexes (113).

Hybridomas with the properties of Ts_2 cells have been prepared in the NP system (113). Such Ts_2 hybridomas have the phenotypic properties of the Ts_2 cell population. Furthermore, these hybridomas constitutively produce soluble factors (TsF_2) with functional properties similar to the cells—i.e. these cells and factors bind to allele-specific idiotypic antibodies (that is, the cells and factors are anti-idiotypic) and they react with anti-I-J alloantisera. Ts_2 cell populations and TsF_2 suppress in the effector phase of the immune response and display I-J and Igh genetic restrictions in their abilty to mediate suppression (113).

It was initially thought that TsF_2 acted directly on Ts_3 cells since the restriction specificity of TsF_2 had to match the I-J and Igh genotype of the Ts_3 donor before suppressive activity was observed. However, some data remained unexplained, suggesting that the mechanism of TsF_2 action was incompletely understood. For example, Ts_3 cells from Igh-compatible donors could completely absorb TsF_2 activity even when the Ts_3 cells were I-J incompatible with the TsF_2 (113,114). Furthermore, the potential role(s) of accessory cells in Ts_3 activation had been overlooked; experiments had not been performed to evaluate the potential contribution of accessory cells to Ts_3 activation. In order to analyze the possible role of accessory cells in the genetic restrictions for Ts_3 activation, $I\text{-}J^b$- or $I\text{-}J^k$-restricted TsF_2 was pulsed onto 5R ($I\text{-}J^k$) or 3R ($I\text{-}J^b$)

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splenic adherent cells, which were then injected i.v. into NP-O-Su-primed 5R or 3R recipients. Although soluble I-J^b-restricted TsF_2 failed to activate Ts_3 cells in 5R recipients, when the factor was pulsed onto I-J-compatible 3R adherent cells suppression was observed (Table 3). However, when the same TsF_2 was pulsed onto I-J-incompatible 5R adherent cells no suppressive activity was generated. These results demonstrate that the I-J restriction of TsF_2 is directed toward determinants on an accessory-cell population. The generation of this TsF_2-presenting cell is sensitive to cyclophosphamide and treatment with 500R irradiation (M. Usui, unpublished data), properties that can distinguish various accessory cells involved in the suppressor-cell cascade (Table 2).

Since the activity of the Ts_2-cell population was shown to be restricted by Igh-linked genes in both T and B cell–mediated responses, it was predicted that these suppressor cells specifically recognized Igh-linked, NP^b-related idiotypic determinants. This prediction was substantiated by the demonstration that Ts_2 effector-phase suppressor T cells, which were induced in vitro by a 4-day culture of Ts_1-containing spleen cells with normal cells, could specifically bind to and be recovered from culture dishes coated with NP^b-bearing anti-NP antibody (115).

Idiotypic systems are composed of a family of idiotypically related but nonidentical molecules (116,117). Thus, it was important to determine whether T-cell receptors recognize the same repertoire of NP^b-idiotypic determinants as anti-idiotypic antiserum. For this purpose, the ability of in vivo induced

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Table 3 I-J restrictions of TsF_2 are directed to an accessory-cell population[a]

Recipients	Cells pulsed with TsF_2	I-J restriction of TsF_2	Percent suppression
5R	none	b	−5
		k	48
	5R	b	−8
	(I-J^k)	k	49
	3R	b	51
	(I-J^b)	k	3
	$B6AF_1$	b	48
	(I-J^b/I-J^k)	k	44
3R	none	b	48
		k	−3
	5R	b	−10
		k	50

[a]Recipients were primed with NP-O-Su. On days 5 and 6 the animals received either soluble or spleen cell–pulsed BW5147 (control), Bs-Ts_2-28 or CKB-Ts_2-59 derived ascitic fluid. On day 6 the recipients were challenged with NP-O-Su. Footpad swelling was measured 24 hr later. The I-J phenotypes of the TsF_2-pulsed cells are indicated in parentheses.

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splenic effector-phase suppressor T cells (Ts_2) to bind monoclonal anti-NP antibodies bearing different classes of NP^b-idiotypic determinants was studied in the PFC system (115). Suppressor T cells were fractionated on petri dishes coated with affinity-purified serum anti-NP antibodies or monoclonal anti-NP antibodies of the IgM class and Igh^b allotype but differing with respect to idiotypic determinants and light-chain class—i.e., carrying the λ_1, λ^2, and K chains (Table 4). Molecular genetic analysis indicated that the λ_1- and λ_2-bearing antibodies expressed the same germ-line V_H genes but used different J_H genes (S-T. Ju, unpublished data). Adherent and nonadherent Ts_2-cell populations were then tested for their ability to suppress when added in the effector phase of an in vitro PFC response. The data indicated that suppressor–Ts_2 cell activity could be detected in the cell population that adhered to anti-NP antibodies from C57BL/6 mice and the λ_2-bearing monoclonal antibody (Table 4). Suppressive activity was also detected to a lesser extent in the nonadherent fraction of the monoclonal λ_2 antibody (Table 4). Thus, splenic Ts_2 cells represent a heterogeneous population only a portion of which recognizes the idiotypic determinants present on monoclonal λ_2 anti-NP antibody. In contrast, no suppressive activity was detected in the cell fraction that adhered to affinity-purified anti-NP antibodies from C3H (Igh^j) mice or the hybridoma λ_1- or K-bearing anti-NP antibody (Table 4). The λ_1-bearing anti-NP antibody expressed the predominant, serologically detected NP^b-idiotypic determinants and shared V_H genes and minor NP^b-related idiotypic determinants with the λ_2-bearing anti-NP antibody (115). From these experiments we concluded that Ts_2 cells did not recognize the predominant, serologically detected NP^b B cell–idiotypic determinants (115), implying that the Ts_2 population interacts with other T-cell populations by recognition of a minor fraction of NP^b-related idiotypic determinants. This interpretation further underscores the differences between T- and B-cell idiotypes. Thus, idiotypic determinants present on T cells appear related but not identical to those expressed on B cells and immunoglobulins. Furthermore, it is increasingly unlikely that T- and B-cell idiotypes are encoded by the same set of germ-line genes, since recent data demonstrate that rearranged Igh-V genes are not expressed in T cells (47,48).

Ts_3 CELLS AND FACTORS

What are the targets of the Ts_2 cells in the effector-suppressor pathway? The answer to this question was provided by older experiments of Sy and associates (40), who analyzed the suppressor–T cell circuit in TNP-specific contact sensitivity. These investigators observed that treatment of the recipients of adoptively transferred TNP-specific afferent suppressor T cells (presumably Ts_1) with cyclophosphamide at the time of immunization rendered these animals

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Table 4 Fine binding specificity of C57BL/6 Ts_2 cells[a]

Affinity purified anti-NP antibodies for Ts_2 panning						Percent suppression	
Source	Class	Igh allotype	V_H gene	J_H gene	% Inhibition of NP^b binding	Adherent	Nonadherent
serum	$\mu + \gamma$ $\lambda + K$	b	probably multiple		95	79 ± 17*	−19 ± 9
serum	$\mu + \gamma$ $\lambda + K$	j	probably multiple		5	0 ± 5	67 ± 9*
hybridoma	μ, λ_1	b	186-2	J_{H4}	75	−8 ± 8	51 ± 9*
hybridoma	μ, λ_2	b	186-2	J_{H2}	35	49 ± 9*	29 ± 9*
hybridoma	μ, K	b	unknown	unknown	5	−3 ± 4	60 ± 17*

[a]C578BL/6 mice received an intravenous injection of 3×10^7 control or NP-coupled syngeneic spleen cells. Seven days later, these spleens were cultured for a 4-day period. Control or suppressor T cells were then enriched for T cells by passage over anti-immunoglobulin plates. Suppressor T cells were further fractionated on affinity-purified serum or hybridoma anti-NP antibodies. $1-5 \times 10^5$ control or fractionated suppressor T cells were then added to responder cultures that had been challenged 4 days previously with 100 ng NP-Ficoll. One day later, duplicate wells were pooled and assayed for direct NP-specific PFC responses. Significant suppression is indicated by an asterisk. The ability of 10 μg of purified antibody to inhibit NP^b binding was used as a measure of NP^b idiotype levels. For further details refer to reference 115.

Annual Reviews

resistant to the suppressive effects of the transferred cells. They interpreted their results to indicate the existence in the suppressor pathway of an effector–T cell subclass, the induction of which occurred in the course of conventional immunization and was cyclophosphamide sensitive. This cell, which they called T auxiliary, is the equivalent of Ts_3 in the NP and ABA systems, as shown below.

Although the effector-phase Ts_2-cell population or factors derived from Ts_2 hybridomas were specific for Igh-linked NP^b-related idiotypic determinants, and the overall effect of the suppression appeared to be idiotype-specific in the PFC system (102,103), no direct evidence proved that the Ts_2 was the final effector-cell population in the suppressor pathway. In fact, the inability of Ts_2 cells to recognize the serologically predominant NP^b determinants may be interpreted as evidence against the direct interaction of Ts_2 cells with NP-specific B cells (115). To further investigate these issues the Ts_2 suppressor cell population was adoptively transferred to normal or cyclophosphamide-treated recipients in the effector phase of the CS response. We observed that the injection of Ts_2 cells or TsF_2 would not suppress an immune animal if the recipient had been treated with low doses of cyclophosphamide shortly after antigen immunization (34). This suggested that the cellular target of TsF_2 or cells involved in its induction were sensitive to cyclophosphamide treatment. Using an adoptive transfer protocol with cyclophosphamide-treated recipients, we demonstrated that Ts_3 cells were induced as a consequence of conventional immunization concomitant with the induction of T cells, mediating helper or CS activity. However, these primed Ts_3 cells appear to remain inactive until appropriately triggered by Ts_2 cells or TsF_2 (118).

The Ts_3 cells in the NP and ABA systems are antigen specific and bind to antigen-coated petri dishes (44,104). They express the Lyt-2 phenotype and also react with anti-I-J alloantisera. Ts_3 cells mediate suppression in the effector phase of the immune response. The activity of Ts_3 cells can be demonstrated in cyclophosphamide-treated recipients and, as is the case for TsF_2, it is restricted by H-2I and Igh genes (34,104).

These results were extended to the PFC system with the demonstration that Ts_2 cells were ineffective when added to cultures of responder cells treated with anti-I-J plus complement (44). The suppressive activity mediated by Ts_2 cells was restored by addition of T cells from NP-KLH-primed, but not TNP-KLH-primed, donors to responder populations depleted of I-J-bearing T cells. With this reconstitution protocol it was shown that the third-order suppressor–T cell population (Ts_3) specifically bound to and could be recovered from NP-BSA-coated petri dishes (44). Since the Ts_3 population specifically bound NP and was activated by anti-NP^b-idiotypic Ts_2 cells, it would be expected that Ts_3 cells express NP^b-related idiotypic determinants. This conclusion was validated by the demonstration that treatment of Ts_3 cells with guinea-pig anti-

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NP^b-idiotype antiserum plus complement ablated suppressor activity in the above reconstitution protocol (44).

Ts_3 hybridomas that constitutively secrete TsF_3 have been obtained in the NP system (119). Five hybridoma T-cell lines were prepared by fusion of Ts_3 cells with the BW5147 thymoma. The culture supernatants from these T-cell hybridomas contain a factor, TsF_3, that specifically suppressed NP-O-Su-induced CS responses. TsF_3 activity was only observed if the factor was administered during the effector phase of the immune response. TsF_3 reacts with allele-specific anti-I-J and anti-NP^b antisera and has binding specificity for the NP hapten. Furthermore, TsF_3 does not suppress I-J-incompatible mice. In addition to this H-2 restriction, the monoclonal TsF_3 factors also demonstrated Igh genetic restrictions. Thus, both the TsF_2 and TsF_3 factors display dual genetic restrictions for I-J- and Igh-linked genes (119).

The ability of one of these cloned T-cell hybridomas and its products to specifically suppress the in vitro PFC reponse to NP-ficoll was studied. TsF_3 was able specifically to suppress in vitro responses when added either in the induction or effector phase of the Mishel-Dutton culture (112). We have shown that TsF_1 also works to suppress NP-ficoll responses, but only when added during the induction phase of the immune response. Comparisons of TsF_1 and TsF_3 indicated that only TsF_1 can induce Ts_2 cells in vitro and only TsF_3 can suppress the response of responder cells treated with anti-I-J plus complement (112). Thus, in the PFC system the ultimate target of TsF_1 is a pre-Ts_2 cell, while TsF_3 appears to act directly or indirectly on B-cell targets in suppression of antibody responses. TsF_1 and TsF_3 factors both bind to NP and can be specifically adsorbed onto columns containing anti-I-J or anti-NP^b antisera. Recent data suggest that although I-J and Igh homology are required for TsF_3 to suppress in vivo CS responses, Igh homology may not be required for the expression of TsF_3 activity in the PFC system (12). The subregion(s) involved in the H-2 restriction of TsF_3 in the PFC system has not yet been identified. Aside from these potential disparities the combined serological and functional data strongly suggest that the same types of TsF_1 and TsF_3 molecules can function to suppress both T and B cell–mediated immune responses. The discrepancies in genetic restrictions may be a function of different mechanisms of TsF_3 interaction with target B or T_{cs} cells or differences resulting from in vivo injection vs in vitro addition of the factor. In either case, it should be noted that the apparent lack of Igh restriction for TsF_3 in the PFC system is the only difference observed to date between the suppressor–T cell pathways as described in the contact-sensitivity and plaque-forming cell systems.

As indicated above, in order to express suppressor activity Ts_3 cells require an additional activation step involving triggering with specific suppressor factors (TsF_2). We have identified two cloned hybridoma cell lines (termed pTs_3) that represent a mature but nonactivated stage in the differentiation of a primed

Annual Reviews

Ts_3 cell (114). These hybridoma cells contain cytoplasmic TsF_3 but do not constitutively secrete this factor. The pTs_3 hybridomas must be specifically activated with ascitic fluid containing TsF_2 in order to release their TsF_3.

Taniguchi and colleagues (120,121) have shown that a KLH-specific TsF_3-like factor consists of a molecular dimer composed of an I-J-bearing chain (28,000 daltons) and an antigen-binding chain. The latter piece may have a membrane (45,000 daltons) and secretory (35,000 daltons) form. We have performed studies to determine the molecular relationship of NP-specific TsF_3 with the suppressor factor described by Taniguchi et al. Thus, NP-specific TsF_3 was reduced with 5 mM DTT and then passed over NP-BSA or anti-I-J immunoabsorbent columns. The fractions were reconstituted in various combinations and then the reducing agent was removed by dialysis. Reduction of NP-specific TsF_3 does not destroy biological activity (105,122). The activity of the reduced TsF_3 could not be recovered in the eluate or filtrate of antigen or anti-I-J immunoabsorbent columns. However, as noted by Taniguchi et al (120), suppressive activity was restored by combining the appropriate filtrate and eluate fractions (105,122). The results are similar to those described by Taniguchi for KLH-specific TsF. The data imply a disulfide-linked heterodimer structure for TsF_3: One chain binds antigen and the other bears I-J-related determinants. To determine if the same class of TsF_3 molecules was responsible for suppression of PFC responses, some of these fractions were also tested for suppressive activity in vitro using NP-ficoll cultures. The data demonstrated that TsF_3 molecules with similar biophysical characteristics were responsible for suppression of both CS and humoral immune responses (112,122).

We have also analyzed the mechanisms responsible for the induction of I-J restrictions on Ts_3 cells in the CS system (74). The I-J phenotype of the antigen-coupled cells used for priming restricted the specificity of the Ts_3 population. Thus, Ts_3 cells were only generated after priming with antigen-coupled I-J homologous cells. Identity at the I-J (and I-E) subregions was sufficient for Ts_3 induction. Furthermore, priming of H-2 heterozygous mice with antigen-coupled parental cells generated Ts_3, which were restricted to the parental haplotype used for priming (74). The splenic cell population responsible for antigen presentation and induction of Ts_3 cells was fractionated. The cells involved in antigen presentation were Thy-1 negative and were primarily found in the firmly (24 hr) adherent, phagocytic, FcR^+ population of normal spleen (Table 2) or in schistosomiasis-induced liver granulomas (78). Ts_3-inducing cells were absent in the fraction containing splenic dendritic or non-adherent cells. Other studies have shown that the cells responsible for Ts_3 inductions are $I\text{-}A^-$, $I\text{-}J^+$, and sensitive to cyclophosphamide (75,81). Thus, the APC involved in Ts_3 induction can be distinguished from the APC involved in Ts_1 induction on the basis of their I-A phenotype and sensitivity to low-dose cyclophosphamide treatment (Table 2). Once Ts_3 cells are generated,

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their reactivation and interaction appear to be restricted by the I-J phenotype of the cell population used for Ts_3 priming (118).

Since TsF_3 is dual restricted and consists of a two-chain structure, we considered the possibility that each chain controls one of the genetic restrictions. Efforts physically to separate and reassociate the chains from different TsF sources have failed in other systems (123). Therefore, we used another approach to analyze this issue. Ts_3 cells from NP-primed (B6 × C3H)F_1 mice (which are heterozygous at both the H-2 and Igh complexes) were fused with the BW5147 thymoma, and four cloned lines were established (124). Supernatants containing TsF_3 from all four Ts_3 hybridomas suppressed NP-specific CS responses of (B6 × C3H)F_1 recipients. In addition, one of these TsF_3 factors suppressed C3H.SW (H-2^b; Igh^j) recipients but not B10.BR (H-2^k; Igh^b), C3H (H-2^k; Igh^j), or B6 (H-2^b; Igh^b) mice. Two of the F_1-derived TsF_3 suppressed B6 mice, while TsF_3 from the fourth clone suppressed C3H recipients but not the other inbred strains. Antigenic determinants present on the TsF_3 correlated with the genetic restrictions (Table 5). Thus, F_1-derived TsF_3 that suppressed H-2^b recipients reacted with anti-I-J^b but not with anti-I-J^k alloantisera. Similarly, those factors that suppressed Igh^b-bearing recipients reacted with anti-NP^b-idiotypic antisera, and the Igh^j-restricted F_1-derived TsF_3 factor reacted with an anti-NP^j-idiotypic antiserum (124). We conclude from these experiments that the Igh and H-2 determinants on TsF_3 are assorted independently in F_1 cells. This further supports the notion that TsF_3 consists of two independent polypeptide chains. Furthermore, we conclude that the molecular structures detected by the anti-I-J and anti-idiotypic antisera control the genetic restrictions of TsF_3 (124). Finally, the expression of these determinants on TsF_3 is clonally restricted and displays at least functional allelic exclusion.

Although we understand how I-J restrictions are induced and the molecular basis for these restrictions, at least for TsF_3, we still fail to understand the reasons for these restrictions and the nature of the cellular targets of TsF_3. One possibility is that an I-J-bearing antigen-presenting cell population is also the target of the I-J restriction. To test this possibility NP-coupled I-J-congenic

Table 5 The antigenic determinants on TsF_3 control the genetic restrictions

(B6 × C3H)F_1 hybridoma-derived TsF_3	Genetic restrictions	Binds immunoadsorbents containing antisera to
F_1-Ts_3-1032	I-J^b and Igh^b	I-J^b and NP^b
F_1-Ts_3-1114	H-2^b and Igh^b	not tested
F_1-Ts_3-1127	H-2^b and Igh^j	I-J^b and NP^j
F_1-Ts_3-1131	H-2^k and Igh^j	I-J^k and NP^j

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(3R or 5R) cells were used for elicitation of CS responses. We noted that the suppressive activity of TsF_3 was restricted by the I-J genotype of the antigen-presenting cells and not of the I-J genotype of the host (83,125). Thus, when NP-O-Su-primed $B6AF_1$ ($I\text{-}J^b/J\text{-}J^k$) mice are challenged with NP-coupled $B6AF_1$ cells the recipients developed CS responses. If these recipients also received either B6- or CKB-derived TsF_3, suppression of the CS response was observed (Table 6). In contrast, when 3R ($I\text{-}J^b$) NP-coupled cells were used for challenge, CS responses were still noted and were suppressed with B6 ($I\text{-}J^b$-restricted) TsF_3, but the suppression mediated by CKB ($I\text{-}J^k$-restricted) TsF_3 was not observed (Table 6). When $I\text{-}J^k$-bearing 5R NP-coupled cells were used for challenge, only CKB-derived TsF_3-mediated suppression was still observed (Table 6). These results strongly suggest that (*a*) appropriate antigen challenge is necessary to elicit suppression, (*b*) both antigen and I-J determinants must be recognized to manifest suppression, and (*c*) the I-J restriction is between the antigen-presenting cell used for elicitation and TsF_3.

The combined data demonstrate that I-J restrictions are involved in the induction, activation, and elicitation of suppressor-T cell responses. In addition, it was noted that anti-I-J alloantisera react with accessory cells, suppressor T cells, and T cell-derived suppressor factors. What do these anti-I-J reagents detect? The alloantisera are generally prepared by reciprocal immunization of 3R and 5R mice with unfractionated spleen cells. Generally, animals are hyperimmunized, and consequently the antisera may contain anti-I-J, anti-idiotype, and/or anti-receptor antibodies. It is not clear which of these activities define the various properties of the anti-I-J antibodies. It is noteworthy that in spite of the availability of monoclonal anti-I-J reagents and

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Table 6 TsF_3 requires interaction with two distinct targets[a]

Host	I-J restriction of TsF_3	Cells used for challenge	Percent suppression
$B6AF_1$	b	NP-$B6AF_1$	67
($I\text{-}J^b/I\text{-}J^k$)	k	NP-$B6AF_1$	69
	b	NP-3R	57
	k	NP-3R	1
	b	NP-5R	−2
	k	NP-5R	64
B6	b	NP-B6	77
(Igh^b)	b	NP-B.C-8	64
B.C-8	b	NP-B6	20
(Igh^a)	b	NP-B.C-8	6

[a]Hosts were primed with NP-O-Su. 24 hr later the mice were treated with 20 mg/kg cyclophosphamide. On days 5 and 6 the animals received either control BW5147-, $Bc\text{-}Ts_3$-8-, or $CKB\text{-}Ts_3$-3-derived ascitic fluid. On day 6 the mice were challenged with NP-coupled cells as indicated. The I-J or Igh genotypes of the hosts are indicated in parentheses. For additional details see reference 83.

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cloned cell lines that possess I-J determinants or release factors with such determinants, definitive characterization of the I-J molecule has not been achieved. Furthermore, molecular genetic attempts to identify the gene corresponding to the I-J region have been fruitless, especially the analysis of suppressor–T cell lines (126). The failure to identify the elusive I-J products has been most frustrating. Nonetheless the differences between the 3R and 5R strains define a polymorphism that allows functional description of the I-J region. The I-J product may represent a portion of the I-E_β chain, although other interpretations cannot be excluded (127). Furthermore, the relationship of the I-J determinants expressed on accessory cells with those detected on T cells requires further evaluation (128). The mysteries concerning I-J can also be extended to those systems in which T cell-specific I-A and/or I-C products are associated with suppressor-cell phenomena (66,82).

The target of the Igh restriction associated with TsF_3 activity is not an antigen-presenting cell. Thus, the Igh restriction specificity of TsF_3 need not match the Igh genotype of the antigen-coupled spleen cells used for challenge (83,125). However, for in vivo expression of TsF_3 activity the Igh genotype of the host must match the TsF_3 restriction specificity. Thus, when Igh-congenic B.C-8 (Igh^a) and B6 (Igh^b) mice were primed with NP-O-Su and then challenged with either NP-coupled B6 or NP-coupled B.C-8 spleen cells, both hapten-coupled cell types are able to elicit equal levels of footpad swelling. Following administration of B6-derived TsF_3, significant levels of suppression were noted in B6 (Igh^b) recipients but not in B.C-8 (Igh^a) recipients (Table 6). These results demonstrate that the Igh restriction exists between TsF_3 and cells in the recipient (83,125). At present we do not know the nature or function of the recipient cells involved in this restriction.

The antigen specificity of each of the cells and factors involved in the NP suppressor cell cascade has been demonstrated by the inability of suppressor cells and factors to suppress contact-sensitivity or plaque-forming cell responses to irrelevant antigens (DNFB or SRBC). In addition, we attempted to evaluate the ability of TsF_3 to mediate bystander suppression in the NP and ABA systems (83,104). For the former experiments, DTH was induced against allogeneic H-2 antigens. B10.BR ($H\text{-}2^k$) mice were primed with B6 ($H\text{-}2^b$) cells and then challenged with either uncoupled or NP-coupled B6 cells $\pm$ NP-specific TsF_3 (Table 7). Nonspecific suppression did not occur when TsF_3 was used to suppress these allo-DTH responses. However, when the mice were challenged with NP-coupled allogenic cells addition of TsF_3 caused marked suppression (Table 7). Additional experiments using similar protocols demonstrated that I-J restrictions existed between the haptenated antigen-presenting cells (APC) used for challenge and the TsF_3 (Table 7). In addition, the hapten had to be physically associated with the I-J-bearing APC, mixtures of uncoupled APC bearing an appropriate I-J genotype and NP-coupled I-J-

Annual Reviews

incompatible cells failed to elicit TsF_3-mediated suppression (Table 7). These results demonstrate that suppression can be mediated by TsF_3 provided that antigen and I-J are both present on the same APC population (83). Once these conditions are satisfied the final suppressive signals can result in bystander suppression of other ongoing T-cell responses.

Using the above system of bystander suppression, we have also analyzed the role of Igh-V and Igh-C genes in controlling the activity of TsF_3. C.B-20 (Igh-V^b,Igh-C^b), BAB/14 (Igh-V^a, Igh-C^b), and BALB/c (Igh-V^a,Igh-C^a) mice were primed with B6 cells and challenged with NP-coupled B6 cells as indicated previously. Following administration of B6 (Igh-V^b,Igh-C^b)-derived TsF_3, suppression was only noted in C.B-20 recipients (Table 7). These results indicate that Igh-V genes control the restriction between TsF_3 and the host. Furthermore, the Igh-V restrictions must involve an unprimed cell in the host, since in the above experiments the hosts were only primed with alloantigen and never received NP prior to TsF_3 administration (83).

ADDITIONAL CELLS OF THE SUPPRESSOR CASCADE

Studies of suppressive mechanisms regulating immune responses to contact-sensitizing agents such as picryl or oxazolone have shown that antigen-specific suppressor T cells and factors can also act indirectly through another subpopulation of T cells called T acceptor cells (Tacc) (85,87). Conventional TsF produced by effector Ts cells arm such Tacc. The armed Tacc subsequently liberate a nonspecific factor that inhibits the transfer of contact sensitivity or in vitro DNA synthesis following exposure to the antigen corresponding to the specificity of the TsF (85,87). To directly compare the cells and factors involved in these terminal suppressive events we exchanged reagents with Dr.

Table 7 Ability of TsF_3 to mediate bystander or cognate suppression[a]

Host	Priming	I-J restriction of TsF_3	Cells used for challenge	Percent suppression
B10.BR	B6	b	B6	-11
		b	NP-B6	73
B10.HTT	B6	b	3R	8
		b	NP-3R	100
		b	NP-5R	-18
		b	3R + NP-5R	-18
BALB/c	B6	b	NP-B6	1
C.B-20	B6	b	NP-B6	39
BAB/14	B6	b	NP-B6	9

[a]Hosts were primed 3×10^7 allogeneic Bc (H-2^b) spleen cells. 24 hrs later the mice were given 20 mg/kg cyclophosphamide, i.p. After 6 days, the mice were challenged with 10^7 uncoupled or NP-coupled spleen cells. B6-derived TsF_3 was given on the day before and the day of antigen challenge. Refer to reference 83 for additional details.

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Asherson and his associates. The resulting experiments determined common points in the suppressor-T cell cascade between the NP and TNP or oxazolone systems (129). Thus, the TsF in the TNP system corresponded to monoclonal TsF_3 in the NP system. The data demonstrated that both factors can act through a Tacc cell that generates nonspecific inhibitors (129). In both cases, the Tacc cell is sensitive to cyclophosphamide and adult thymectomy. In addition, the interaction of antigen and MHC with the TsF_3 on the surface of the Tacc is genetically restricted, and the restriction maps to the I-J subregion (129). It appears that accessory cells are the target of this I-J restriction. Further evidence for the equivalence of NP-specific TsF_3 and conventional oxazolone-specific TsF was provided by experiments in which the T acceptor cell was armed by both factors. The release of nonspecific inhibitor was then triggered by the mixed hapten NP-oxazolone-lysine, which is univalent in respect to both haptens (129). This finding suggests that cross-linking of molecules of oxazolone- and NP-specific TsF_3 were required for suppression [see (130) for further discussion].

The combined observations indicate the equivalence of the suppressor factors involved in these well-characterized systems. They directly demonstrate that the factors have identical functional properties in terms of their ability to arm the T acceptor cell. These properties are consistent with other data demonstrating that these antigen-specific suppressor factors have similar properties, including I-J determinants, antigen-binding sites, susceptibility to dithioerythrital, and action at the expression stage of the immune response (105,130). The data further suggest that a fourth T-cell population may be involved in the NP-suppressor circuit. Thus, it appears TsF_3 can either act through multiple mechanisms involving Tacc or, as in the PFC system, without involvement of additional T cells.

It should be noted that there are no apparent Igh genetic restrictions when NP-specific TsF_3 are used to arm Tacc (129). This finding is consistent with the above observations indicating that the target of the Igh restriction is distinct.

Whether the initial phases of the suppressor-cell cascade are also equivalent in the two systems is unresolved. However, several studies have indicated homologies between the afferent suppressors of the TNP system and Ts_1 (131). Recent data also suggest that ABA-specific suppressor factor may be the equivalent of the inducer-suppressor factor described by Gershon and his associates (M. I. Greene, unpublished data).

Igh–V RESTRICTIONS AND MOLECULAR ORGANIZATION OF TsF

The presence of idiotypic specificities detected serologically on T-cell and T cell–suppressor factors, with reagents against B-cell idiotypes, has presented a considerable challenge, in the light of: (*a*) the lack of evidence of immu-

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noglobulin gene rearrangement in T cells, and (*b*) the realization that the specificities expressed on T cells differ significantly from the classical B-cell idiotypes, in those systems where they have been compared. Nevertheless, it has been clearly demonstrated in both the extensively studied ABA and NP systems that Igh genes impose certain restrictions in the interactions particularly of Ts_1 and Ts_2 cells and factors, and of Ts_2 with Ts_3 cells, in vivo (125).

Therefore, we concluded that the Igh-controlled idiotypic specificities detected on T cells and their factors were the result of the special ability of suppressor T cells to develop repertoires directed to reactivity with the immunoglobulins they are often meant to regulate. If this were indeed the case, and if T cells were selected with receptors active with class-II MHC antigens and/or immunoglobulin idiotypic specificities, then, according to a variant of the Jerne idiotypic network hypothesis (132), such receptors (which need not be coded by Igh genes) might also express B-cell cross-reactive idiotypes as observed in the many systems where they have been detected.

Using both the ABA and NP systems, we have begun to explore whether the expression of T-cell idiotypic restriction on suppressor T cells is controlled by the genotype of the B cells and not of the T cells. Preliminary results, to be reported elsewhere, agree with this hypothesis. The purpose of such internal images of idiotypes might be to permit regulatory network interactions to proceed among the various T- and B-cell elements during the antigen-specific immune response, even when the concentration of antigen is limiting. Since as stated earlier such mimicry molecules on T cells need not be encoded by Igh genes, this hypothesis can account for the expression of idiotype-like determinants on Ts cells without the rearrangement of Igh genes in these T cells. Furthermore, it can account for the ability of idiotype or anti-idiotype to induce suppressor T cells in a variety of experimental systems and the apparent idiotype specificity in these suppressor-cell systems. As previously noted, Igh restrictions of TsF_3 are not always critical under selected in vitro conditions. This suggests that the Igh restrictions may be related to interactions with anti-idiotypic elements under in vivo conditions. The identification and characterization of these elements are vital to understanding the physiological interactions that occur among lymphocytes under in vivo conditions. Efforts are under way to explore these interactions.

Ultimately one must define the T-cell receptor in molecular terms. Recently, considerable progress has been made in defining clonotypic markers on clones of both human and murine T-cell lines (133-135). Monoclonal antibodies have been prepared to these products. These antibodies presumably detect antigen-specific receptors on proliferating, IL-2 releasing, or killer T-cell clones. Under nonreducing conditions the antibodies specifically precipitate molecules of approximately 80,000 daltons. Reducing conditions identify two molecules of approximately 40,000-45,000 daltons (133-135). These data suggest that the

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T-cell receptor on these antigen-specific clones is a disulfide-linked heterodimer. At least one and probably both of the molecular chains display clonal heterogeneity (133). Similar studies have not yet been extended to clonotypic determinants of suppressor T cells. However, several previous reports suggested that the soluble suppressor factors have a similar overall structure. Thus, as indicated previously, TsF_3 factors of the KLH, NP, and other systems also exist as disulfide-linked heterodimers (120,123,129). The antigen-binding chain has been shown to be 40,000-45,000 daltons while the I-J chain appears smaller, approximately 20,000-25,000 daltons. TsF_3 may also exist as a noncovalently associated molecular complex, since other reports have identified noncovalently linked forms with similar functional properties (33).

The relationship between the TsF_3-like factors and those derived from Ts_1-like cells is not clear. Some reports have indicated that TsF_1-like inducer factors are also disulfide-linked heterodimers or noncovalently linked heterodimer structures (123). However, other reports have described a TsF_1-inducer factor that appears to consist of a single antigen-binding, I-J-bearing glycoprotein chain of 18,000-28,000 daltons (136,137). It is possible that a second chain is provided elsewhere in the system, thereby bringing this example in line with other observations. Although it is tempting to extend the homology between the clonotypic molecules identified in various antigen-specific clones with the factors derived from suppressor cells, such speculations on homology are still without experimental support. This will undoubtedly be a topic of extensive investigation as additional reagents and molecular probes become available.

SUMMARY

We have described a model system of immunoregulation in which gene products associated with both the major histocompatibility complex and the heavy-chain immunoglobulin gene complex guide a series of cellular interactions. The Igh genetic restrictions may represent the use of internal images of antigen and idiotype as suppressor–T cell receptors. The data indicate that the T-cell and B-cell Igh products are distinct. The T cell–derived idiotype-like determinants are used for suppressor–T cell communications.

The MHC restrictions generally involve the I-J subregion. These restrictions are imposed by the presentation of antigen or suppressor factor by specialized populations of I-J-bearing accessory cells. The role of MHC products in the induction of suppressor cells has several homologies with the mechanisms responsible for the induction of H-2-restricted helper cells. First, I-region products on specialized presenting cells determine the specificity and genetic restrictions of the T cells. Thus, recognition of antigen in the context of I-A and I-E products is required for helper-T cell induction, and similarly the various suppressor–T cell subsets recognize antigen or suppressor factor pre-

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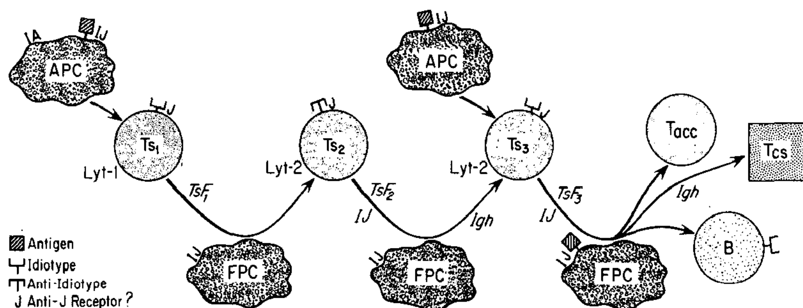

Figure 1 A summary of the NP suppressor-cell cascade. Intravenous administration of NP on I-A$^+$, I-J$^+$ APC induces an Lyt-1$^+$ Ts_1 population. Ts_1 release a factor (TsF_1) that is presented on I-J-bearing factor-presenting cells (FPC) to induce Lyt-2$^+$ Ts_2. In turn, Ts_2 releases TsF_2, which is also presented by FPC. The TsF_2 activates previously primed Ts_3 cells in an I-J- and Igh-restricted fashion. Ts_3 are generated by antigen priming on I-J$^+$ APC. Once Ts_3 cells are activated, they release TsF_3, which, after binding antigen on an I-J$^+$ FPC, mediates nonspecific suppression of CS responses, either directly or via T acceptor cells (Tacc). In the CS system TsF_3 is dually restricted: The target of the I-J restriction is the FPC and the target of the Igh restriction resides in the host. Igh restrictions are not required between TsF_3 and Tacc or B cells. The receptors on the Ts have specificity for antigen or idiotype as indicated. Furthermore, the Ts appear to have anti-self I-J receptors.

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sented in the context of I-J subregion-encoded antigens. Furthermore, the data suggest that the suppressor cells bear receptors for self I-J products. As an additional analogy between suppressor and helper cells, we have shown that in H-2-heterozygous F_1 animals at least two populations of suppressor cells can be induced, one specific for each parental H-2 haplotype.

The NP and ABA suppressor-cell pathways consist of multiple cellular elements, including at least three and possibly four distinct T-cell populations and two or more distinctive accessory cell populations. These are summarized in Figure 1. The specific soluble suppressor factors produced by each suppressor–T cell subset are involved in cellular communication processes. The terminal phases of the suppressor-cell cascade are antigen dependent but involve nonspecific bystander effects. In this review we have indicated the numerous homologies between the data in the hapten systems studied in our laboratories and the various other suppressor-cell models previously described in the literature.

Literature Cited

1. Jandinski, J., Cantor, H., Tadakuma, T., Peavy, D. L., Pierce, C. W. 1976. Separation of helper T cells from suppressor T cells expressing different Ly components. I. Polyclonal activation: Suppressor and helper activities are inherent properties of distinct T-cell subclasses. *J. Exp. Med.* 143:1382–90
2. Katz, D. H., Benacerraf, B. 1972. The regulatory influence of activated T cells on B cell responses to antigens. *Adv. Immunol.* 15:1–94
3. Rabourdin-Combe, C., Dorf, M. E., Guimezanes, A., Fridman, W. H. 1979. T cell produced immunoglobulin binding factor (IBF) bears determinants coded for

Annual Reviews

by the I region of the major histocompatibility complex and lacks allogeneic restriction. *Eur. J. Immunol.* 9:237–42

4. Gilbert, K. M., Hoffmann, M. K. 1983. Suppressor B lymphocytes. *Immunol. Today* 4:253–55
5. Allison, A. C. 1978. Mechanisms by which activated macrophages inhibit lymphocyte responses. *Immunol. Rev.* 40:3–27
6. Kirchner, H., Holden, H. T., Herberman, R. B. 1975. Splenic suppressor macrophages induced in mice by injection of *Corynebacterium parvum*. *J. Immunol.* 115:1212–16
7. Ptak, W., Zembala, M., Asherson, G. L., Marcinkiewicz, J. 1981. Inhibition of contact sensitivity by macrophages. The separate phenomena of the production of nonspecific macrophage suppressor factor by macrophages armed with specific T suppressor factor, and the nonspecific inhibition of passive transfer by high density macrophages. *Int. Arch. Allergy Appl. Immunol.* 65:121–28
8. Gershon, R. K., Kondo, K. 1970. Cell interactions in the induction of tolerance. *Immunology* 18:723–37
9. Abbas, A. K., Takaoki, M., Greene, M. I. 1982. T lymphocyte-mediated suppression of myeloma function in vitro. IV. Generation of effector suppressor cells specific for myeloma idiotypes. *J. Exp. Med.* 155:1216–21
10. Braley-Mullen, H. 1980. Direct demonstration of specific suppressor T cells in mice tolerant to type III pneumococcal polysaccharide: Two-step requirement for development of detectable suppressor cells. *J. Immunol.* 125:1849–54
11. Rohrer, J. W., Odermatt, B., Lynch, R. G. 1979. Immunoregulation of murine myeloma: isologous immunization with M315 induces idiotype-specific T cells that suppress IgA secretion by MOPC-315 cells in vivo. *J. Immunol.* 122:2011–19
12. Sherr, D. H., Onyon, M. J., Dorf, M. E. 1984. Role of idiotypes in the 4-hydroxy-3-nitrophenyl acetyl (NP) specific suppressor T cell pathway. In *Idiotypes—Structure and Function*, ed A. Nisonoff, M. I. Greene. NY: Plenum. In press
13. Suemura, M., Ishizaka, A., Kobatake, S., Sugimura, K., Maeda, K., Nakanishi, K., Kishimoto, S., Yamamura, Y., Kishimoto, T. 1983. Inhibition of IgE production in B hybridomas by IgE class-specific suppressor factor from T hybridomas. *J. Immunol.* 130:1056–60
14. Sherr, D. H., Cheung, N-K. V., Heghinian, K. M., Benacerraf, B., Dorf, M. E. 1979. Immune suppression *in vivo* with antigen-modified syngeneic cells. II. T-cell mediated nonresponsiveness to fowl gamma globulin. *J. Immunol.* 122:1899–1904
15. Tada, T., Okumura, K. 1980. The role of antigen specific T cell factors in the immune response. *Adv. Immunol.* 28:1–87
16. Bach, B. A., Sherman, L., Benacerraf, B., Greene, M. I. 1978. Mechanisms of regulation of cell-mediated immunity. II. Induction and suppression of delayed-type hypersensitivity to azobenzenearsonate coupled syngeneic cells. *J. Immunol.* 121:1460–68
17. Weinberger, J. A., Germain, R. N., Ju, S. T., Greene, M. I., Benacerraf, B., Dorf, M. E. 1979. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. *J. Exp. Med.* 150:761–76
18. Asherson, G. L., Zembala, M. 1974. Suppression of contact sensitivity by T cells in the mouse. I. Demonstration that suppressor cells act on the effector phase of contact sensitivity; and their induction following in vitro exposure to antigen. *Proc. R. Soc. Lond. Ser. B* 187:329–48
19. Battisto, J. R., Bloom, R. B. 1966. Dual immunological unresponsiveness induced by cell membrane coupled hapten or antigen. *Nature* 212:156–57
20. Finberg, R., Burakoff, S. J., Benacerraf, B., Greene, M. I. 1979. The cytolytic T lymphocyte response to trinitrophenyl-modified syngeneic cells. II. Evidence for antigen-specific suppressor T cells. *J. Immunol.* 123:1210–14
21. Greene, M. I., Ratnofsky, S., Takaoki, M., Sy, M. S., Burakoff, S., Finberg, R. W. 1982. Antigen-specific suppression of cytotoxic T cell responses: An idiotype-bearing factor regulates the cytotoxic T cell response to azobenzenearsonate-coupled cells. *J. Immunol.* 128:1188–91
22. Araneo, B. A., Kapp, J. A. 1981. Genetic analysis of immune suppression. I. Gene complementation is required for suppression of antigen-specific proliferative responses by T-cell derived factors. *Immunogenetics* 14:221–30
23. Baxevanis, C. N., Ishii, N., Nagy, Z. A., Klein, J. 1982. H-2-controlled suppression of T cell response to lactate dehydrogenase B: Characterization of the lactate dehydrogenase B suppressor pathway. *J. Exp. Med.* 156:822–33
24. Fujimoto, S., Greene, M. I., Sehon, A. H. 1976. Regulation of the immune response to tumor antigens. I. Immuno-

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Annual Reviews

suppressor cells in tumor-bearing hosts. *J. Immunol.* 116:791–800
25. Yamauchi, K., Fujimoto, S., Tada, T. 1979. Differential activation of cytotoxic and suppressor T cells against syngeneic tumors in the mouse. *J. Immunol.* 123:1653–58
26. Arnon, R. 1981. Experimental allergic encephalomyelitis—susceptibility and suppression. *Immunol. Rev.* 55:5–30
27. Doughty, B. L., Phillips, S. M. 1982. Delayed hypersensitivity granuloma formation and modulation around *Schistosoma mansoni* eggs in vitro. II. Regulatory T cell subsets. *J. Immunol.* 128:37–42
28. Rose, N. R., Kong, Y. C. M., Okayasu, I., Giraldo, A. A., Beisel, K., Sundick, R. S. 1981. T-cell regulation in autoimmune thyroiditis. *Immunol. Rev.* 55:299–314
29. Lanier, L. L., Engleman, E. G., Gatenby, P., Babcock, G. F., Warner, N. L., Herzenberg, L. A. 1983. Correlation of functional properties of human lymphoid cell subsets and surface marker phenotypes using multiparameter analysis and flow cytometry. *Immunol. Rev.* 74:143–60
30. Meuer, S. C., Cooper, D. A., Hodgdon, J. C., Hussey, R. E., Morimoto, C., Schlossman, S. F., Reinherz, E. L. 1983. Immunoregulatory human T lymphocytes triggered as a consequence of viral infection: Clonal analysis of helper, suppressor inducer and suppressor effector cell populations. *J. Immunol.* 131:1167–72
31. Nishimura, Y., Sasazuki, T. 1983. Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man. *Nature* 302:67–69
32. Germain, R. N., Benacerraf, B. 1981. A single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. *Scand. J. Immunol.* 13:1–10
33. Green, D. R., Flood, P. M., Gershon, R. K. 1983. Immunoregulatory T-cell pathways. *Ann. Rev. Immunol.* 1:439–63
34. Sunday, M. E., Benacerraf, B., Dorf, M. E. 1981. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. *J. Exp. Med.* 153:811–22
35. Benacerraf, B., Germain, R. N. 1979. Specific suppressor responses to antigen under I region control. *Fed. Proc.* 38:2053–57
36. Claman, H. N., Miller, S. D., Sy, M-S., Moorhead, J. W. 1980. Suppressive mechanisms involving sensitization and tolerance in contact allergy. *Immunol. Rev.* 50:105–32
37. Yamauchi, K., Murphy, D., Cantor, H., Gershon, R. 1981 Analysis of antigen-specific, Ig-restricted cell-free material made by I-J$^+$ Ly-1 cells (Ly-1 $T_{si}F$) that induces Ly-2$^+$ cells to express suppressive activity. *Eur. J. Immunol.* 11:905–12
38. Asano, Y., Hodes, R. J. 1983. T cell regulation of B cell inactivation. I-A restricted T suppressor cells inhibit the major histocompatibility complex-restricted interactions of T helper cells with B cells and accessory cells. *J. Exp. Med.* 157:1867–84
39. Sugimura, K., Kishimoto, T., Maeda, K., Yamamura, Y. 1981. Demonstration of T15 idiotype-positive effector and suppressor T cells for phosphorylcholine-specific delayed-type hypersensitivity response in CBA/N or (CBA/N × BALB/c)F_1 male mice. *Eur. J. Immunol.* 11:454–61
40. Sy, M. S., Miller, S. D., Moorhead, J. W., Claman, H. N. 1979. Active suppression of 1-fluoro-2,4-dinitrobenzene-immune T cells. Requirement of an auxiliary T cell induced by antigen. *J. Exp. Med.* 149:1197–1207
41. Dietz, M. H., Sy, M. S., Benacerraf, B., Nisonoff, A., Greene, M. I., Germain, R. N. 1981. Antigen and receptor driven regulatory mechanisms. VII. H-2 restricted anti-idiotypic suppressor factor from efferent suppressor T cells. *J. Exp. Med.* 153:450–63
42. Weinberger, J. Z., Germain, R. N., Benacerraf, B., Dorf, M. E. 1980. Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenyl acetyl. V. Role of idiotypes in the suppressor pathway. *J. Exp. Med.* 152:161–69
43. Lewis, G. K., Goodman, J. W. 1978. Purification of functional determinant specific idiotype-bearing murine T cells. *J. Exp. Med.* 148:915–24
44. Sherr, D. H., Dorf, M. E. 1982. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XIII. Characterization of a third order T cell (Ts_3) involved in suppression of in vitro PFC responses. *J. Immunol.* 128:1261–66
45. Taniguchi, M., Miller, J. F. A. P. 1977. Enrichment of specific suppressor T cells and characterization of their surface markers. *J. Exp. Med.* 146:1450–54
46. Cramer, M., Krawinkel, U., Melchers, I., Imanishi-Kari, T., Ben-Neriah, Y., Givol, D., Rajewski, K. 1979. Isolated hapten-binding receptors of sensitized lymphocytes. IV. Expression of immu-

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noglobulin variable regions in (4-hydroxy-3-nitrophenyl) acetyl (NP)-specific receptors isolated from murine B and T lymphocytes. *Eur. J. Immunol.* 9:332–38

47. Kronenberg, M., Kraig, E., Siu, G., Kapp, J. A., Kappler, J., Marrack, P., Pierce, C. W., Hood, L. 1983. Three T-cell hybridomas do not contain detectable heavy chain variable gene transcripts. *J. Exp. Med.* 158:210–27
48. Nakanishi, K., Sugimura, K., Yaunta, Y., Maeda, K., Kashiwamura, S. I., Honjo, T., Kishimoto, T. 1982. A T15-idiotype-positive T suppressor hybridoma does not use the T15 V_H gene segment. *Proc. Natl. Acad. Sci. USA* 79:6984–88
49. Okuda, K., Minami, M., Ju, S-T., Dorf, M. E. 1981. Functional association of idiotypic and I-J determinants on the antigen receptor of suppressor T cells. *Proc. Natl. Acad. Sci. USA* 78:4557–61
50. Germain, R. N., Ju, S-T., Kipps, T. J., Benacerraf, B., Dorf, M. E. 1979. Shared idiotypic determinants on antibodies and T cell derived suppressor factor specific for the random terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}. *J. Exp. Med.* 149:613–22
51. Harvey, M. A., Adorini, L., Miller, A., Sercarz, E. E. 1979. Lysozyme-induced T-suppressor cells and antibodies have a predominant idiotype. *Nature* 281:594–96
52. Hirai, Y., Nisonoff, A. 1980. Selective suppression of the major idiotypic component of an anti-hapten response by soluble T cell–derived factors with idiotypic or anti-idiotypic receptors. *J. Exp. Med.* 151:1213–31
53. Kapp, J. A., Araneo, B. A., Ju, S-T., Dorf, M. E. 1981. Immunogenetics of monoclonal suppressor T cell products. In *Immunoglobulin Idiotypes*, ed. C. Janeway, E. Sercarz, H. Wigzel, pp. 387–96. New York: Academic
54. Gershon, R. K., Maurer, P. H., Merryman, C. F. 1973. A cellular basis for genetically controlled immunologic-unresponsiveness in mice: tolerance induction in T cells. *Proc. Natl. Acad. Sci. USA* 70:250–54
55. Kapp, J. A., Pierce, C. W., Schlossman, S., Benacerraf, B. 1974. Genetic control of immune response *in vitro*. V. Stimulation of suppressor T cells in nonresponder mice by the terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *J. Exp. Med.* 140:648–59
56. Dorf, M. E., Plate, J. M. D., Stimpfling, J. H., Benacerraf, B. 1975. Characterization of immune response and mixed leukocyte reactions in selected intra-H-2 recombinant strains. *J. Immunol.* 114:602–5
57. Kapp, J. A., Pierce, C. W., Benacerraf, B. 1974. Genetic control of immune responses in vitro. III. Tolerogenic properties of the terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}(GAT) for spleen cells from nonresponder H-2^s and H-2^q)mice. *J. Exp. Med.* 140:172–84
58. Benacerraf, B., Dorf, M. E. 1976. Genetic control of specific immune responses and immune suppression by I-region genes. *Cold Spring Harbor Symp. Quant. Biol.* 41:465–75
59. Debre, P., Kapp, J. A., Dorf, M. E., Benacerraf, B. 1975. Genetic control of specific immune suppression. II. H-2 linked dominant genetic control of immune suppression by the random copolymer L-glutamic acid^{50}-L-tyrosine^{50} (GT). *J. Exp. Med.* 142:1447–54
60. Murphy, D. B. 1978. The I-J subregion of the murine H-2 gene complex. *Springer Sem. Immunopathol.* 1:111–31
61. Okumura, K., Herzenberg, L. A., Murphy, D. B., McDevitt, H. O., Herzenberg, L. A. 1976. Selective expression of H-2 (I-region) loci controlling determinants on helper and suppressor T lymphocytes. *J. Exp. Med.* 144:685–98
62. Tada, T., Taniguchi, M., David, C. S. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* 144:713–25
63. Thèze, J., Waltenbaugh, C., Dorf, M. E., Benacerraf, B. 1977. Immunosuppressive factor(s) specific for L-glutamic acid^{50}-L-tyrosine^{50} (GT). II. Presence of I-J determinants on the GT suppressive factor. *J. Exp. Med.* 146:287–92
64. Chensue, S. W., Boros, D. L., David, C. S. 1983. Regulation of granulomatous inflammation in murine schistosomiasis. II. T suppressor cell-derived, I-C subregion-encoded soluble suppressor factor mediates regulation of lymphokine production. *J. Exp. Med.* 157:219–30
65. Liew, F. Y., Sia, D. Y., Parish, C. R., McKenzie, I. F. C. 1980. Major histocompatibility gene complex (MHC)-coded determinants on antigen-specific suppressor factor for delayed-type hypersensitivity and surface phenotypes of cells producing the factor. *Eur. J. Immunol.* 10:305–9
66. Rich, S. S., David, C. S., Rich, R. R. 1979. Regulatory mechanisms in cell-

Annual Reviews

mediated immune responses. VII. Presence of I-C subregion determinants on mixed leukocyte reaction suppressor factor. *J. Exp. Med.* 149:114–26
67. Basten, A., Miller, J. F. A. P., Johnson, P. 1975. T-cell-dependent suppression of an anti-hapten antibody response. *Transplant. Rev.* 26:130–48
68. Frei, P. C., Benacerraf, B., Thorbecke, G. J. 1965. Phagocytosis of the antigen, a crucial step in the induction of the primary response. *Proc. Natl. Acad. Sci. USA* 53:20–23
69. Weigle, W. O., Sieckmann, D. G., Doyle, M. U., Chiller, J. M. 1975. Possible roles of suppressor cells in immunologic tolerance. *Transplant. Rev.* 26:186–205
70. Daynes, R. A., Spellman, C. W. 1977. Evidence for the generation of suppressor cells by ultraviolent radiation. *Cell. Immunol.* 31:182–87
71. Greene, M. I., Sy, M-S., Kripke, M., Benacerraf, B. 1979. Impairment of antigen-presenting cell function by ultraviolet radiation. *Proc. Natl. Acad. Sci. USA* 76:6591–95
72. Spellman, C. W., Daynes, R. A. 1978. Ultraviolet light induced murine suppressor lymphocytes dictate specificity of anti-ultraviolet tumor immune responses. *Cell. Immunol.* 38:25–34
73. Czitrom, A. A., Sunshine, G. H., Mitchison, N. A. 1980. Suppression of the proliferative response to H-2D by I-J subregion gene products. *Immunogenetics* 11:97–102
74. Minami, M., Honji, N., Dorf, M. E. 1982. The mechanism responsible for the induction of I-J restrictions on Ts_3 suppressor cells. *J. Exp. Med.* 156:1502–15
75. Nakamura, R. M., Tanaka, H., Tokunaga, T. 1982. In vitro induction of suppressor T cells in delayed-type hypersensitivity to BCG and an essential role of I-J positive accessory cells. *Immunol. Lett.* 4:295–99
76. Sherr, D. H., Heghinian, K. M., Benacerraf, B., Dorf, M. E. 1980. Immune suppression in vivo with antigen-modified syngeneic cells. IV. Requirement for Ia^+ adherent cells for induction. *J. Immunol.* 124:1389–95
77. Zembala, M., Asherson, G. L., Colizzi, V. 1982. Hapten-specific T suppressor factor recognized both hapten and I-J region products on haptenized spleen cells. *Nature* 297:411–13
78. Sunday, M. E., Stadecker, M. J., Wright, J. A., Aoki, I., Dorf, M. E. 1983. Induction of immune responses by schistosome granuloma macrophages. *J. Immunol.* 130:2413–17
79. Usui, M., Aoki, I., Dorf, M. E. 1984. A role for antigen presenting cells in suppressor cell induction. *J. Immunol.* In press
80. Aoki, I., Minami, M., Dorf, M. E. 1983. A mechanism responsible for the induction of H-2 restricted second order suppressor T cells. *J. Exp. Med.* 157:1726–35
81. Lowy, A., Tominaga, A., Drebin, J. A., Takaoki, M., Benacerraf, B., Greene, M. I. 1983. Identification of an $I\text{-}J^+$ antigen-presenting cell required for third order suppressor cell activation. *J. Exp. Med.* 157:353–58
82. Ikezawa, Z., Baxevanis, C. N., Nonaka, M., Abe, R., Tada, T., Nagy, Z. A., Klein, J. 1983. Monoclonal suppressor factor specific for lactate dehydrogenase B. I. Mechanism of interaction between the factor and its target cells. *J. Exp. Med.* 157:1855–66
83. Aoki, I., Usui, M., Minami, M., Dorf, M. E. 1984. Activity of a genetically restricted suppressor factor requires interactions with two distinct targets. *J. Immunol.* In press
84. Fresno, M., Mcvay-Boudreau, L., Cantor, H. 1982. Antigen-specific T lymphocyte clones. III. Papain splits purified T suppressor molecules into two functional domains. *J. Exp. Med.* 155:981–93
85. Malkovsky, M., Asherson, G. L., Chandler, P., Colizzi, V., Watkins, M. C., Zembala, M. 1983. Nonspecific inhibitor of DNA synthesis elaborated by T acceptor cells. I. Specific hapten- and I-J-driven liberation of an inhibitor of cell proliferation by $Lyt\text{-}1^-2^+$ cyclophosphamide-sensitive T acceptor cells armed with a product of $Lyt\text{-}1^+2^+$-specific suppressor cells. *J. Immunol.* 130:785–90
86. Taniguchi, M., Tokuhisa, T. 1980. Cellular consequences in the suppression of antibody response by the antigen-specific T cell factor. *J. Exp. Med.* 151:517–27
87. Zembala, M. A., Asherson, G. L., James, B. M. B., Stein, V. E., Watkins, M. C. 1982. Anti-haptene T suppressor factor acts through an $I\text{-}J^+$, $Ly1^-2^+$, T acceptor cell that releases a nonspecific inhibitor of the transfer of contact sensitivity when exposed to antigen. *J. Immunol.* 129:1823–29
88. Asano, Y., Hodes, R. J. 1983. T cell regulation of B cell activation: Antigen-specific and antigen-nonspecific suppressor pathways are mediated by distinct T cell subpopulations. *J. Immunol.* 130:1061–65
89. Goodman, J. W., Sercarz, E. 1983. The complexity of structures involved in T-cell activation. *Ann. Rev. Immunol.* 1:465–98

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Annual Reviews

90. Benacerraf, B., Kapp, J. A., Debre, P., Pierce, C. W., DelaCroix, F. 1975. The stimulation of specific suppressor T cell in genetic nonresponder mice by linear random copolymers of L-amino acids. *Transplant. Rev.* 26:21–38
91. Germain, R. N., Theze, J., Kapp, J. A., Benacerraf, B. 1978. Antigen-specific T-cell-mediated suppression. I. Induction of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} specific suppressor T cells in vitro requires both antigen-specific T-cell-suppressor factor and antigen. *J. Exp. Med.* 147:123–36
92. Binz, H., Wigzell, H. 1977. Antigen-binding idiotypic T-lymphocyte receptors. *Contemp. Top. Immunol.* 7:113–77
93. Bona, C., Paul, W. E. 1979. Cellular basis of regulation of expression of idiotype. I. T-suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-trinitrophenyl antibodies bearing 460 idiotype. *J. Exp. Med.* 149:592-600.
94. Cosenza, J., Julius, M. H., Augustin, A. A. 1977. Idiotypes as variable region markers: analogues between receptors on phosphorylcholine specific T and B lymphocytes. *Immunol. Rev.* 34:3–33
95. Eichmann, K. 1974. Idiotype suppression. I. Influence of the dose and of the effector functions of anti-idiotype antibody on the production of an idiotype. *Eur. J. Immunol.* 4:296–302
96. Nisonoff, A., Ju, S-T., Owen, F. L. 1977. Studies of structure and immunosuppression of a cross-reactive idiotype in strain A mice. *Immunol. Rev.* 34:89–118
97. Ju, S-T., Benacerraf, B., Dorf, M. E. 1978. Idiotypic analysis of antibodies to poly(Glu^{60}Ala^{30}Tyr^{10}): Interstrain and interspecies idiotypic cross-reactions. *Proc. Natl. Acad. Sci. USA* 75:6192–96
98. Ju, S-T., Dorf, M. E. 1981. Idiotypic analysis of anti-GAT antibodies. IX. Genetic mapping of the Gte idiotypic markers within the Igh–V locus. *J. Immunol.* 126:183–86
99. Jack, R. S., Imanishi-Kari, T., Rajewski, K. 1977. Idiotypic analysis of the response of C57BL/6 mice to the (4-hydroxy-3 nitrophenyl) acetyl group. *Eur. J. Immunol.* 7:559–65
100. Makela, O., Karjalainen, K. 1977. Inherited Ig idiotypes of the mouse. *Immunol. Rev.* 34:119–38
101. Sunday, M. E., Weinberger, J. Z., Benacerraf, B., Dorf, M. E. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. IV. Specificity of cutaneous sensitivity responses. *J. Immunol.* 125:1601–5
102. Sherr, D. H., Ju, S-T., Weinberger, J. Z., Benacerraf, B., Dorf, M. E. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VII. Idiotype specific suppression of plaque forming cell responses. *J. Exp. Med.* 153:640–52
103. Sherr, D. H., Dorf, M. E. 1981. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. IX. Characterization of idiotype specific effector phase suppressor cells on plaque forming cell responses in vitro. *J. Exp. Med.* 153:1445–56
104. Benacerraf, B., Greene, M. I., Sy, M. S., Dorf, M. E. 1982. Suppressor T cell circuits. *Ann. N.Y. Acad. Sci.* 392:300–8
105. Dorf, M. E., Minami, M., Usui, M., Aoki, I. 1984. The NP suppressor cell cascade. In *Progress in Immunology*, ed. T. Tada, Volume 5. NY: Academic. In press
106. Sy, M-S., Brown, A., Bach, B. A., Benacerraf, B., Gottlieb, P., Nisonoff, A., Greene, M. I. 1980. Genetic and serological analysis of the expression of cross-reactive idiotypic determinants on anti-p-azobenzenearsonate antibodies and p-azobenzenearsonate specific suppressor T cell factors. *Proc. Natl. Acad. Sci. USA* 78:1143–47
107. Miller, S. D., Sy, M-S., Claman, H. N. 1977. The induction of hapten-specific T cell tolerance using hapten-modified lymphoid membranes. II. Relative roles of suppressor T cells and clone inhibition in the tolerant state. *Eur. J. Immunol.* 7:165–70
108. Taniguchi, N., Usui, M., Okuda, K., Matuhasi, T. 1982. Hapten-Pullulan conjugate-induced CMI suppression: Demonstration of a common pathway of suppressor cells involving idiotypic interactions. *J. Immunol.* 129:1816–22
109. Whitaker, R. B., Nepom, J. T., Sy, M-S., Takaoki, M., Gramm, C. F., Fox, I., Germain, R. N., Nisonoff, A., Greene, M. I., Benacerraf, B. 1981. Suppressor factor from a T cell hybrid inhibits delayed type hypersensitivity responses to azobenzenearsonate (ABA). *Proc. Natl. Acad. Sci. USA* 78:6441–45
110. Okuda, K., Minami, M., Sherr, D. H., Dorf, M. E. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XI. Pseudogenetic restrictions of hybridoma suppressor factors. *J. Exp. Med.* 154:468–79
111. Dorf, M. E., Okuda, K., Minami, M. 1982. Dissection of a suppressor cell cascade. *Cur. Top. Microbiol. Immunol.* 100:61–67

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112. Sherr, D. H., Minami, M., Okuda, K., Dorf, M. E. 1983. Analysis of T cell hybridomas. III. Distinctions between two types of hapten specific suppressor factors which affect plaque-forming cell responses. *J. Exp. Med.* 157:515–29
113. Minami, M., Okuda, K., Furusawa, S., Benacerraf, B., Dorf, M. E. 1981. Analysis of T cell hybridomas. I. Characterization of H-2 and Igh restricted monoclonal suppressor factors. *J. Exp. Med.* 154:1390–1402
114. Minami, M., Okuda, K., Furusawa, S., Dorf, M. E. 1983. Analysis of T cell hybridomas. IV. Characterization of inducible suppressor cell hybridomas. *J. Exp. Med.* 157:1379–95
115. Sherr, D. H., Ju, S-T., Dorf, M. E. 1981. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XII. Fine specificity of anti-idiotypic suppressor T cells (Ts_2). *J. Exp. Med.* 154:1382–89
116. Ju, S-T., Karjalainen, K., Dorf, M. E. 1981. A common idiotype on SJL and C57BL/6 anti-NP antibodies and its relationship with chain production. *J. Exp. Med.* 154:1369–81
117. Reth, M., Imanishi-Kari, T., Rajewsky, K. 1979. Analysis of the repertoire of anti-(4-hydroxy-3-nitrophenyl) acetyl (NP) antibodies in C57BL/6 mice by cell fusion. II. Characterization of idiotypes by monoclonal anti-idiotype antibodies. *Eur. J. Immunol.* 9:1004–13
118. Minami, M., Furusawa, S., Dorf, M. E. 1982. I-J restrictions on the activation and interaction of parental and F_1-derived Ts_3 suppressor cells. *J. Exp. Med.* 156:465–79
119. Okuda, K., Minami, M., Furusawa, S., Dorf, M. E. 1981. Analysis of T cell hybridomas. II. Comparisons among three distinct types of monoclonal suppressor factors. *J. Exp. Med.* 154:1838–51
120. Taniguchi, M., Saito, T., Takei, I., Tokuhisa, T. 1981. Presence of interchain disulfide bonds between two gene products that compose the secreted form of an antigen-specific suppressor factor. *J. Exp. Med.* 153:1672–77
121. Taniguchi, M., Tokuhisa, T., Kanno, M., Yaoita, Y., Shimizu, A., Honjo, T. 1982. Reconstitution of antigen-specific suppressor activity with translation products of mRNA. *Nature* 298:172–74
122. Furusawa, S., Minami, M., Sherr, D. H., Dorf, M. E. 1984. Analysis of the suppressor T cell cascade with products derived from T cell hybridomas. In *Cell Fusion: Gene Transfer and Transformation*, ed. R. F. Beers, Jr., E. G. Basset, pp. 299–313. NY: Raven Press
123. Lei, H. Y., Ju, S-T., Dorf, M. E., Waltenbaugh, C. 1983. Regulation of immune response by I-J gene products. III. GT-specific suppressor factor is composed of separable I-J and idiotype-bearing chains. *J. Immunol.* 130:1274–79
124. Minami, M., Aoki, I., Honji, N., Waltenbaugh, C. R., Dorf, M. E. 1983. The role of I-J and Igh determinants on F_1 derived suppressor factor in controlling restriction specificity. *J. Exp. Med.* 158:1428–43
125. Dorf, M. E., Usui, M., Sherr, D. H. 1984. Use of suppressor cell hybridomas to dissect T cell interactions. In *T Cell Clones*, ed. H. von Boehmer, W. Hass. NY: Elsevier. In press.
126. Kronenberg, M., Steinmetz, M., Kobori, J., Kraig, E., Kapp, J. A., Pierce, C. W., Sorensen, C. M., Suzuki, G., Tata, T., Hood, L. 1983. RNA transcripts for I-J polypeptides are apparently not encoded between the I-A and I-E subregions of the murine major histocompatibility complex. *Proc. Natl. Acad. Sci. USA* 80:5704–8
127. Steinmetz, M., Minard, K., Horvath, S., McNicholas, J., Frelinger, J., Wake, C., Long, E., Mach, B., Hood, L. 1982. A molecular map of the immune response region from the major histocompatibility index of the mouse. *Nature* 300:35–42
128. Murphy, D. B., Yamauchi, K., Habu, S., Eardley, D. D., Gershon, R. K. 1981. T cells in a suppressor circuit and non T:Non B cells bear different I-J determinants. *Immunogenetics* 13:205.
129. Asherson, G. L., Dorf, M. E., Colizzi, V., Zembala, M., James, B. M. B. 1983. Equivalence of conventional anti-picryl T suppressor factor in the contact sensitivity system and monoclonal anti-NP TsF_3: Their final nonspecific effect via the T acceptor cell. *Immunology.* In press
130. Asherson, G. L., Colizzi, V., Zembala, M., James, B. M. B., Watkins, M. C. 1983. Nonspecific inhibitor of contact sensitivity made by T acceptor cells. Triggering of T cells armed with antigen specific T suppressor factor (TsF) requires both occupancy of the major histocompatibility complex recognition site by soluble I-J product and crosslinking of the antigen recognition sites of the TsF. *J. Exp. Med.* In press
131. Tsurufuji, M., Benacerraf, B., Sy, M. S. 1983. An antigen-specific signal is required for the activation of second-order suppressor T cells in the regulation of delayed-type hypersensitivity to 2,4,6–trinitrobenzene sulfonic acid. *J. Exp. Med.* 158:932–45

Annual Reviews

132. Jerne, N-K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris)* 125C:373–89
133. Acuto, O., Meuer, S. C., Hodgdon, J. C., Schlossman, S. F., Reinherz, E. L. 1983. Peptide variability exists within alpha and beta subunits of the T cell receptor for antigen. *J. Exp. Med.* 158:1368–73
134. Allison, J. P., McIntyre, B. W., Bloch, D. 1982. Tumor-speific antigen of a murine T-lymphoma defined with monoclonal antibody. *J. Immunol.* 129:2293–2300
135. Haskins, K., Kubo, R., White, J., Pigeon, M., Kappler, J., Marrack, P. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149–69
136. Krupen, K., Araneo, B. A., Brink, L., Kapp, J. A., Stein, S., Wieder, K. J., Webb, D. R. 1982. Purification and characterization of a monoclonal T cell suppressor factor specific for L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). *Proc. Natl. Acad. Sci. USA* 79:1254–58
137. Sorensen, C. M., Pierce, C. W., Webb, D. R. 1983. Purification and characterization of an L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT)-specific suppressor factor from genetic responder mice. *J. Exp. Med.* 158:1034–47

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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REGULATION OF IgE SYNTHESIS

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INTRODUCTION

The IgE antibody response to protein antigens shares mechanisms with the IgM and IgC antibody responses to T-dependent antigens. However, the IgE response in experimental animals has several characteristics not easily demonstrated in the IgG response. The IgE antibody response is obtained under restricted conditions and is highly dependent on the adjuvant employed. *Bordetella pertussis* vaccine (BP) and aluminum hydroxide gel (alum) are effective adjuvants for the IgE antibody response, while complete Freund's adjuvant (CFA) is less effective. Even when a high-responder mouse strain is immunized with a potent immunogen together with an appropriate adjuvant, an increase in the dose of immunogen makes the IgE antibody response transient and causes a dissociation between the IgG and IgE antibody responses (1). Thus, a persistent IgE antibody response is obtained only when a high-responder strain is immunized with a minute dose of a potent immunogen together with an appropriate adjuvant.

The IgE response is controlled not only by Ir-genes but also by another gene. Some strains, such as SJL, cannot form IgE antibodies to conventional antigens, in spite of a substantial IgG antibody response (2). Breeding experiments showed that this genetic control is not linked to H-2 complex.

Another unique aspect of the IgE antibody response is that the antibody response is selectively enhanced by low-dose X irradiation of rodents or by treatment of the animals with cyclophosphamide (3, 4). Even low-responder mouse strains, such as SJL, produce IgE antibodies if they are irradiated prior to immunization. In both humans and experimental animals, infection with some nematodes enhances the IgE synthesis (5, 6). Infection of antigen-primed

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Annual Reviews

rats with the nematode *Nippostrongylus brasiliensis* (Nb) results in an enhancement of IgE antibody formation against priming antigens (7, 8). Augmentation of the antibody response after the nematode infection is directed to IgE antibodies; neither IgG_1 nor IgG_2 antibody response to the same antigen was affected by the infection (8). These findings suggest that the IgE antibody response is controlled not only by antigen-specific helper and suppressor T cells but also by some additional mechanisms selective for IgE isotype.

Since IgE antibodies are responsible for hay fever and are probably involved in other allergic diseases, several means of regulating the IgE antibody response have been tried. In this review, I briefly summarize attempts to suppress IgE antibody formation through antigen-specific mechanisms and then describe current developments in research on isotype-specific regulation.

ANTIGEN-SPECIFIC REGULATION OF THE IgE RESPONSE

Regulation of the IgE response by means of antigen-specific mechanisms has been attempted either through (*a*) tolerization of B cells or (*b*) manipulation of the population of T cells that regulate the differentiation of precursor B cells to IgE-forming cells. Thus Katz et al (9) induced B-cell tolerance by injecting hapten coupled to a nonimmunogenic carrier, d-glutamic acid d-lysine copolymer (dGL). Injections of dinitrophenyl (DNP) derivatives of dGL before immunization with DNP-ovalbumin (OA) completely suppressed the primary and secondary IgE anti-hapten antibody responses, and an injection of the dGL conjugates into immunized animals terminated the ongoing anti-hapten antibody formation. The effect of the treatment was persistent and specific for the haptenic group. Suppression by DNP-dGL is applied to all immunoglobulin isotypes, including IgE, and is due to inactivation of hapten-specific B cells. Hapten-specific suppressor T cells were not detectable in the treated animals. Subsequently, Lee & Sehon (10) employed DNP-conjugates of polyvinyl alcohol (PVA) to terminate the anti-DNP IgE antibody response. Like DNP-dGL, DNP-PVA conjugates inactivate hapten-specific B cells. In addition, this material can induce DNP-specific suppressor T cells that regulate the antibody response of all isotypes.

Another approach to regulation of the anti-hapten IgE antibody response is to induce anti-idiotypic antibodies. If the anti-hapten antibodies are restricted to certain idiotypes, idiotype-specific regulation may be applied for suppression of the IgE antibody response. Blaser et al (11) reported that anti-idiotypic antibody suppresses primary IgE antibody response and ongoing antibody formation to the benzyl penicilloyl (BPO) group. The same investigators have shown in BALB/c mice that the production of anti-idiotypic antibodies against

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Annual Reviews

syngeneic anti-ovalbumin (OA) antibodies resulted in depression of both anti-hapten and anti-OA IgE antibody formation against DNP-OA (12).

An important practical problem involves regulation of the IgE antibody response to allergens and protein antigens. In view of the tolerizing effect of DNP-dGL, Liu et al (13) prepared dGL conjugates with ovalbumin and ragweed antigen E and studied the effect of the conjugates on the IgE response. Treatment of mice with dGL-conjugates of ovalbumin suppressed both the primary and secondary IgE antibody responses to ovalbumin. However, mechanisms of the suppression appear to be entirely different from those obtained by DNP-dGL. Ovalbumin-dGL conjugates failed to tolerize ovalbumin-specific B cells or to induce ovalbumin-specific suppressor T cells, but the suppression obtained by OA-dGL was confined to IgE isotype.

In the second approach to regulation of the IgE response to protein antigens, antigen-specific suppressor T cells are induced. This approach is based on the fact that antigenic determinants recognized by T cells are not necessarily the same as the major antigenic determinants in some antigens, such as ragweed antigen E and ovalbumin. For example, neither urea-denatured antigen E nor ovalbumin reacts with antibodies against native antigens or with B cells specific for the major antigenic determinants. However, the modified antigens can stimulate T cells specific for the native antigen (14, 15). Alum-absorbed urea-denatured antigens prime helper T cells specific for the native antigen, and an injection of a large dose of urea-denatured antigen without adjuvant results in the induction of antigen-specific suppressor T cells (16). Ovalbumin-polyethylene glycol (PEG) conjugates prepared by Lee & Sehon (17) have immunological effects similar to those of urea-denatured antigen. Injections of the conjugates without adjuvant induce suppressor T cells specific for the native antigen (18). Treatment of mice with the modified antigen either prior to or immediately after immunization with the native antigen suppresses the primary IgE and IgG antibody responses, and treatment of immunized mice with the antigen suppresses the secondary antibody response. However, the treatment has only slight effects on the ongoing IgE antibody formation.

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ISOTYPE-SPECIFIC T CELLS AND REGULATORY T-CELL FACTORS

Dissociation between the IgE antibody response and IgG response under a variety of experimental conditions suggested the possibility that T helper cells for IgE responses may be distinct from T helper cells for IgG-B cells. In the in vitro antibody response of rabbit mesenteric lymph node (MLN) cells, the helper function of carrier-primed cells for the IgE response was highly dependent on the adjuvant employed for carrier priming (19). Thus, T cells primed

Annual Reviews

by immunization with alum-absorbed carrier had helper activities for both IgG and IgE antibody responses, whereas T cells primed by the same antigen included in CFA enhanced only IgG antibody responses. This suggests that helper T cells for IgE might be distinct from those for IgG isotype. Subsequently, Kishimoto et al (20) extended this hypothesis to antigen-specific suppressor T cells. In view of the fact that CFA is a poor adjuvant for the IgE antibody response, they primed BALB/c mice with DNP derivatives of mycobacteria (DNP-Myc) and then immunized these mice with alum-absorbed DNP-OA. Priming of mice with DNP-Myc suppressed the anti-hapten IgE antibody response to DNP-OA but enhanced the IgG antibody response. Furthermore, transfer of splenic T cells from DNP-Myc-primed mice suppressed the primary IgE antibody response of the recipients to DNP-OA, without affecting the IgG antibody response. Suppressor T cells obtained from DNP-Myc-treated animals were specific for DNP group, and selectively regulated the IgE antibody response. These investigators incubated spleen cells from DNP-Myc-primed animals with DNP-heterologous carrier such as DNP-HSA and obtained IgE-specific suppressive factor (IgE-TsF) (21). This T-cell factor was not DNP-specific, but treatments of B cells with the factor selectively suppressed the IgE response. The effect of IgE-TsF appears to be MHC-restricted, and the factor could be absorbed with alloantibodies specific for the products of K and I region of H-2 complex (22). Based on these findings, the same investigators constructed a T-cell hybridoma that spontaneously secreted IgE-TsF (23). The physicochemical properties of IgE-TsF are not known. However, the factor has a molecular weight of approximately 60,000 or higher and binds to lentil lectin Sepharose (24). Recent experiments by this group demonstrated that IgE-TsF has an antigenic determinant coded for MHC complex and has affinity for IgE (25) (see Table 1).

Katz et al (26) and Ovary et al (27) carried out another line of investigations on IgE-specific regulatory T cells, based on the selective enhancement of the IgE response by low-dose X irradiation. Thus, Watanabe et al (28) as well as Chiorazzi et al (4) found that irradiation-enhanced IgE response in low IgE responder (SJL) mice was suppressed by the transfer of normal syngeneic thymocytes or splenic T cells. Nonspecific suppressor T cells were Lyt 1^+ cells rather than Lyt 2^+ cells (29). Tung et al (30) discovered that repeated injections of CFA induced selective suppression of IgE antibody responses to unrelated antigens, and demonstrated that T cells from CFA-treated animals were more effective than normal T cells in the suppression of irradiation-enhanced IgE response. They also found that serum of CFA-treated animals contained a soluble factor that selectively suppressed the IgE response. This factor they called the suppressive factor of allergy (SFA). Administration of serum of CFA-treated animals to irradiated SJL mice reversed the irradiation-enhanced IgE response to the normal low or undetectable levels. SFA could

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Table 1 Properties of IgE-specific regulatory factors

	SFA	EFA	IgE-TsF	IgE-potentiating factor	IgE-suppressive factor
Cell source	Lyt 1^+	T cells	Lyt 2^+	Lyt1$^+$, $Fc_\epsilon R^{+a}$	Lyt 1^{+a}, $Fc_\gamma R^+$
Molecular weight	150,000	?	60,000 (150,000)	15,000	15,000
Affinity for IgE	−	−	+	+	+
Affinity for lentil lectin	−	+	+	+	−
MHC-restriction	−	−	+	−	−
Target cells	T cells	T cell	sIgE$^+$ cells[b]	sIgE$^+$ B cells	sIgE$^+$ B cells plasma cells

[a]w 3/25$^+$ T cells in the rat
[b]Suppress IgE synthesis by IgE-forming hybridoma

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be obtained in mixed lymphocyte cultures of allogenic lymphocytes as well (31). The factor was formed not only in low IgE responder strains but also in high IgE responders (32), and it exerted a selective effect on the IgE response across the strain and species barriers (33). SFA has a molecular weight range of 150,000 to 200,000, does not have either immunoglobulin determinants or H-2 (or Ia) determinants, but bound to anti-β2 microglobulin (34) (see Table 1).

Katz et al (32) detected in the serum of CFA-treated animals another soluble factor that selectively enhanced the IgE response. They called it an enhancing factor of allergy (EFA). The nature of EFA is not known, except that it has affinity for lentil lectin and can be separated from SFA that lacks affinity for the lectin.

The third approach to IgE-specific regulation makes use of rats infected with Nb that selectively enhances the IgE synthesis. Since the IgE-specific potentiation following the infection is dependent on T cells (35), it was anticipated that T cells from Nb-infected rats would selectively enhance the differentiation of IgE-B cells to IgE-forming cells. Indeed, T cells from the infected rats selectively enhanced the in vitro IgE-forming cell response of DNP-OA primed MLN cells to homologous antigen. Furthermore, culture supernatants of MLN cells (or T cells) from Nb-infected animals selectively enhanced the in vitro IgE response without affecting the IgG response (36). The T-cell factor(s) responsible for the selective potentiation of IgE response has affinity for IgE and can be purified by absorption with IgE-coupled Sepharose followed by elution at acid pH (37, 38). The factor could be detected by its ability to inhibit rosette formation of $Fc_\epsilon R^+$ lymphocytes with IgE-coated erythrocytes (E′-IgE). IgE-potentiating factor derives from $Fc_\epsilon R^+$ T cells,

Annual Reviews

which increase during Nb-infection (37). The IgE-potentiating factor is a glycoprotein of 13,000–15,000 daltons; it has an affinity for lentil lectin, Con A, and *Limulus polyphemus* agglutinin (LPA), the lectin specific for terminal sialic acid (38, 39) (see Table 1).

T-cell factors having the affinity for IgE—i.e. IgE-binding factors—exhibit heterogeneous biologic activities and physicochemical properties. When MLN cells obtained from rats 8 days after Nb-infection were incubated with IgE, the cells produced two species of IgE-binding factors with molecular weights of 15,000 (15K) and 30,000 (30K). The 15K IgE-binding factor selectively suppressed, rather than enhanced, the IgE response. The 30K IgE-binding factor could be detected by its ability to inhibit rosette formation of $Fc_{\epsilon}R^{+}$ cells, but it neither suppressed nor enhanced the IgE response (40). Both the 15K IgE-suppressive factor and 30K "inactive" IgE binding factor lacked affinity for lentil lectin and Con A, but they bound to peanut agglutinin (PNA) (39), which is specific for terminal galactose residue. Unlike the IgE-TsF obtained by Kishimoto et al (22), the 15K IgE-suppressive factor overcame the MHC-barrier. Indeed, rat IgE-binding factors have affinity not only for rat IgE but also for mouse IgE, and the rat 15K IgE-suppressive factor suppresses the IgE response of DNP-KLH-primed BALB/c mouse spleen cells to homologous antigen (41) (Table 1).

The effect of IgE-binding factors on the IgE response is probably due to the binding of the factors to IgE-bearing B cells. Both the IgE-potentiating factor and IgE-suppressive factor could be absorbed by IgE-bearing ($sIgE^{+}$) B cells. Recent experiments demonstrated a direct effect of IgE-suppressive factor on IgE-forming plasma cells. We have established a rat-mouse T-cell hybridoma that produces IgE-binding factors upon incubation with IgE (42). One of the hybridomas, 23B6, produces two species of IgE-binding factors, of 30,000 and 15,000 daltons. The 15K IgE-binding factor selectively suppressed the IgE response of rat MLN cells, while the 30K IgE-binding factor was biologically inactive. Because the factors had affinity not only for rat IgE but also for mouse IgE, we determined possible effects of the factors on mouse IgE-forming cells. In order to obtain a sufficient number of IgE-producing plasma cells, DNP-KLH-primed spleen cells of $B_6D_2F_1$ mice were transferred into irradiated syngeneic mice, and the recipients were boosted with alum-absorbed antigen (43). Incubation of a plasma cell–enriched fraction of their spleen cells with purified IgE-suppressive factor resulted in a marked decrease in the number of IgE-bearing cells, IgE-plaque forming cells (PFC), and IgE-containing cells; but the same factor did not affect the number of IgG_1-secreting cells (41). In contrast, the 30K "inactive" IgE-binding factor failed to suppress both the IgE and IgG_1 formation. It was also shown that $sIgE^{+}$ B cells, which did not contain cytoplasmic IgE, lost surface IgE after incubation with the 15K IgE-suppressive factor, while the 30K inactive IgE-binding factor failed

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to affect the expression of cell surface IgE. The 15K IgE-suppressive factor also suppressed IgE formation by an IgE-producing hybridoma that bore sIgE. It appears that the suppressive factor binds to sIgE and suppresses the formation of IgE by the cells. These findings agree with a recent report by Suemura et al (44), who demonstrated that their IgE-TsF from BALB/c mice suppressed IgE-formation by another IgE-producing hybridoma.

CORRELATION BETWEEN THE IgE RESPONSE AND THE NATURE OF IgE-BINDING FACTORS

Formation of IgE-potentiating factor or IgE-suppressive factor is not restricted to Nb-infection. Repeated injection of CFA into rats, which is known to selectively suppress the IgE response to an unrelated antigen (30), induced the formation of IgE-suppressive factor (45). Serum of CFA-treated rats contained IgE-suppressive factor, and their $Fc_{\gamma}R$ T cells spontaneously released IgE-suppressive factor in vitro. In contrast, an injection of BP, which is the best adjuvant for the IgE antibody response in the rat, induced the formation of IgE-potentiating factor (46). Serum obtained 5–7 days after BP-treatment contained IgE-potentiating factor, and culture supernatants of peripheral blood T lymphocytes of BP-treated animals contained IgE-potentiating factor.

Formation of IgE-binding factors was associated with immune response as well. Spleen cells of rats immunized with bacillus Calmette-Guérin (BCG) formed IgE-binding factors upon incubation with tuberculin PPD (47). The factors formed by BCG-primed spleen cells consisted of two species, one of approximately 60,000, the other of 13,000–15,000 daltons. Both the 60K IgE-binding factor and the 15K IgE-binding factor selectively suppressed the IgE response of DNP-OA-primed rat lymphocytes to homologous antigen. The 15K IgE-suppressive factor lacked affinity for Con A and lentil lectin but bound to PNA, indicating that the factor is identical to IgE-suppressive factor obtained by CFA treatment (45). The 60K IgE-suppressive factor is similar to IgE-TsF obtained by incubation of DNP-Myc-primed mouse spleen cells with DNP-HSA (24).

Formation of IgE-binding factor by antigen-primed spleen cells is not unique to the BCG system. Immunization of rats with keyhole limpet hemocyanin (KLH) (included in either alum or CFA) and stimulation of their spleen cells with KLH resulted in the formation of IgE-binding factors (48). When spleen cells were obtained 2–4 weeks after priming, the IgE-binding factors formed by the spleen cells differed depending on the adjuvant employed for priming. Spleen cells of rats primed with alum-absorbed KLH formed IgE-potentiating factor upon antigenic stimulation, while those of rats primed with the antigen included in CFA formed IgE-suppressive factor. Both the IgE-potentiating factor and IgE-suppressive factor formed by KLH-primed spleen cells have a

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molecular weight of 15K; they are identical to those produced by BP-treatment and CFA treatment, respectively. In addition to these factors, KLH-primed spleen cells produced the 60K and 30K IgE-binding factors upon antigenic stimulation. However, these factors neither potentiated nor suppressed the IgE response.

The IgE-binding factors were formed by mouse T cells as well. Spleen cells of mice immunized with alum-absorbed antigen formed IgE-binding factors upon incubation with homologous antigen. However, the IgE-binding factors formed by the antigen-primed spleen cells differ depending on mouse strains. When high IgE responder $B_6D_2F_1$ mice were immunized with a minimum dose of alum-absorbed ovalbumin for a persistent IgE antibody formation, their spleen cells formed IgE-potentiating factor upon incubation with ovalbumin. In contrast, an immunization of low IgE responder SJL mice with alum-absorbed ovalbumin gave the IgG antibody response but essentially no IgE antibody response. Incubation of their spleen cells with ovalbumin resulted in the formation of IgE-suppressive factor (41).

It is apparent that IgE-potentiating factor is detected whenever the IgE synthesis and/or IgE antibody response is enhanced. On the other hand, IgE-suppressive factor is formed in various conditions in which IgE-response is suppressed. The correlation between enhancement of the IgE response and the formation of IgE-potentiating factor, and that between suppression of the IgE-response and the formation of IgE-suppressive factor, strongly suggest that IgE-binding factors are involved in the IgE response in vivo.

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RELATIONSHIP AMONG IgE-SPECIFIC REGULATORY FACTORS

Role of Carbohydrate Moieties in IgE-Binding Factors

The 15K rat IgE-potentiating factor is a glycoprotein and appears to contain both N-linked, mannose-rich oligosaccharide and O-linked oligosaccharide with sialic acid as terminal sugar residues (39). Biologic activity of the factor was lost by treatment of the factor with neuraminidase, indicating that the terminal sialic acid is required for its potentiation of the IgE response (38). Evidence was obtained that the N-linked oligosaccharide in IgE-potentiating factor is essential for its biologic activity. When normal rat MLN cells were precultured with 1 μg/ml Con A and the cells were incubated with IgE, essentially all IgE-binding factors formed by the cells had affinity for PNA, and the factors selectively suppressed the IgE response. In contrast, the same MLN cells activated by 10 μg/ml Con A formed IgE-potentiating factor upon incubation with IgE (49). The factor has affinity for lentil lectin, Con A, and LPA. However, if the cells activated with 10 μg/ml Con A were incubated with IgE in the presence of tunicamycin, which inhibits the assembly of oligosaccharide

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linked N-glycosidically to asparagine residue (50), IgE-binding factors formed by the cells no longer bound to Con A or lentil lectin, and this factor selectively suppressed the IgE response (49). The "IgE-suppressive factor" formed by the cells activated by 10 μg/ml Con A in the presence of tunicamycin retained their affinity for LPA. However, neuraminidase treatment of the factors generated in the presence of tunicamycin resulted in loss of their affinity for LPA and the appearance of affinity for PNA with a slight increase in IgE-suppressive activity. The biologic activity of the latter factors after neuraminidase treatment and their affinity for lectins are identical to those of IgE-suppressive factor (39).

The presence of tunicamycin during the biosynthesis of IgE-suppressive factor affected neither the affinity of the factor for PNA nor its biologic activity. It appears that IgE-suppressive factor contains O-glycosidically linked oligosaccharide, of which assembly to a peptide is not affected by tunicamycin (51). The affinity of the IgE-suppressive factor for PNA suggests that the terminal sugar residue in the oligosaccharide is possibly galactose → N acetylgalactosamine. Recent experiments suggest that the O-linked oligosaccharide is required for the biologic activities of the IgE-suppressive factor. Culture supernatants from a T cell hybridoma (23B6) incubated with IgE for 1–2 hr did not contain a detectable amount of IgE-binding factor. However, extracts of the cells contained both the 13K–15K-IgE-suppressive factor that has affinity for PNA and the 11K IgE-binding factor that has no affinity for PNA. This factor may represent the IgE-suppressive factor before glycosylation. Failure of the 11K IgE-binding factor to suppress the IgE response strongly suggests that O-linked oligosaccharides are essential for the biologic activity of the 15K suppressive factor.

Pharmacologic Modulation of IgE-Binding Factors

Switching of T cells from the formation of IgE-potentiating factor to the formation of IgE-suppressive factor was achieved by treating Con A–activated cells with glucocorticoids. As described above, normal rat MLN cells cultured with 10 μg/ml Con A produced IgE-potentiating factor upon incubation with rat IgE. However, if the Con A–activated cells were precultured for 12 hr in the presence of 1–5 μM dexamethasone and then incubated with IgE, IgE-binding factors formed by the cells lacked affinity for lentil lectin and suppressed, rather than potentiated, the IgE response (52). An IgE-induced $Fc_{\epsilon}R$ expression on the cells was also suppressed in the dexamethasone-treated cells. Effect of glucocorticoid treatment on the nature of IgE-binding factors may be due to an enhanced formation of phospholipase inhibitory protein—i.e. lipomodulin—in glucocorticoid-treated cells (53). Indeed, T cells activated by 10 μg/ml Con A produced IgE-suppressive factor when they were incubated with IgE in the presence of lipomodulin (54). As described, rat MLN cells

Annual Reviews

activated by 1 μg/ml Con A formed IgE-suppressive factor upon incubation with IgE. However, if the same cells, activated by 1 μg/ml Con A, were incubated with IgE in the presence of melittin or monoclonal anti-lipomodulin, which activates phospholipase (55, 56), IgE-potentiating factor was formed (57). Similar results were obtained when the cells activated by 1 μg/ml Con A were incubated with IgE in the presence of lysolecithin, a product of phosphatidylcholine cleaved by phospholipase A_2. Switching of the Con A–activated cells from the formation of IgE-suppressive factor to the formation of IgE-potentiating factor was observed even when the cells had been treated with mitomycin C. These findings suggested that the same cells have the capacity to form both the IgE-potentiating factor and the IgE-suppressive factor, and that the nature of IgE-binding factors formed by the activated T cells is decided by the process of glycosylation of the same precursor molecules (57). This hypothesis was supported by subsequent experiments with T cell hybridoma cells (23B6). Although the hybridomas formed the 15K IgE-suppressive factor upon incubation with IgE, the same cells formed IgE-potentiating factor when they were incubated with IgE in the presence of lysolecithin or anti-lipomodulin (58).

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Presence of Common Antigen Determinants in IgE-Binding Factors and $Fc_\epsilon R$

A close relationship between the IgE-potentiating factor and IgE-suppressive factor was shown by using antibodies against IgE-binding factors (59). Polyclonal guinea-pig antibodies against rat IgE-potentiating factor bound not only the immunizing antigen but also IgE-suppressive factor. Some monoclonal antibodies against "inactive" 30K IgE-binding factor bound all of the 15K IgE-potentiating factor, IgE-suppressive factor, and the 30K IgE-binding factor. Both the polyclonal antibodies and monoclonal antibodies bound intracellular unglycosylated IgE-binding factor, suggesting that the antibodies are directed towards the peptide portion of IgE-binding factors. The antibodies against IgE-binding factors cross-reacted to $Fc_\epsilon R$ on both B and T cells but not to $Fc_\gamma R$. Pretreatment of MLN cells from Nb-infected rats with the antibodies inhibited rosette formation of $Fc_\epsilon R^+$ cells with E′-IgE but did not inhibit rosette formation of $Fc_\gamma R^+$ cells with IgG-coated erythrocytes (EA_γ). Specific binding of anti-IgE-binding factor antibodies to $Fc_\epsilon R$ on both B and T cells was confirmed by immunofluorescence. The proportions of $Fc_\epsilon R^+$ lymphocytes in MLN cells and those stained by the antibody were comparable, and they increased in parallel after infection of the animals with Nb. Depletion of $Fc_\epsilon R^+$ cells in MLN cells of the Nb-infected rats by the rosetting technique resulted in a parallel decrease in the cells stained by anti-IgE-binding factor. Furthermore, incubation of normal MLN cells with IgE resulted in an increase

Annual Reviews

in both $Fc_{\epsilon}R^+$ cells and those stained by anti-IgE-binding factor. Since IgE specifically induces the expression of $Fc_{\epsilon}R$ (60), parallel increases in IgE-RFC and in the cells stained by anti-IgE-binding factor provide strong evidence that $Fc_{\epsilon}R$ are involved in the staining. It would appear that IgE-binding factors and $Fc_{\epsilon}R$ on both T and B cells share a common antigenic determinant.

Possible Relationship Among Various T-Cell Factors Involved in Isotype-Specific IgE Regulation

Since the IgE-TsF described by Suemura has affinity for IgE (25), this factor probably belongs to the family of IgE-binding factors. Binding of IgE-TsF to lentil lectin Sepharose, in spite of its suppressive activity, may be due to the presence of additional peptide(s) in the molecule. Suemura et al (25) reported that the factor from BALB/c mice bound alloantibodies against $H\text{-}2^d$ products. It is not known whether IgE-TsF is composed of multiple polypeptide chains. However, recent experiments in our laboratory have shown that rat 30K and 60K IgE-binding factors bound monoclonal antibody specific for rat Ia-determinant, MRC-OX3 (61), while the 15K IgE-suppressive factor failed to do so. Furthermore, reduction and alkylation of the 30K "inactive" IgE-binding factor, followed by gel filtration of the materials, yielded the 13K and 11K IgE-binding factor that did not bind to the monoclonal anti-Ia. These findings indicate that the 30K binding factor consists of two polypeptide chains—i.e. (*a*) 13K or 11K IgE-binding factor and (*b*) a peptide containing Ia determinant—and that the two peptide chains are linked by disulfide bond(s).

Both the SFA and the EFA described by Katz et al (32) have no affinity for IgE. However, a recent report by Katz (62) indicated that SFA induces the formation of IgE-suppressive factor and that EFA is involved in the formation of IgE-potentiating factor. The effector molecules obtained in their system had affinity for IgE. Thus, it appears that SFA and EFA regulate the IgE response through the formation of IgE-suppressive factor and IgE-potentiating factor, respectively (see Table 1).

MECHANISMS FOR THE FORMATION OF IgE-BINDING FACTORS

Formation of IgE-Binding Factors by Specific Ligand

In the course of studies on $Fc_{\epsilon}R^+$ lymphocytes, we realized that incubation of normal MLN cells with homologous IgE resulted in a substantial increase in the proportion of $Fc_{\epsilon}R^+$ cells (60) and the formation of IgE-binding factors (63). An increase in $Fc_{\epsilon}R^+$ cells after incubation with homologous IgE was confirmed in mouse spleen cells (64). In the rat MLN cells, most of $Fc_{\epsilon}R^+$

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cells induced by IgE were B lymphocytes, but some T cells also expressed $Fc_\epsilon R$. Depletion of $Fc_\epsilon R^+$ cells in MLN cells prior to incubation with IgE affected neither the increase in $Fc_\epsilon R^+$ cells nor the formation of IgE-binding factors. However, IgE failed to induce $Fc_\epsilon R$ expression and IgE-binding factor formation, if $Fc_\gamma R^+$ cells had been depleted prior to incubation with IgE (65). Since the induction of $Fc_\epsilon R$ expression by IgE was prevented by a high concentration of IgG, it was anticipated that IgE might have weak affinity for $Fc_\gamma R$ and that the binding of IgE to the receptors might have induced the expression of $Fc_\epsilon R$ and the formation of IgE-binding factors (65). However, recent experiments with antibodies specific for IgE-binding factors have changed our interpretation. Incubation of a $Fc_\epsilon R^+$ cell–depleted fraction with the $F(ab')_2$ fragments of anti-IgE-binding factor antibodies, which cross-react to $Fc_\epsilon R$ but not to $Fc_\gamma R$, resulted in the induction of IgE-binding factor formation and the expression of $Fc_\epsilon R$ (59). Most $Fc_\epsilon R^+$ cells co-express $Fc_\gamma R$ (66). It appears that a substantial portion of $Fc_\gamma R^+$ cells co-express a minimum number of $Fc_\epsilon R$. This number is not sufficient for rosette formation with E′-IgE, but the binding of IgE or anti-IgE-binding factor antibodies to the receptors apparently induces the formation of IgE-binding molecules.

When MLN cells of Lewis rats or BALB/c spleen cells were incubated with IgE, IgE-binding factors formed by the cells neither potentiated nor suppressed the IgE response. Fractionation of IgE-binding factors on a lentil lectin Sepharose showed that approximately half the IgE-binding factors had affinity for the lectin, while the rest had affinity for PNA. The factors having affinity for lentil lectin selectively potentiated the IgE response, while those having the affinity for PNA suppressed the response. Thus, failure of unfractionated IgE-binding factors to affect the IgE response is probably due to the presence of approximately equal amounts of IgE-potentiating and IgE-suppressive factors in the preparation. However, the nature of IgE-binding factors induced by IgE differs depending on mouse strains. When normal spleen cells from $B_6D_2F_1$ mice were incubated with mouse IgE, the majority of IgE-binding factors formed by the cells had affinity for lentil lectin, and the factors selectively potentiated the IgE response. In contrast, incubation of SJL spleen cells with mouse IgE resulted in the formation of IgE-suppressive factor (67). As already described, a similar difference in the nature of IgE-binding factors was observed when $B_6D_2F_1$ mice and SJL mice were immunized with alum-absorbed OA and their spleen cells were stimulated with homologous antigen (41). However, recent experiments demonstrated that spleen cells of $B_6D_2F_1$ mice formed IgE-suppressive factor when they were incubated with IgE in the presence of lipomodulin, while SJL spleen cells produced IgE-potentiating factor in the presence of lysolecithin. It appears that lymphocytes from the two strains differ in the process of glycosylation of IgE-binding factors.

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Cellular Mechanisms for the Formation of IgE-Binding Factors by Adjuvant Treatment

Formation of IgE-binding factors after repeated injections of CFA or a single injection of BP does not involve IgE. Culture supernatants of macrophages and monocytes from the adjuvant-treated animals contained soluble factors that induce normal T cells to form IgE-binding factors (68, 69). When normal MLN cells were incubated with the "inducer" factor, approximately half the IgE-binding factors formed by the cells bound to lentil lectin, and unfractionated IgE-binding factors neither potentiated nor suppressed the IgE response. The selective formation of IgE-suppressive factor by CFA-treatment is due to another T-cell factor derived from W $3/25^-$ OX 8^+ T cells in CFA-treated animals. This soluble factor prevents the assembly of N-linked oligosaccharide (glycosylation) to IgE-binding factors during their biosynthesis. Thus, "inducer" factor from macrophages in combination with glycosylation inhibiting factor (GIF) from OX 8^+ T cells stimulates normal W $3/25^+$ T cells to form IgE-suppressive factor (69). In contrast, W $3/25^+$ T cells in BP-treated animals spontaneously release another soluble factor that enhances the assembly of N-linked oligosaccharide to IgE-binding factors during their biosynthesis. A mixture of inducer factor from monocytes and glycosylation enhancing factor (GEF) from W $3/25^+$ T cells of BP-treated animals induced normal T cells to form IgE-potentiating factor (68). IgE-suppressive factor in CFA-treated spleen cells derived from W $3/25^+Fc_\gamma R^+$ T cells (45, 69), while IgE-potentiating factor in BP-treated rats derived from W $3/25^+Fc_\epsilon R^+$ T cells (46). Thus, the main difference between the CFA-treatment and BP-treatment is that T cells of different subsets (OX 8^+ T cells in CFA-treated rats and W $3/25^+$ T cells in BP-treated rats) are activated by the adjuvant to form either GIF or GEF, and these T-cell factors determine biologic activities of IgE-binding factors formed.

Cellular Mechanisms for the Selective Formation of IgE-Suppressive Factor or IgE-Potentiating Factor by Antigenic Stimulation

Mechanisms for the formation of IgE-binding factors by antigen-primed lymphoid cells are similar to those involved in the formation of the factors by adjuvant treatment. Culture supernatants of BCG-primed spleen cells with PPD contained not only IgE-binding factors but also inducer and GIF, which induce normal MLN cells to form IgE-suppressive factors (47). Both inducers and GIF derive primarily from W $3/25^-$OX 8^+ T cells, while the IgE-suppressive factor is derived from W $3/25^+Fc_\gamma R^+$ T cells. It appears that presentation of antigen (PPD) to antigen-primed OX 8^+ T cells through macrophages results

Annual Reviews

in the release of both inducer factor and GIF, and that these factors, in turn, stimulate unprimed W $3/25^+Fc_\gamma R^+$ T cells to form IgE-suppressive factors (47). The same principle may apply to the formation of IgE-TsF by stimulation of DNP-Myc-primed mouse spleen cells with DNP-HSA (21). Sugimura et al (70) reproduced Kishimoto's observations on DNP-Myc-primed spleen cells by using phosphatidylcholine-derivatives of mycobacteria (PC-Myc) as a priming antigen. They constructed T-cell hybridomas from splenic T cells of PC-Myc-primed mice. One type of hybridoma was specific for PC; it released PC-specific suppressive factor that regulated both IgE and IgG responses to PC-KLH. Another type of hybridoma spontaneously secreted IgE-TsF but did not express PC-specific molecules on its cell surface. From these findings, Sugimura et al concluded that splenic T cells in PC-Myc-primed mice contained both PC-specific suppressor T cells and IgE-specific but antigen nonspecific T cells; they suggested that IgE-TsF may be secreted from IgE-specific T cells by interaction with antigen-specific suppressor T cells. They predicted that PC-specific suppressive factor may be released by antigenic stimulation of the PC-specific suppressor T cells, and may activate IgE-class specific suppressor T cells for the formation of IgE-TsF (70). It is not settled whether the antigen-specific suppressive factor or nonspecific factors such as inducer plus GIF are involved in the formation of IgE-TsF. Nevertheless, these findings agree with our conclusion that IgE-suppressive factor does not derive from antigen-specific suppressor T cells but that the formation of the factors is controlled by the antigen-specific cells (47).

Similar mechanisms apply for the formation of IgE-binding factors from KLH-primed spleen cells. As shown in Figure 1, stimulation of KLH-alum-primed spleen cells with KLH resulted in the formation of "inducers" and GEF, while antigenic stimulation of KLH-CFA primed cells resulted in the formation of inducers and GIF (71). "Inducer" factor derived primarily from W $3/25^+Fc_\gamma R^-$ T cells, which have the same surface markers as helper T cells (72), while IgE-binding factors were derived from W $3/25^+Fc_\gamma R^+$ T cells. For the formation of inducers by antigenic stimulation, adherent cells are required. It appears that antigen-primed helper T cells release inducers upon antigenic stimulation, and this factor stimulates unprimed $Fc_\gamma R^+$ W $3/25^+$ (Ly 1^+) T cells to form IgE-binding factors (Figure 1). Analysis of cellular mechanisms for the selective formation of IgE-potentiating factor or IgE-suppressive factor revealed that the nature of IgE-binding factors is determined by antigen-primed cells. Upon antigenic stimulation, OX 8^+ $Fc_\gamma R^-$ T cells in KLH + CFA-primed spleen cells formed GIF, while $Fc_\gamma R^+$ W $3/25^+$ T cells in KLH + alum-primed spleen cells formed GEF (Figure 1). One may speculate that immunization with KLH-alum results in priming of not only $Fc_\gamma R^-$ W $3/25^+$ T helper cells but also $Fc_\gamma R^+$ W $3/25^+$ T cell subset, while KLH-CFA immunization primed $Fc_\gamma R^-$ OX 8^+ T cells in addi-

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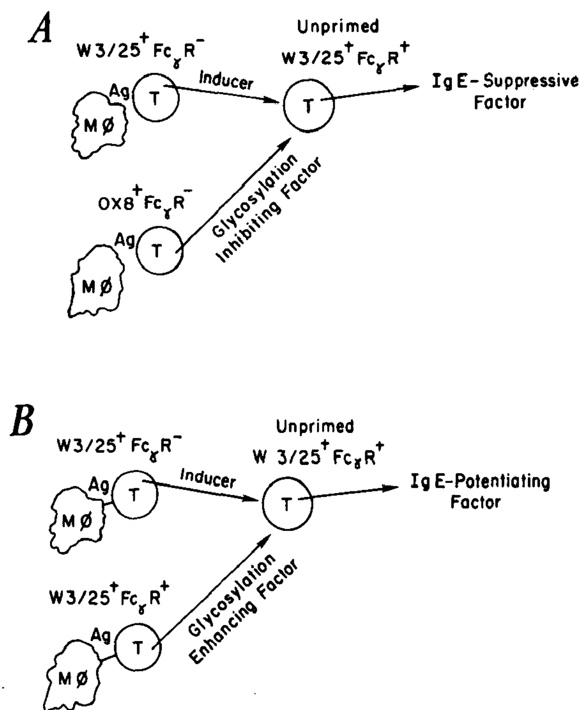

Figure 1 Schematic models for the formation of IgE-suppressive factor by spleen cells of KLH + CFA-primed spleen cells (A) and the formation of IgE-potentiating factor by spleen cells of KLH + alum-primed spleen cells (B). Presentation of antigen to antigen-primed W $3/25^+$ $Fc_\gamma R^-$ T cells results in the formation of inducers. Stimulation of antigen-primed OX 8^+ T cells in (A) or W $3/25Fc_\gamma R^+$ T cells in (B) results in the formation of glycosylation-inhibiting factor or glycosylation-enhancing factor. IgE-binding factors are formed by unprimed W $3/25^+$ $Fc_\gamma R^+$ T cells.

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tion to helper T cells; and it may be for this reason that antigenic stimulation of KLH-alum-primed spleen cells and KLH-CFA-primed spleen cells yielded different "modulators" (GIF or GEF), which determine the nature of IgE-binding factors.

T-CELL FACTORS INVOLVED IN THE SELECTIVE FORMATION OF IgE-POTENTIATING FACTOR OR IgE-SUPPRESSIVE FACTOR

Inducers of IgE-Binding Factors

Macrophage-derived inducers of IgE-binding factors in adjuvant-treated animals appear to be interferon-like substances. An injection of polyinosinic-poly-cytidylic acid (pI:pC), a well known inducer of interferon, induces the

Annual Reviews

formation of IgE-binding factors in vivo (73). Incubation of normal rat spleen cells or mouse spleen cells with pI:pC resulted in the formation of IgE-binding factors. It was found that pI:pC stimulated adherent cells to form soluble factors that in turn stimulated T cells to form IgE-binding factors. Culture supernatants of co-culture of normal mouse spleen cells with measles virus–infected Hela cells, which contained Type I interferon, induced normal rat lymphocytes to form IgE-binding factors; "inducers" in the culture supernatant were neutralized by a minute amount of anti-Type I interferon (73). Furthermore, purified mouse β interferon induced normal mouse spleen cells to form IgE-binding factors (67). These results do not exclude the possibility that monokines other than interferon might also induce the formation of IgE-binding factors. However, inducer factors from macrophages and monocytes of adjuvant-treated rats as well as those obtained by stimulation of adherent cells with pI:pC had affinity for Poly U Sepharose and were not affected by exposure to pH 2.0 (68, 69). These properties of inducer factors are similar to those of Type I interferon (74, 75).

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Inducer factors obtained by antigenic stimulation of antigen-primed T cells may be γ-interferon. The factors derived from both BCG-primed spleen cells and KLH-primed spleen cells had affinity for Poly U Sepharose but were inactivated by exposure to acid pH (47). BCG-primed Lyt 2^+ T cells form γ-interferon upon stimulation with PPD (76, 77). However, the cell source of γ-interferon does not appear to be restricted to Lyt 2^+ cells. Landolfor et al (78) reported that cell sources of γ-interferon produced in mixed lymphocyte cultures are both Lyt 1^+ T cells and Lyt $1^+2^+3^+$ T cells. These researchers proposed that the ability to produce γ-interferon is not restricted to one phenotypically defined T cell subset. Although inducers in KLH-primed spleen cells derived from W $3/25^+$ (Lyt 1^+) T cells, the soluble factor may well be a γ-interferon-like substance.

Glycosylation Inhibiting Factor (GIF)

Our experiments indicate that GIF is related to lipomodulin, a phospholipase inhibitory protein, which is induced by treatment of a variety of cells with glucocorticoid (53, 79). GIF inhibits the assembly of N-linked oligosaccharide to IgE-binding factor for the selective formation of IgE-suppressive factor. It suppressed IgE-induced expression of $Fc_\epsilon R$ on lymphocytes. The factor has the molecular weight of 15,000–16,000 and bound to a monoclonal antibody against rat lipomodulin (54). Since purified lipomodulin from rabbit neutrophils also inhibits the assembly of N-linked oligosaccharide to IgE-binding factors and prevents IgE-induced expression of $Fc_\epsilon R$, we speculated that GIF is related to lipomodulin (54). Rabbit lipomodulin has a molecular weight of 40,000 (53). However, recent experiments by Hirata (80) have shown that lipomodulin is inactivated by phosphorylation before secretion from the cells,

Annual Reviews

and that phosphorylated lipomodulin is cleaved into 30,000-dalton and 15,000-dalton fragments. Gel filtration of culture filtrate of spleen cells from CFA-treated rats showed that most of the "lipomodulin," detected by radioimmunoassay, was eluted together with GIF. Although this fraction did not inhibit phospholipase A_2, the same fraction treated with alkaline phosphatase inhibited the enzyme, indicating that GIF is phosphorylated lipomodulin (54). Blackwell et al (81) reported that glucocorticoids induce macrophages to form phospholipase inhibitory protein, "macrocortin," with a molecular weight of ~15,000. Hirata et al (82) have shown that monoclonal anti-lipomodulin bound to macrocortin. These findings indicate that GIF is macrocortin.

GIF was released by antigenic stimulation of OX 8^+ T cells from both BCG-primed and KLH + CFA-primed rats. A rat-mouse T-cell hybridoma, which formed IgE-suppressive factor upon incubation with IgE, spontaneously released GIF (83). The factor derived from antigen-primed OX 8^+ T cells, and the hybridoma cell was also absorbed by anti-lipomodulin and had a molecular weight comparable to that of macrocortin. It appears that a fragment of phosphorylated lipomodulin is involved in the selective formation of IgE-suppressive factor in all of these systems.

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Glycosylation Enhancing Factor

Analysis of mechanisms for the selective formation of IgE-potentiating factor by BP-treatment revealed that lymphocytosis promoting factor (LPF or pertussigen) from the BP organism (84, 85) was responsible for the formation of GEF. Incubation of normal spleen cells with pertussigen resulted in the release of this factor. GEF was derived from W $3/25^+$ T cells, having a molecular weight of approximately 25,000 and an isoelectric point of pH 6.6 or higher (86). This T-cell factor has lectin-like properties, bound to acid-treated Sepharose, and could be recovered from the beads by elution with galactose or lactose. Incubation of normal MLN cells or a T-cell hybridoma (23A4) with GEF and IgE resulted in the selective formation of IgE-binding factors with N-linked oligosaccharide (glycosylated form), which selectively enhanced the IgE response (58). Switching of 23A4 cells by GEF from the formation of unglycosylated IgE-binding factor to the formation of glycosylated form was inhibited by D-galactose, lactose, or N-acetylgalactosamine, but not by either glucose or N-acetylglucosamine. This indicated that the binding of GEF to galactose residues on the cells is essential for the modulation of IgE-binding factors (87).

Another unique property of GEF is that the T-cell factor is a trypsin-like enzyme. The factor is inactivated by irreversible inhibitors of serine protease, such as phenylmethylsulfonylfluoride (PMSF) and diisopropylfluorophosphate (DFP). Benzamidine, a competitive and reversible inhibitor of trypsin, also inhibited the GEF-induced glycosylation of IgE-binding factors. Furthermore,

Annual Reviews

the factor could be absorbed by p-aminobenzamidine agarose and recovered by elution with benzamidine (87). Synthetic substrates of trypsin, but not the substrates of chymotrypsin, inhibited GEF-induced glycosylation of IgE-binding factors. Furthermore, trypsin, kallikrein, and plasmin switched 23A4 cells from the formation of unglycosylated IgE-binding factor to the formation of the glycosylated form. Since N-α-p-tosyl L-lysine chloromethylketone (TLCK) inhibited both trypsin and plasmin (88) but failed to inhibit kallikrein and GEF, GEF is most likely a kallikrein-like enzyme. It was also found that bradykinin, a biologically active cleavage product of kininogen by kallikrein (88), enhanced the glycosylation of IgE-binding factors at the concentration of 10 ng/ml. Bradykinin activates phospholipase in fibroblasts and induces the release of arachidonic acid and its derivatives (89, 90). It is reasonable to speculate that GEF cleaves a substrate in/on lymphocytes to form a kinin-like substance that in turn enhances the glycosylation of IgE-binding factors through the activation of phospholipase. Activators of phospholipase, such as melittin or monoclonal anti-lipomodulin, enhance the glycosylation of IgE-binding factors (57).

The GEF is released from T cells not only by LPF but also by antigenic stimulation of KLH + alum-primed spleen cells (71). T cells in MLN of 14-day Nb-infected rats spontaneously released GEF (83). The T-cell factor obtained in all three experimental conditions had affinity for p-amino-benzamidine Sepharose and was inactivated by PMSF, indicating that the factor is trypsin(kallikrein)-like enzyme. In contrast, GEF was not obtained from MLN cells of 8-day Nb-infected rats, spleen cells of CFA-treated rats, or KLH-CFA-primed animals. Thus, the formation of GEF was accompanied by the selective formation of IgE-potentiating factor in various experimental conditions but was not detected when IgE-suppressive factor was formed. Concomitant formation of IgE-suppressive factor and GIF, along with the formation of IgE-potentiating factor and GEF, indicates that the nature and biologic activities of IgE-binding factors formed by the same T cells are determined by the balance between GEF and GIF (83).

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CONCLUSION

Selective enhancement or suppression of IgE response by various immunological maneuvers probably share common mechanisms—i.e. selective formation of either IgE-potentiating factor or IgE-suppressive factor. The two IgE-binding factors are comparable in molecular weight, have affinity for IgE, and share a common antigenic determinant with $Fc_{\epsilon}R$. The main differences between the IgE-potentiating factor and IgE-suppressive factor are their carbohydrate moieties, which play an essential role in their biologic activities. Accumulated evidence suggests that the same T cells have the capacity to form

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both IgE-potentiating factor and IgE-suppressive factor and that the nature and biologic activities of IgE-binding factors are decided by posttranslational glycosylation processes of the same precursor molecules. Mechanisms for the selective formation of either IgE-potentiating factor or IgE-suppressive factor are shown schematically in Figure 2. In all systems employed in our laboratory, IgE-binding factors derived from W 3/25$^+$ (Lyt 1$^+$) T cells that bore either $Fc_\epsilon R$, $Fc_\gamma R$, or both. Even when $Fc_\epsilon R$ are not detectable by the rosetting technique, the source cells of IgE-binding factor probably comprise a minimum number of $Fc_\epsilon R$. Stimulation of these T cells by either IgE or interferon-like substances (inducers) results in the formation of both IgE-potentiating factor and IgE-suppressive factor. The selective formation of IgE-potentiating factor requires GEF, which enhances the assembly of N-linked oligosaccharide during the synthesis of IgE-binding factors. On the other hand, induction of IgE-binding-factor formation in the presence of GIF results in the selective formation of IgE-suppressive factor, which lacks N-linked oligosaccharide. GIF is a fragment of phosphorylated lipomodulin, and GEF is a kallikrein-like enzyme. The cell sources of GIF and GEF were OX 8$^+$ (Lyt 2$^+$) T cells and W 3/25$^+$ (Lyt 1$^+$) T cells, respectively, in antigen-primed animals and adjuvant-treated rats. Considering the nature of these factors, however, similar substances may be released from the other cells as the results of inflammation and may participate in the modulation of IgE-binding factors. Nevertheless, the balance between GEF and GIF in the environment of FcR$^+$ T cells will

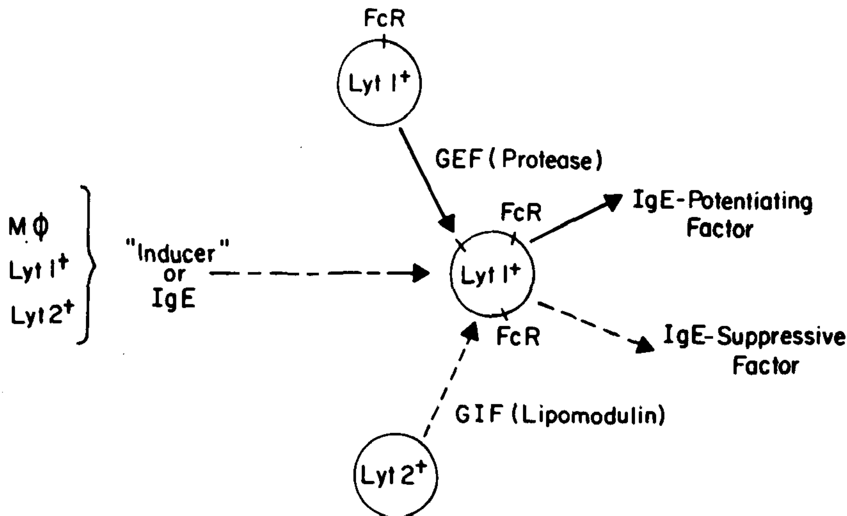

Figure 2 Schematic models for the selective formation of IgE-potentiating factor or IgE-suppressive factor. FcR$^+$ Lyt 1$^+$ T cells form IgE-potentiating factor in the presence of GEF, but the same cells form IgE-suppressive factor in the presence of GIF.

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determine the nature of IgE-binding factors formed by the cells, and these factors in turn either enhance or suppress the IgE response.

Formation of IgE-binding factors is not confined to rodent lymphocytes. Activated human T cells form IgE-binding factors upon incubation with IgE (91). Recently we constructed human T-cell hybridomas that produce IgE-binding factors (92). Saryan et al (93) reported that peripheral blood T cells of patients with hyper-IgE syndrome or atopic dermatitis produced IgE-specific potentiating factor, which appears to have affinity for IgE. It is hoped that basic information obtained in rodent systems will be applied for regulation of the IgE response in atopic patients in the future.

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Literature Cited

1. Ishizaka, K. 1976. Cellular events in the IgE antibody response. *Adv. Immunol.* 23:1–75
2. Levine, B. B. 1971. Genetic factors in reagin production in mice. In *Biochemistry of Acute Allergic Reactions*, ed. K. F. Austen, E. L. Becker, pp. 1–11. Oxford: Blackwell
3. Tada, T., Taniguchi, M., Okumura, K. 1971. Regulation of homocytotropic antibody formation in the rat. II. Effect of X-irradiation. *J. Immunol.* 106:1012–18
4. Chiorazzi, N., Fox, D. A., Katz, D. H. 1976. Hapten-specific IgE antibody response in mice. VI. Selective enhancement of IgE antibody production by low doses of X-irradiation and cyclophosphamide. *J. Immunol.* 117:1629–37
5. Johansson, S. G. O., Melbin, T., Vahlquist, B. 1968. Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E. *Lancet* 1:1118–21
6. Rousseaux-Prevost, R., Bazin, H., Capron, A. 1977. IgE in experimental schistosomiasis. I. Serum IgE levels after infection by *Schistosoma mansoni* in various strains of rats. *Immunology* 33: 501–5
7. Jarrett, E. E. E., Stewart, D. D. 1972. Potentiation of rat reaginic (IgE) antibody by helminth infection. Simultaneous potentiation of separate reagins. *Immunology* 23:749–55
8. Block, M. K., Ohman, J. L., Waltin, J., Cygan, R. W. 1973. Potentiated reagin response: initiation with minute doses of antigen and alum followed by infection with *Nippostrongylus brasiliensis*. *J. Immunol.* 110:197–204
9. Katz, D. H., Hamaoka, T., Benacerraf, B. 1973. Induction of immunological tolerance in bone-marrow-deprived lymphocytes of the IgE antibody class. *Proc. Natl. Acad. Sci. USA* 7:2776–80
10. Lee, W. Y., Sehon, A. H. 1981. Suppression of the anti-DNP IgE response with tolerogenic conjugates of DNP with polyvinyl alcohol. II. Induction of hapten-specific suppressor T cells. *J. Immunol.* 126:414–18
11. Blaser, K., Nakagawa, T., deWeck, A. L. 1980. Suppression of the benzylpenicilloyl (BPO)-specific IgE formation by isologous anti-idiotypic antibodies in BALB/c mice. *J. Immunol.* 125:24–30
12. Blaser, K., Nakagawa, T., deWeck, A. L. 1981. Suppression of anti-hapten IgE and IgG antibody responses by isologous anti-idiotypic antibodies against purified anti-carrier (ovalbumin) antibodies in BALB/c mice. *J. Immunol.* 126:1180–84
13. Liu, F. T., Bogowitz, C. A., Bargatze, R. F., Binnecker, M., Katz, L. R., Katz, D. H. 1979. Immunological tolerance to allergic protein determinants:properties of tolerance induced in mice treated with conjugates of protein and a synthetic

Annual Reviews

copolymer of D-glutamic acid and D-lysine (D-GL). *J. Immunol.* 123:2456–65
14. Takatsu, K., Ishizaka, K. 1975. Reaginic antibody formation in the mouse. VI. Suppression of IgE and IgG antibody response to ovalbumin following the administration of high dose urea-denatured antigen. *Cell. Immunol.* 20:276–89
15. Ishizaka, K., Okudaira, H., King, T. P. 1975. Immunogenic properties of modified antigen E. II. Ability of urea-denatured antigen and a polypeptide chain to prime T cells specific for antigen E. *J. Immunol.* 114:110–15
16. Takatsu, K., Ishizaka, K. 1976. Reaginic antibody formation in the mouse. VII. Induction of suppressor T cells for IgE and IgG antibody responses. *J. Immunol.* 116:1257–64
17. Lee, W. Y., Sehon, A. H. 1978. Suppression of reaginic antibodies with modified allergens. II. Abrogation of reaginic antibodies with allergens conjugated to polyethylene glycol. *Int. Arch. Allergy Appl. Immunol.* 56:193–206
18. Lee, W. Y., Sehon, A. H., Akerblom, E. 1981. Suppression of reaginic antibodies with modified allergens. IV. Induction of suppressor T cells by conjugates of polyethylene glycol (PEG) and monomethyl PEG with ovalbumin. *Int. Arch. Allergy Appl. Immunol.* 64:100–14
19. Kishimoto, T., Ishizaka, K. 1973. Regulation of antibody response *in vitro*. VI. Carrier-specific helper cells for IgG and IgE antibody response. *J. Immunol.* 111:920–32
20. Kishimoto, T., Hirai, Y., Suemura, M., Yamamura, Y. 1976. Regulation of antibody response in different immunoglobulin classes. I. Selective suppression of anti-DNP IgE antibody responses by preimmunization of DNP-coupled mycobacterium. *J. Immunol.* 117:396–404
21. Suemura, M., Kishimoto, T., Hirai, Y., Yamamura, Y. 1977. Regulation of antibody responses in different immunoglobulin classes. III. *In vitro* demonstration of IgE-class specific suppressor function of DNP-mycobacterium primed T cells and the soluble factor released from these cells. *J. Immunol.* 119:159–55
22. Kishimoto, T., Hirai, Y., Suemura, M., Nokanishi, K., Yamamura, Y. 1978. Regulation of antibody response in different immunoglobulin classes. IV. Properties and functions of IgE-class specific suppressor factors released from DNP-mycobacterium-primed T cells. *J. Immunol.* 121:2106–12
23. Watanabe, T., Kimoto, M., Maruyama, S., Kishimoto, T., Yamamura, Y. 1978. Regulation of antibody response in different immunoglobulin classes. V. Establishment of T cell hybrid cell line secreting IgE class-specific suppressor factor. *J. Immunol.* 121:2113–17
24. Kishimoto, T., Sugimura, K., Suemura, M., Shiho, O., Maeda, K., et al. 1981. T-15-idiotype positive IgE class-specific suppressor T-cells and characterization of the suppressor factor. *Int. Arch. Allergy Appl. Immunol.* 66 (Suppl. 1):31–38
25. Suemura, M., Shiho, O., Deguchi, H., Yamamura, Y., Böttcher, I., Kishimoto, T. 1981. Characterization and isolation of IgE class-specific suppressor factor (IgE-TsF). I. The presence of the binding sites for IgE and of the H-2 gene products in IgE-TsF. *J. Immunol.* 127:465–71
26. Katz, D. H. 1980. Recent studies on the regulation of IgE antibody synthesis in experimental animals and man. *Immunology* 41:1–24
27. Ovary, Z. 1981. IgE production and suppression in mice. *Int. Arch. Allergy Appl. Immunol.* 66 (Suppl. 1):8–18
28. Watanabe, N., Kojima, S., Ovary, Z. 1976. Suppression of IgE antibody production in SJL mice. I. Nonspecific suppressor T cells. *J. Exp. Med.* 143:833–45
29. Watanabe, N., Kojima, S., Shen, F. W., Ovary, Z. 1977. Suppression of IgE antibody production in SJL mice. II. Expression of Ly-1 antigen on helper and nonspecific suppressor T cells. *J. Immunol.* 118:485–88
30. Tung, A. S., Chiorazzi, N., Katz, D. H. 1978. Regulation of IgE antibody production by serum molecules. I. Serum from complete Freund's adjuvant–immune donors suppresses irradiation-enhanced IgE production in low responder mouse strains. *J. Immunol.* 120:2050–59
31. Katz, D. H. 1979. Regulation of IgE antibody production by serum molecules. III. Induction of suppressive activity of allogenic lymphoid cell interactions and suppression of IgE synthesis by the allogenic effect. *J. Exp. Med.* 149:539–44
32. Katz, D. H., Bargatze, R. F., Bogowitz, C. A., Katz, L. R. 1979. Regulation of IgE antibody production by serum molecules. IV. Complete Freund's adjuvant induces both enhancing and suppressive activities detectable in the serum of low and high responder mice. *J. Immunol.* 122:2184–90
33. Katz, D. H., Bargatze, R. F., Bogowitz, C. A., Katz, L. R. 1980. Regulation of IgE antibody production by serum molecules. VII. The IgE selective damping activity of suppressive factor of allergy

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Annual Reviews

(SFA) is exerted across both strain and species restriction barriers. *J. Immunol.* 124:819–24
34. Katz, D. H., Tung, A. S. 1979. Regulation of IgE antibody production by serum molecules. VI. Preliminary biochemical and immunological characterization of serum molecules active in suppressing IgE antibody production. *Immunopharmacology* 1:103–14
35. Jarrett, E. E. E., Ferguson, A. 1974. Effect of T cell depletion on the potentiated reagin response. *Nature* 250:420–22
36. Suemura, M., Ishizaka, K. 1979. Potentiation of IgE response *in vitro* by T cells from rats infected with *Nippostrongylus brasiliensis*. *J. Immunol.* 123:918–24
37. Suemura, M., Yodoi, J., Hirashima, M., Ishizaka, K. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. I. Mechanisms of enhancement of IgE response by IgE-potentiating factor. *J. Immunol.* 125:148–54
38. Yodoi, J., Hirashima, M., Ishizaka, K. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. II. Glycoprotein nature and source of IgE-potentiating factor. *J. Immunol.* 125:1436–41
39. Yodoi, J., Hirashima, M., Ishizaka, K. 1982. Regulatory role of IgE-binding factors from rat T lymphocytes. V. The carbohydrate moieties in IgE-potentiating factors and IgE-suppressive factors. *J. Immunol.* 128:289–95
40. Hirashima, M., Yodi, J., Ishizaka, K. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. III. IgE-specific suppressive factor with IgE-binding activity. *J. Immunol.* 125:1442–48
41. Ishizaka, K. 1984. Isotype specific regulation of IgE response. *Progr. Immunol.* 5. In press
42. Huff, T. F., Uede, T., Ishizaka, K. 1982. Formation of rat IgE-binding factors by rat-mouse T cell hybridomas. *J. Immunol.* 129:509–14
43. Okudaira, H., Ishizaka, K. 1981. Reaginic antibody formation in the mouse. XI. Participation of long-lived antibody-forming cells in persistent antibody formation. *Cell. Immunol.* 58:188–201
44. Suemura, M., Ishizaka, A., Kobataka, S., Sugimura, K., Maeda, K., et al. 1983. Inhibition of IgE production in B hybridomas by IgE-class-specific suppressor factor from T hybridomas. *J. Immunol.* 130:1056–60
45. Hirashima, M., Yodoi, J., Ishizaka, K. 1980. Regulatory role of IgE-binding factors from rat T lymphocytes. IV. Formation of IgE-binding factors in rats treated with complete Freund's adjuvant. *J. Immunol.* 125:2154–60
46. Hirashima, M., Yodoi, J., Ishizaka, K. 1981. Regulatory role of IgE-binding factors from rat T lymphocytes. V. Formation of IgE-potentiating factors by T lymphocytes from rats treated with *Bordetella pertussis* vaccine. *J. Immunol.* 126:838–42
47. Hirashima, M., Uede, T., Huff, T. F., Ishizaka, K. 1982. Formation of IgE-binding factors by rat T lymphocytes. IV. Mechanisms for the formation of IgE-suppressive factors by antigenic stimulation of BCG-primed spleen cells. *J. Immunol.* 128:1909–16
48. Uede, T., Huff, T. F., Ishizaka, K. 1982. Formation of IgE-binding factors by rat T lymphocytes. V. Effect of adjuvant for the priming immunization on the nature of IgE-binding factors formed by antigenic stimulation. *J. Immunol.* 129:1384–90
49. Yodoi, J., Hirashima, M., Ishizaka, K. 1981. Lymphocytes bearing Fc receptors for IgE. V. Effect of tunicamycin on the formation of IgE-potentiating factor and IgE-suppressive factor by Con A activated lymphocytes. *J. Immunol.* 126:877–82
50. Leavitt, R., Schlesinger, S., Kornfeld, S. 1977. Tunicamycin inhibits glycosylation and multiplication of Sindbis and vesicular stomatitis virus. *J. Virol.* 21:375–85
51. Hanover, J. A., Lennarz, W. J., Young, J. D. 1980. Synthesis of N- and O-linked glycopeptides in oviduct membrane preparations. *J. Biol. Chem.* 255:6713
52. Yodoi, J., Hirashima, M., Ishizaka, K. 1981. Lymphocytes bearing Fc receptors for IgE. VI. Suppressive effect of glucocorticoids on the expression of Fc_ϵ receptors and glycosylation of IgE-binding factors. *J. Immunol.* 127:471–76
53. Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D., Axelrod, J. 1980. A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci. USA* 77:2533–36
54. Uede, T., Hirata, F., Hirashima, M., Ishizaka, K. 1983. Modulation of the biologic activities of IgE-binding factors. I. Identification of glycosylation inhibiting factor as a fragment of lipomodulin. *J. Immunol.* 130:878–84
55. Shier, W. T. 1979. Activation of high levels of endogenous phospholipase A_2 in cultured cells. *Proc. Natl. Acad. Sci. USA* 76:195–99
56. Hirata, F., delCarmine, R., Nelson, C.

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A., Axelrod, J., Schiffmann, E., et al. 1981. Presence of autoantibody for the phospholipase inhibitory protein, lipomodulin in patients with rheumatic diseases. *Proc. Natl. Acad. Sci. USA* 78:3190–94
57. Yodoi, J., Hirashima, M., Hirata, F., DeBlas, A. L., Ishizaka, K. 1981. Lymphocytes bearing Fc receptors for IgE. VII. Possible participation of phospholipase A_2 in the glycosylation of IgE-binding factors. *J. Immunol.* 127:477–82
58. Huff, T. F., Uede, T., Iwata, M., Ishizaka, K. 1983. Modulation of the biologic activities of IgE-binding factors. III. Switching of a T cell hybrid clone from the formation of IgE-suppressive factor to the formation of IgE-potentiating factor. *J. Immunol.* 131:1090–95
59. Huff, T. F., Yodoi, J., Uede, T., Ishizaka, K. 1984. Presence of an antigenic determinant common to rat IgE-potentiating factor, IgE-suppressive factor and Fc_ϵ receptors on T and B lymphocytes. *J. Immunol.* 132:406–12
60. Yodoi, J., Ishizaka, T., Ishizaka, K. 1979. Lymphocytes bearing Fc receptors for IgE. II. Induction of Fc_ϵ receptor bearing rat lymphocytes by IgE. *J. Immunol.* 123:455–62
61. McMaster, W. R., Williams, A. F. 1979. Identification of Ia glycoproteins in rat thymus and purification from rat spleen. *Eur. J. Immunol.* 9:426–33
62. Katz, D. H., Marcelletti, J. F. 1984. Regulation of the IgE antibody system in humans and experimental animals. *Progr. Immunol.* 5. In press
63. Yodoi, J., Ishizaka, K. 1980. Lymphocytes bearing Fc receptors for IgE. IV. Formation of IgE-binding factor by rat T lymphocytes. *J. Immunol.* 124:1322–29
64. Chen, S-S., Bohn, J. W., Lin, F-T., Katz, D. H. 1981. Murine lymphocytes expressing Fc receptors for IgE ($Fc_\epsilon R$). I. Conditions for inducing $Fc_\epsilon R^+$ lymphocytes and inhibition of the inductive events by suppressive factor of allergy (SFA). *J. Immunol.* 127:166–73
65. Yodoi, J., Ishizaka, K. 1979. Lymphocytes bearing receptors for IgE. III. Transition of $Fc_\gamma R(+)$ cells to $Fc_\epsilon R(+)$ cells by IgE. *J. Immunol.* 123:2004–10
66. Vander-Mallie, R., Ishizaka, T., Ishizaka, K. 1982. Lymphocytes bearing Fc receptors for IgE. VIII. Affinity of mouse IgE for $Fc_\epsilon R$ on mouse B lymphocytes. *J. Immunol.* 128:2306–11
67. Uede, T., Sandberg, K., Bloom, B. R., Ishizaka, K. 1983. IgE-binding factors from mouse T lymphocytes. I. Formation of IgE-binding factors by stimulation with homologous IgE and interferon. *J. Immunol.* 130:649–54
68. Hirashima, M., Yodoi, J., Ishizaka, K. 1981. Formation of IgE-binding factor by rat T lymphocytes. II. Mechanisms of selective formation of IgE-potentiating factors by treatment with *Bordetella pertussis* vaccine. *J. Immunol.* 127:1804–10
69. Hirashima, M., Yodoi, J., Huff, T. F., Ishizaka, K. 1981. Formation of IgE-binding factors by rat T lymphocytes. III. Mechanisms of selective formation of IgE-suppressive factors by treatment with complete Freund's adjuvant. *J. Immunol.* 127:1810–16
70. Sugimura, K., Nakanishi, K., Maeda, K., Kashiwamura, S., Suemura M., et al. 1982. The involvement of two distinct subsets of T cells for the expression of the IgE class-specific suppression: Establishment and characterization of PC-specific T 15-idiotype-positive T hybridomas and IgE class-specific, antigen-nonspecific T-hybridomas. *J. Immunol.* 128:1637–43
71. Uede, T., Ishizaka, K. 1982. Formation of IgE-binding factors by rat T lymphocytes. VI. Cellular mechanisms for the formation of IgE-potentiating factor and IgE-suppressive factor by antigenic stimulation of antigen-primed spleen cells. *J. Immunol.* 129:1391–97
72. Stout, R. D., Herzenberg, L. A. 1974. The Fc receptors in thymus derived lymphocytes. I. Detection of subpopulation of murine T lymphocytes bearing Fc receptor. *J. Exp. Med.* 142:611–21
73. Yodoi, J., Hirashima, M., Bloom, B. R., Ishizaka, K. 1981. Formation of IgE-binding factors by rat lymphocytes. I. Induction of IgE-binding factors by poly I:C and interferon. *J. Immunol.* 127:1579–85
74. Mayer-Gnignard, J. D., Thang, M. N., Maeyer, E. D. 1977. Binding of mouse interferon to polynucleotides. *Proc. Natl. Acad. Sci. USA* 74:3787
75. Stewart, W. E. II. 1981. Interferons:their purification and characterization. In *The Interferon System,* Wien/New York: Springer, p. 134–83
76. Youngner, J. S., Salvin, S. B. 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. *J. Immunol.* 111:1919–22
77. Sonnenfeld, G., Mandel, A. D., Mergian, T. C. 1979. *In vitro* production and cellular origin of murine Type II interferon. *Immunology* 36:883–90
78. Landolfo, S., Kirchner, H., Simon, M. M. 1982. Production of immune inter-

Annual Reviews

feron is regulated by more than a T cell subset:Ly 1,2,3 and IaT-5 phenotypes of murine T lymphocytes involved in IFN-γ production in primary and secondary mixed lymphocyte reaction. *Eur. J. Immunol.* 12:295–99

79. Flower, R. J., Blackwell, G. J. 1979. Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature* 278:456–59
80. Hirata, F. 1981. The regulation of lipomodulin, a phospholipase inhibitory protein in rabbit neutrophils by phosphorylation. *J. Biol. Chem.* 246:7730–33
81. Blackwell, G. J., Cornnuccio, R., Rosa, M. D., Flower, R. J., Parente, L., Persico, P. 1980. Macrocortin:a polypeptide causing the antiphospholipase effect of glucocorticoids. *Nature* 287:147–49
82. Hirata, F., Notsu, Y., Iwata, M., Parente, L., DiRosa, M., Flower, R. J. 1982. Identification of several species of phospholipase inhibitory proteins by radioimmunoassay for lipomodulin. *Biochem. Biophys. Commun.* 109:223–30
83. Iwata, M., Huff, T. F., Ishizaka, K. 1984. Modulation of the biologic activities of IgE-binding factor. V. The role of glycosylation enhancing factor and glycosylation inhibiting factor in determining the nature of IgE-binding factors. *J. Immunol.* 132. In press
84. Morse, J. H., Kong, A. S., Lindenbaum, J., Morse, S. I. 1977. The mitogenic effect of the lymphocytosis-promoting factor from *Bordetella pertussis* on human lymphocytes. *J. Clin. Invest.* 50:683–92
85. Munoz, J. A., Bergman, R. K. 1977. *Bordetella pertussis:* Immunological and other biological activities. In *Immunology, Ser. 4,* ed. N. Rose, p. 123. New York: Marcel-Decker
86. Iwata, M., Huff, T. F., Uede, T., Munoz, J. J., Ishizaka, K. 1983. Modulation of the biologic activities of IgE-binding factor. II. Physicochemical properties and cell sources of glycosylation enhancing factor. *J. Immunol.* 130:1802–8
87. Iwata, M., Munoz, J. J., Ishizaka, K. 1983. Modulation of the biologic activities of IgE-binding factor. IV. Identification of glycosylation-enhancing factor as a kallikrein-like enzyme. *J. Immunol.* 131:1954–60
88. Pisano, J. J. 1974. Chemistry and biology of the kallikrein-kinin system. In *Proteases and Biological Control,* ed. E. Reich, D. B. Rifkin, E. Shaw, p. 199–222. Cold Spring Harbor, NY: Cold Spring Harbor Conf. on Cell Proliferation
89. Bell, R. L., Baenziger, N. L., Majerns, P. W. 1980. Bradykinin-stimulated release of arachidonate from phosphatidyl inositol in mouse fibrosarcoma cells. *Prostaglandins* 20:269–74
90. Bareis, D. L., Manganiello, V. C., Hirata, F., Vaughan, M., Axelrod, J. 1983. Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation and cAMP accumulation in human fibroblasts. *Proc. Natl. Acad. Sci. USA* 80:2514–18
91. Ishizaka, K., Sandberg, K. 1981. Formation of IgE-binding factors by human T lymphocytes. *J. Immunol.* 126:1692–96
92. Huff, T. F., Ishizaka, K. 1984. Formation of IgE-binding factors by human T cell hybridomas. *Proc. Natl. Acad. Sci. USA* . In press
93. Saryan, J. A., Leung, D. Y., Geha, R. S. 1983. Induction of human IgE synthesis by a factor from T cells of patients with hyper-IgE states. *J. Immunol.* 130:242–47

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:159-182. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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TRANSPLANTATION OF PANCREATIC ISLETS

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INTRODUCTION

The complications in diabetes mellitus develop over many years. They involve the eye, kidney, and cardiovascular system and may lead to blindness, renal failure, and early development of arteriosclerosis. Apparently these complications are secondary to the diabetic state and are due to the inability of our present forms of therapy to maintain the blood sugar within normal limits at all times. The insulin-producing cells (beta cells) in the islets of Langerhans of insulin-dependent diabetics have either been destroyed or altered by mechanisms unknown at present. Thus, a possible therapeutic approach would be to transplant normal islets into diabetic patients with the hope that the transplants would maintain normoglycemia and prevent or arrest the development of diabetic complications. This approach requires that rejection of the islet transplants be prevented by procedures other than continuous immunosuppressive therapy.

During the past few years, several procedures have been developed that prevent rejection of islet allografts and xenografts in rats and mice without requiring continuous immunosuppression of the recipient animals. In this review we describe the islet transplant model as developed with isografts, the concepts and procedures used for prevention of islet rejection, and the present status of islet transplantation as a possible therapeutic approach to human diabetes.

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Annual Reviews

ISLET ISOGRAFTS

The islets of Langerhans are small, round structures (150–250 μm) scattered throughout the pancreas. They contain a mixture of four different types of endocrine cells, each secreting a specific hormone. The beta cells secrete insulin and form 75–80% of the islet cell population; the remaining endocrine cells secrete glucagon, pancreatic polypeptide, or somatostatin. The total mass of islet tissue forms only 2–3% of the pancreas. Several years ago, our laboratory developed a technique for the isolation of islets from the mammalian pancreas (1,2,3). In this procedure the exocrine pancreas is disrupted by injection of Hanks' solution into the pancreatic ductal system. The pancreas is then chopped and incubated with collagenase, which results in the further destruction of the acinar tissue and liberation of the individual islets. Islets isolated by this procedure are viable and will form, store, and release insulin and other intra-islet hormones in vitro.

The diabetic model used in most islet transplant studies is produced by injecting either streptozotocin or alloxan into the recipient animals. These two agents have a specific cytotoxic action on the beta cells, and their injection produces a stable form of diabetes in the experimental animal. The success or failure of the islet transplants can then be measured easily by monitoring either the blood sugar or the 24-hr urine glucose level before and after transplantation.

Transplants of isografts of rat islets reverse the diabetic state to normal in the recipients and maintain normoglycemia in these animals for more than 1.5 years (4,5). The sites used successfully for transplantation of the islets are the spleen (6,7), the intraperitoneal cavity (8), the subrenal capsule (9), and embolization of the islets into the liver via the portal vein (5). The latter site requires fewer islets to reverse the diabetic state to normal than do the other transplant sites (10), probably because of the intravascular location of the islets, the acquisition of a dual blood supply from the portal vein and hepatic artery, and the liberation of insulin into the liver (the primary site of action of the hormone). Immunohistochemical studies have shown that the transplanted adult islets contain all four types of specialized islet cells found in normal islets (11).

Isografts of either dispersed neonatal rat pancreases from 35 donors or 3–4 intact fetal rat pancreases have also been successful in maintaining normoglycemia in diabetic recipients (12,13). The fetal pancreas is of particular interest because the islet tissue forms 10–15% of the mass of the pancreas, and fetal islet cells have the ability to replicate. Brown and his associates (14) demonstrated that transplantation of a single fetal rat pancreas would achieve normoglycemia in the recipients when they first transplanted the pancreas beneath the renal capsule of a normal syngeneic rat, left it for two weeks, and then transplanted the kidney bearing the fetal pancreas as an isograft into a

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Annual Reviews

diabetic recipient. Subsequently it was shown that either treatment of the recipients with insulin for a few days following transplantation or culturing the fetal pancreas in the presence of nonstimulatory levels of glucose for 3–4 weeks would permit the successful reversion of the diabetic state by an isograft of a single fetal rat pancreas (15,16). These findings indicate that the diabetic state has an adverse effect on the growth and differentiation of the fetal tissue. If the fetal pancreas is permitted to replicate and differentiate in a normoglycemic environment, it is capable of forming a sufficient mass of endocrine tissue for reversal of the diabetic state.

Only the early stages of diabetic complications involving the eye, kidney, and the autonomic nervous system occur in the experimental animal. After the successful transplantation of islet isografts, the immediate question was whether islet transplants would affect these early diabetic complications. Mauer et al (17) demonstrated the presence of immunoglobulins and an increase in mesangial matrix in the glomeruli of diabetic rats after several months. Islet isografts in these animals caused the immunoglobulin deposits to disappear and the mesangial-matrix volume to return to normal.

The retinal capillaries of the eye have been shown to be leaky in both human and experimental diabetes. By using ocular fluorophotometry to measure the accumulation of intravascularly injected fluorescein in the eyes of rats, Krupin et al (18) demonstrated that this leakage returned to normal baseline values following reversal of the diabetic state with isografts. Further, islet isografts into diabetic rats either prevent the development of the neurologic lesions of diabetic autonomic nephropathy or restore them to normal (19).

These studies indicate that at least early diabetic complications involving the microvascular system and autonomic nerves in animals can be prevented or reversed by islet transplantation and maintenance of normoglycemia in the recipients.

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ALLOGRAFTS OF ADULT ISLETS

After the successful transplantation of islets as isografts, it was quickly demonstrated that allografts of islets were rejected rapidly regardless of whether they were transplanted across a major or a minor histocompatibility barrier. The susceptibility to rejection appeared to be greater than with other tissues. The rate of rejection was more rapid, and immunosuppressive therapy was only moderately successful in prolonging the survival of the allografts (8).

Several years ago we initiated studies to determine whether pretreatment of the islets prior to transplantation would affect the rejection of islet allografts. Our rationale for the development of these approaches was based on that originally suggested by Snell in 1957 (20). He proposed that lymphoid cells present within a graft might play a significant role in the induction of rejection.

Annual Reviews

Elkins & Guttmann (21) coined the term passenger leukocyte for these crucial stimulatory cells. The passenger leukocyte concept has been reviewed in detail recently (22).

The ultimate test of the passenger leukocyte concept would be the abrogation of allograft rejection by procedures that either removed or altered passenger leukocytes prior to the transplantation of the grafts. Jacobs (23) obtained suggestive evidence for prolongation of ovarian allograft survival following in vitro culture of the ovarian tissue for 6–12 days prior to transplantation. In 1975, Lafferty et al (24) obtained definitive evidence that in vitro culture of donor mouse thyroids in 95% O_2 for three weeks prior to transplantation resulted in an 80–90% survival of the thyroid allografts at 40 days after transplantation. In contrast, the uncultured control thyroids were rejected sooner than 15 days after transplantation. If 10^3 donor peritoneal exudate cells were injected intravenously at the time of transplanting the cultured thyroids, then the grafts were rejected within 15 days (25). Based upon these observations, Lafferty et al suggested that culture in high oxygen had either inactivated or killed passenger leukocytes and that these cells played a significant role in the induction of rejection.

Our initial attempts to determine whether the passenger leukocyte concept was applicable to islet allografts involved pretreating donor rats with agents that diminished the lymphoid content of their tissues, such as total body irradiation and intravenous injections of silica. These procedures resulted in moderate prolongation of islet allograft survival across major and minor histocompatibility barriers (26). Although these findings suggested that the passenger leukocyte concept might apply to islet allografts, they were not definitive.

Subsequently, we found that in vitro culture of rat islets at 24°C for 7 days prior to transplantation, in conjunction with a single injection of antilymphocyte serum (ALS) into the diabetic recipients, resulted in a 90–100% survival of allografts at 100 days after transplantation across major histocompatibility barriers (27). Neither a single injection of ALS alone or in vitro culture of the islets at 24°C for 7–21 days alone produced a marked prolongation of islet allograft survival (28). Both the low-temperature culture and the single injection of ALS were required to prevent rejection of the allografts.

Opelz & Terasaki (29) had reported previously that in vitro maintenance of lymphocytes at 22°C for more than four days abolished the ability of the lymphocytes to stimulate allogeneic lymphocytes in mixed lymphocyte cultures. However, the cells retained their ability to respond to allogeneic lymphocytes. It appears that culture at low temperature diminished or altered the lymphoid elements in the islets prior to transplantation. Fortunately, the nonlymphoid elements of the islets not only survived under these conditions but remained morphologically and functionally intact for as long as three weeks of in vitro culture at 24°C.

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It was possible that culture at low temperature somehow altered the expression of histocompatibility antigens on the islet cells rather than depleting passenger cells. This is unlikely because rejection of established islet allografts at six months after transplantation could be induced by the intravenous injection of $>10^7$ donor peritoneal exudate cells (28). These findings indicated that the normoglycemic status of the recipient animals was dependent upon the islet transplants and that the allogeneic islets were identifiable immunologically once the immune system was properly activated. It was surprising, therefore, when Parr (30), using an immunoferritin labeling technique, reported a complete absence of H–2 antigens on the dispersed mouse islet cells. If rejection occurred by the induction of specific cytotoxic T lymphocytes, then how could the foreign islet cells be recognized if they did not express histocompatibility antigens?

Studies using more conventional microcytotoxicity assays for histocompatibility antigens demonstrated that mouse islet cells do indeed express H-2K and H-2D antigens but do not express Ia antigens (31). These results have been confirmed by Baekkeskov et al (32), using biochemical techniques for the isolation and characterization of histocompatibility antigens from the islets. They demonstrated the presence of class I antigens in islets from mice, rats, and humans and an absence of class II (Ia) antigens on these cells. Paar's inability to demonstrate H-2 antigens with the immunoferritin technique may have been due to the loss of antigen after treatment with trypsin, the type of antibodies employed, or the sensitivity of the immunoferritin labeling technique.

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Pretreatment of Islets with Donor Specific Anti–Ia Antibody

Cells bearing Ia antigens have been shown to have particularly important roles in the initiation of immune responses. T lymphocytes of the Ly 1 helper phenotype recognize antigens only in the context of class II antigens. This also pertains to alloantigens, because mixed lymphocyte reactions between H-2 incompatible lymphocytes are dependent on stimulation of the response by Ia-bearing cells. Thus, it was logical to expect the passenger cell of islets to bear Ia. The lack of expression of Ia antigens by the bulk of murine islet cells made it possible to determine whether pretreatment of islets with donor-specific anti-Ia antibody and complement would prevent rejection of these islets when transplanted as allografts. Islets were isolated from B10.BR/SgSnJ(H-2^k) mice, incubated with anti-I^k serum followed by complement, and transplanted into C57BL/6J(H-2^b) recipients made diabetic by the intravenous injection of streptozotocin. This pretreatment regimen produced 100% survival of the islet allografts for at least 200 days after transplantation (33).

Rejection of islet allografts established by pretreatment of the islets with anti-Ia serum and complement could be induced by the intravenous injection

Annual Reviews

of $>10^7$ donor splenocytes at 3–4 months after transplantation. These findings not only achieved the ultimate test of the passenger leukocyte concept—the prevention of rejection by removal or alteration of passenger leukocytes—but they also indicated that the lymphoid cell responsible for induction of rejection was Ia^+.

Morrow et al (34) have confirmed these findings and demonstrated that pretreatment of the islets with only donor-specific anti-Ia serum and omission of complement would prevent rejection of mouse islet allografts. This group also performed islet allografts in congeneic donor-recipient combinations differing at the H-2K, D, or I region only or with combinations of these disparities (35). It is most interesting that disparity in the K region resulted in an islet allograft survival of approximately 30% at 100 days after transplantation, 80% with a D-region disparity only, and >90% with an I-region disparity only. In addition, the remaining islet allografts in these three groups, still surviving after 100 days, differed in their sensitivity to the induction of rejection by the intravenous injection of $>10^7$ donor specific splenocytes. Rejection was induced in these recipients with established islet allografts with K-region or D-region disparity, whereas rejection was not induced in those with only I-region disparity. The surprising finding in these investigations was the high percentage of islet allograft survival obtained where only an I- or D-region disparity existed. Therefore in future considerations of human islet transplants it may be of importance to match as closely as possible for class I antigens, since this may permit prolonged survival of the islet allografts. It will also be of interest to determine whether disparity of the I region only will permit as high a percentage of success with allografts of fetal pancreases transplanted between appropriate strains of congeneic mice.

In Vitro Culture of Mega-islets in 95% O_2

We had found that in vitro culture of individual rat islets in the presence of 95% O_2 caused the islets to disintegrate within two to four days. Bowen et al (36) found this same toxicity to oxygen for individual mouse islets; however, they developed a simple in vitro procedure to produce aggregates of mouse islets that would withstand exposure to 95% O_2 in vitro. Approximately 50 individual mouse islets were swirled in a culture dish and allowed to aggregate. These individual aggregates remained morphologically intact and could be transplanted successfully beneath the renal capsule of either normal or diabetic recipients. Incubation of the mouse mega-islets for a period of seven days in the presence of 95% O_2 prevented rejection of the allografts when evaluated both histologically and functionally. Apparently the high oxygen tension in these cultures destroyed or altered passenger leukocytes within the islets, permitting successful transplants across major histocompatibility barriers in mice.

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Induction of Specific Immunologic Unresponsiveness

Several findings suggest that a specific immunologic unresponsiveness or tolerance was induced in recipient animals bearing established endocrine allografts. Determination of the number of donor lymphoid cells required to induce rejection of these grafts indicated that they became more resistant to rejection over a period of several months following transplantation. Allografts of thyroids or islets pretreated to remove passenger leukocytes could be rejected by injecting 10^3–10^4 donor peritoneal exudate cells into the recipients at the time of transplantation, whereas 10^6–10^7 donor peritoneal exudate cells were required to induce rejection of these established endocrine grafts six months after transplantation (25,28). During this six-month interval, a change obviously occurred either in the immune system of the recipient animals or in the endocrine grafts. A change in the endocrine grafts was unlikely since rejection could still be induced at six months after transplantation by injecting a large number of donor lymphoid cells. This indicated that the transplanted tissue could still be recognized as foreign and rejected by cytotoxic T lymphocytes. In addition, when thyroid allografts were removed at 350 days after transplantation and retransplanted into syngeneic donors, rejection could be induced by a small number of donor peritoneal exudate cells (37).

Evidence of a change in the immune system of the recipients follows: Transplants of untreated islets from an unrelated strain into recipients with established islet allografts were rejected acutely, whereas transplants of untreated islets from the original donor strain were not rejected (38). Donohoe et al (37) reported that recipients with long-established thyroid allografts would accept a second uncultured thyroid allograft from the original donor strain of mice. We attempted to induce rejection of established islet allografts in mice by daily injections of recipient-specific anti-I-J serum, reasoning that the antiserum might eliminate possible suppressor cells in the recipients and result in rejection (39). This treatment by itself did not result in rejection of the islets. However, half of the animals treated with anti-I-J serum became exceedingly sensitive to the induction of rejection by small numbers of donor splenocytes (10^4), whereas $>10^7$ donor splenocytes were required to induce rejection of islet allografts in comparable recipients that had not been treated with anti-I-J serum. These findings strongly suggest that a tolerant status was induced in recipient animals bearing established endocrine allografts.

If the transplantation of islets devoid of Ia-positive passenger lymphoid cells induces tolerance, then it should be possible to preimmunize recipient animals with Ia-negative cells, induce a partial state of tolerance, and thus be able to transplant untreated donor islets. This was attempted in the mouse by using donor blood treated with anti–Ia antiserum and complement, since red

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blood cells are Ia-negative in the mouse but express H-2K and D antigens similar to mouse islet cells (40). Mice pretreated with Ia–depleted donor blood nearly always accepted untreated islet allografts for more than 100 days after transplantation, whereas preimmunization of the recipients with untreated donor blood caused an acceleration of rejection as compared to controls. This immunologic unresponsiveness was specific, since preimmunization of the recipients with Ia-depleted blood from an irrelevant strain (H-2^{s}) did not prevent rejection.

Preimmunization with donor Ia-negative blood apparently raises the threshold level of contaminating passenger lymphoid cells that can be present in untreated islet grafts and yet permit successful transplantation. Thus, it appears that either transplantation of Ia-negative mouse islet cells or immunization with Ia-negative donor blood cells induces specific unresponsiveness in the recipients.

Lafferty et al (41) have also shown that injection of donor spleen cells irradiated with ultraviolet light does not induce rejection of islet allografts. In contrast, the irradiated spleen cells appear to enhance the rate of induction of tolerance in the recipient animals. Recently, Hardy's group (42) has shown that pretreatment of recipient rats with UV-irradiated donor blood permits successful transplantation of untreated islet allografts across both major and minor histocompatibility barriers.

In the past, several models for the induction of immunologic unresponsiveness in the recipients were developed that permitted survival of the allografts of skin and other organs. These pretreatment regimens were complex and involved the use of donor spleen cells, blood, or extracts of donor organs such as the liver or spleen in conjunction with ALS or other immunosuppressive agents (43–47).

We do not know the mechanism involved in the induction of unresponsiveness by either preimmunization with donor blood depleted of Ia-bearing cells or the induction and maintenance of tolerance by transplants of islets depleted of Ia-bearing passenger lymphoid cells, nor do we know which cells induce the unresponsive state. Regardless of mechanism, the finding that preimmunization of recipient mice with donor blood depleted of Ia-positive cells may be important in the application of islet transplantation to human diabetes. If tolerance could be induced in human recipients by preimmunization with appropriate Ia-negative cells, then it should be possible to transplant human islets that were not completely free of Ia-positive passenger lymphoid cells. Pretransplant blood transfusions have been shown to have a beneficial effect on the survival of kidney grafts in humans (48). The mechanisms responsible for the prolongation of kidney graft survival by pretransplant blood transfusions are unknown. Human red blood cells apparently do not express major histocompatibility antigens.

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Intra-islet Ia–Positive Cells

The prevention of rejection of mouse islet allografts by treatment of the islets with Ia–antibody and complement indicates that the antigen-presenting cells in the islet grafts responsible for the induction of rejection are Ia-positive. Our laboratory has demonstrated Ia-bearing cells within isolated canine islets using a monoclonal anti-human-Ia antibody that cross-reacts with canine Ia antigens (49). Approximately 2–3 Ia^+ cells were found per islet.

Steinman (50) has demonstrated that dendritic cells in the lymph nodes and spleens of mice are Ia–bearing and have distinct cytologic characteristics that distinguish them from macrophages and other lymphoid cells. Further, enriched populations of mouse dendritic cells were shown to be potent stimulators of mixed leukocyte reactions (51). Recently, Steinman's group (52) has developed a specific monoclonal antibody for mouse dendritic cells. The removal of dendritic cells by the monoclonal antibody and complement dramatically reduced the stimulatory capacity of spleen cells in a mixed leukocyte reaction (53).

Dendritic cells morphologically similar to those described by Steinman were shown by Hart & Fabre (54) to be present in the heart, liver, pancreas, thyroid, skin, skeletal muscle, and kidney of the rat but were absent from the brain. Hart & Fabre also demonstrated the presence of similar cells within islets in histologic sections of the rat pancreas.

A similar type of Ia-positive cell with finger-like projections was demonstrated in isolated mouse islets using alloantisera to mouse Ia antigens (Figure 1). Treatment of isolated mouse islets with anti-Ia antisera and complement caused a complete loss of these cells. In addition, the specific monoclonal antibody for mouse dendritic cells plus complement also destroyed the intra-islet Ia-positive cells.

Most importantly, pretreatment of the islets with the antidendritic cell antibody prevented rejection of the islet allografts (Figure 2), a result similar to our previous findings on pretreating the islets with anti-Ia antibody and complement prior to transplantation. Thus in the mouse, at least, the Ia-positive passenger lymphoid cell responsible for the induction of rejection of islet allografts appears to be a dendritic cell.

FETAL PANCREAS ALLOGRAFTS

Prevention of rejection of allografts of the fetal pancreas by culture in vitro has not been as successful as that with allografts of isolated adult islets (55). In vitro culture of donor fetal mouse pancreas in the presence of 95% O_2 for three weeks produced only a slight to moderate reduction in immunogenicity of the fetal allografts. Pretreatment of donor rats with a donor liver extract, procarbazine hydrochloride, and a single injection of ALS has prevented rejec-

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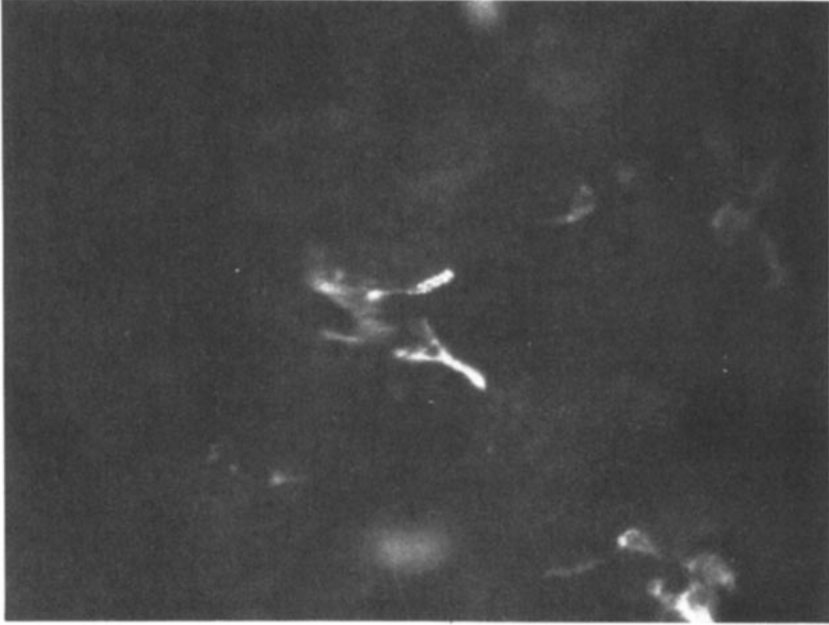

Figure 1 Fluorescent photomicrograph of an Ia$^+$ cell (dendritic cell) in an isolated mouse islet.

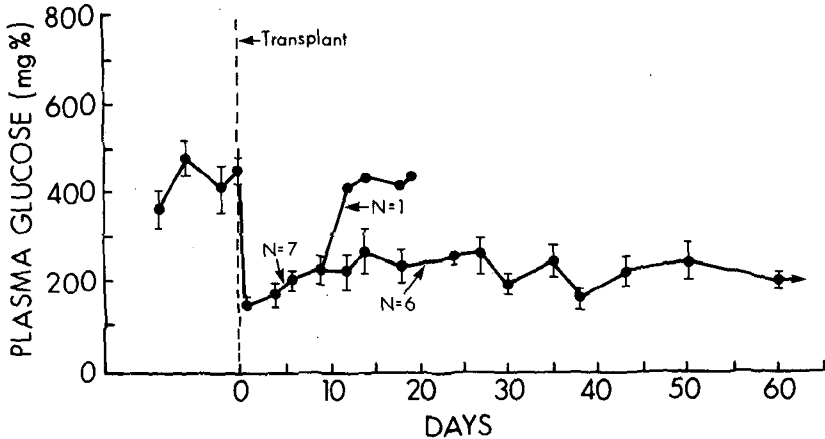

Figure 2 Plasma glucose levels in diabetic recipient mice (C57BL/6J) following transplantation of donor islets (B10.BR/SgSnJ) pretreated with a monoclonal antidendritic cell antibody and complement.

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tion of fetal pancreas allografts transplanted across a minor histocompatibility barrier, but this regimen did not prevent rejection across a major barrier (56). Further, treatment of fetal mouse pancreas with anti-Ia antiserum and complement did not prevent rejection when transplanted across major histocompatibility barriers.

This difference between adult islets and the fetal pancreas is probably due to the large amount of immature lymphoid tissue present in the fetal pancreas. Large masses of lymphoid tissue have been observed in established isografts of fetal mouse pancreas (55). Apparently the in vitro culture conditions using 95% O_2 or treatment with anti-Ia antisera do not eliminate the lymphoid cells below the critical level required for prevention of rejection.

Lafferty's group has developed a method for the isolation of pro-islets from fetal mouse pancreas (57). Pro-islets are composed of undifferentiated tissue elements that differentiate into islets following transplantation as isografts. In vitro culture of fetal pro-islets for ten days in 95% O_2 resulted in approximately a 50% survival of allografts at four weeks after transplantation, whereas intact fetal pancreases cultured under the same conditions were rejected at this time (58). The use of fetal pro-islets is one approach to the problem of rejection of fetal islet tissue, but the yield of islet tissue is low: Pro-islets from eight donor fetal pancreases were required to induce normoglycemia in diabetic recipients. The prevention of rejection of fetal pancreas allografts is an important area of investigation, particularly because the fetal pancreas may be usable as a source of islet tissue for human transplants.

ISLET XENOGRAFTS

After Lafferty et al demonstrated that culture of donor mouse thyroids in 95% O_2 would prevent allograft rejection, Sollinger et al (59) used the same approach to prolong survival of thyroid xenografts (rat to mouse). We found that low-temperature culture of donor rat islets and a single injection of ALS into diabetic mice would prevent rejection of the xenografts with a 70% survival at 100 days (60). We also found that high-oxygen culture of rat mega-islets for seven days prior to transplanting the xenografts beneath the renal capsule of diabetic mice would produce approximately a 45% survival of the xenografts at 60 days (61).

A surprising histologic finding in established rat islet xenografts in mice was the presence of focal accumulation of lymphocytes immediately adjacent to the islet xenografts regardless of whether they were transplanted into the liver via the portal vein or beneath the renal capsule of the recipients (61,62). This reaction was unusual because lymphocytes were not observed in or around established islet allografts.

Lymphocytes were isolated from established islet xenografts and character-

Annual Reviews

ized by immunofluorescence (63). The lymphoid reaction was composed of 80% T lymphocytes and 20% B lymphocytes, with a 3-fold predominance of Lyt–2$^+$ cells as compared to Lyt–1$^+$ cells. These Lyt–2$^+$ cells were not cytotoxic T lymphocytes since the recipients were normoglycemic when they obtained the lymphocytes (70 days after transplantation) and removal of the islet xenograft at this time resulted in a prompt return to a diabetic state. Thus it is possible that the Lyt–2$^+$ cells were suppressor T cells located immediately adjacent to the established islet xenografts.

CANINE ISLET ALLOGRAFTS

The collagenase technique for islet isolation works effectively in small animals. In large animals, however, the yield with the procedure is extremely low because the pancreases of large animals contain wide bands of collagen that become partially digested and entrap the islets. Recently, two methods have been developed that permit mass isolation of islets from the canine pancreas. We found that strips of Velcro in the digestion tubes would retain the partially digested collagen and thus permit the isolation of 40,000–60,000 islets from a single canine pancreas (64). Scharp's group (65) has developed an automated method for mass isolation of canine islets. Intrasplenic autografts of islets isolated by this automated procedure would maintain normoglycemia in total-pancreatectomized dogs.

These techniques for islet isolation from the pancreases of large animals have enabled studies to determine whether the procedures that prevented islet allograft rejection in rats and mice would also prevent rejection of massive transplants of islets in the dog. Culture of donor canine islets at 24°C for seven days prior to transplantation into the mesentery of normal recipients, in conjunction with a single injection of goat anti-dog lymphocyte serum, resulted in a 75% survival of the islet allografts at 2–3 weeks after transplantation; 25% survived in controls. These encouraging findings suggest that the passenger leukocyte concept also applies to islet allografts in dogs.

As indicated above, Ia$^+$ dendritic cells were demonstrated in canine islets using a monoclonal anti-human-Ia antibody that cross-reacts with canine Ia antigens. Islet cells, pancreatic ducts, and acini were Ia$^-$. We can thus attempt to destroy the Ia$^+$ dendritic cells with specific antibodies and complement and determine whether rejection of canine islet allografts, like that of mouse islet allografts, can be prevented. Since massive numbers of islets are transplanted into the dog, investigations on the prevention of rejection of canine islet allografts will serve as an excellent prototype for subsequent islet transplantation in human diabetes.

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HUMAN ISLET TRANSPLANTS

The barriers to the application of islet transplantation to human diabetes are now not conceptual but technical. Methods for the prevention of rejection of islet allografts and xenografts in rats and mice without the continuous use of immunosuppressive drugs, and their initial encouraging application to large animals, suggest that the passenger leukocyte concept should also apply to human islet transplants. The technical problems remaining are the development of techniques for the mass isolation of human islets, determination of the optimum procedure for prevention of rejection of islet allografts in large animals, and selection of the optimum and safest site for islet transplantation.

Recently developed techniques for the mass isolation of islets from the canine pancreas provide the basis for the mass isolation of human islets. In the past, human islet transplants were attempted without success; the transplanted tissue failed to function. This is not surprising since the technology did not exist for isolating massive quantities of intact, viable islets from the human pancreas.

Transplantation of islets in human diabetes requires maintenance of the islet allografts without continuous immunosuppression of the recipients. This has been accomplished in preventing rejection of islet allografts in small animals. Five procedures have been used successfully in rats and mice: low-temperature culture of donor islets and a single injection of ALS; culture of donor mega-islets in 95% O_2; treatment of donor islets with anti-Ia antibody and complement; preimmunization of recipients with donor blood depleted of Ia-bearing cells; and preimmunization of recipients with UV-irradiated donor blood. Since they involve minimal or no immunosuppressive treatment of the recipients, these procedures could be used in treating humans. The successful application of these procedures in prevention of rejection of massive islet allografts in dogs should demonstrate their applicability to humans.

Preventing rejection of fetal pancreas allografts is more difficult, but procedures developed to obtain pro-islets from the mouse fetal pancreas may eliminate lymphoid elements from the transplants and permit success.

The site of islet implantation in humans must be safe and easily accessible during implantation or removal of the graft. Insulin secreted at the site should be released into the portal venous system. The omentum and the mesentery of the small intestine meet these criteria. We have found that isografts of rat islets placed on the surface of the omentum and encased by a small strip of peritoneum maintain normoglycemia in diabetic recipients, and removal of the grafts cause a return to diabetic status (66). If such results can also be accomplished in the dog, then the omentum would be the ideal site for human islet transplantation.

Annual Reviews

Once these three remaining technical problems have been resolved, we can determine whether the passenger leukocyte concept applies to human islet allografts and, if successful, whether the complications of diabetes can be prevented or arrested by islet transplantation.

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Literature Cited

1. Lacy, P. E., Kostianovsky, M. 1967. A method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35
2. Lacy, P. E., Walker, M. M., Fink, C. J. 1972. Perifusion of isolated rat islets *in vitro*. The participation of the microtubular system in the biphasic release of insulin. *Diabetes* 21:987
3. Scharp, D. W., Kemp, C. B., Knight, M. J., Ballinger, W. F., Lacy, P. E. 1973. The use of Ficoll in the preparation of viable islets of Langerhans from the rat pancreas. *Transplantation* 16:686
4. Ballinger, W. F., Lacy, P. E. 1972. Transplantation of intact pancreatic islets in rats. *Surgery* 72:175
5. Kemp, C. B., Knight, M. J., Scharp, D. W., Lacy, P. E., Ballinger, W. F. 1973. Transplantation of isolated pancreatic islets into the portal vein of diabetic rats. *Nature* 244:447
6. Mirkovitch, V., Campiche, M. 1976. Successful intrasplenic autotransplantation of pancreatic tissue in totally pancreatectomized dogs. *J. Surg. Res.* 24:125
7. Feldman, S. D., Hirshberg, G. E., Dodi, G., Raizman, M. E., Scharp, D. W., Ballinger, W. F., Lacy, P. E. 1977. Intrasplenic islet isografts. *Surgery* 82:386
8. Reckard, C. R., Barker, C. F. 1973. Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. *Transplant. Proc.* 5:761
9. Bowen, K. M., Andrus, L., Lafferty, K. J. 1980. Successful allotransplantation of mouse pancreatic islets to nonimmunosuppressed recipients. *Diabetes* 29:98
10. Reckard, C. R., Franklin, W., Schulak, J. A. 1978. Intrasplenic versus intraportal pancreatic islet transplants: quantitative, qualitative and immunological aspects. *Trans. Am. Soc. Artif. Intern. Organs* 24:232
11. Griffith, R. C., Scharp, D. W., Hartman, B. K., Ballinger, W. F., Lacy, P. E. 1977. A morphologic study of intrahepatic portal-vein isografts. *Diabetes* 26:201
12. Leonard, R. J., Lazarow, A., Hegre, O. D. 1973. Pancreatic islet transplantation in the rat. *Diabetes* 22:413
13. Brown, J., Molnar, I. G., Clark, W., Mullen, Y. 1974. Control of experimental diabetes mellitus in rats by transplantation of fetal pancreases. *Science* 184:1377
14. Mullen, Y. S., Clark, W. R., Molnar, I. G., Brown, J. 1977. Complete reversal of experimental diabetes mellitus in rats by a single fetal pancreas. *Science* 195:68
15. Brown, J., Heininger, D., Kuret, J., Mullen, Y. 1981. Islet cells grow after transplantation of fetal pancreas and control of diabetes. *Diabetes* 30:9
16. Mandel, T. E., Collier, S., Carter, W., Higginbotham, L., Martin, F. I. R. 1980. Effect of *in vitro* glucose concentration on fetal mouse pancreas cultures used as grafts in syngeneic diabetic mice. *Transplantation* 30:231
17. Mauer, S. M., Sutherland, D. E. R., Steffes, M. W., Leonard, R. J., Najarian, J. S., Michael, A. F., Brown, D. M. 1974. Pancreatic islet transplantation. Effects on the glomerular lesions of experimental diabetes in the rat. *Diabetes* 23:748
18. Krupin, T., Waltman, S. R., Scharp, D. W., Oestrich, C., Feldman, S. D., Becker, B., Ballinger, W. F., Lacy, P. E. 1979. Ocular fluorophotometry in experimental diabetes mellitus. The effect of pancreatic islet isografts. *Invest. Ophthal. Vis. Sci.* 18:1185
19. Schmidt, R. E., Plurad, S. B., Olack, B. J., Scharp, D. W. 1983. The effect of pancreatic islet transplantation and insulin therapy on experimental diabetic autonomic neuropathy. *Diabetes* 32:532

Annual Reviews

20. Snell, G. D. 1957. The homograft reaction. *Ann. Rev. Microbiol.* 2:439
21. Elkins, W. L., Guttmann, R. D. 1968. Pathogenesis of a local graft versus host reaction: Immunogenicity of circulating host leukocytes. *Science* 159:1250
22. Lafferty, K. J., Prowse, S. J., Simeonovic, C. J., Warren, H. S. 1983. Immunobiology of tissue transplantation: A return to the passenger leukocyte concept. *Ann. Rev. Immunol.* 1:143
23. Jacobs, B. 1974. Ovarian allograft survival. *Transplantation* 18:454
24. Lafferty, K. J., Cooley, M. A., Woolnough, J., Walker, K. Z. 1975. Thyroid allograft immunogenicity is reduced after a period in organ culture. *Science* 188:259
25. Talmage, D. W., Dart, G., Radovich, J., Lafferty, K. J. 1976. Activation of transplant immunity: Effect of donor leukocytes on thyroid allograft rejection. *Science* 191:385
26. Lacy, P. E., Davie, J. M., Finke, E. H., Scharp, D. W. 1979. Prolongation of islet allograft survival. *Transplantation* 27:171
27. Lacy, P. E., Davie, J. M., Finke, E. H. 1979. Prolongation of islet allograft survival following in vitro culture (24°C) and a single injection of ALS. *Science* 204:312
28. Lacy, P. E., Davie, J. M., Finke, E. H. 1979. Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. *Transplantation* 28:415
29. Opelz, G., Terasaki, P. I. 1974. Lymphocyte antigenicity loss with retention of responsiveness. *Science* 184:464
30. Parr, E. L. 1979. The absence of H–2 antigens from mouse pancreatic ß-cells demonstrated by immunoferritin labeling. *J. Exp. Med.* 150:1
31. Faustman, D., Hauptfeld, V., Davie, J. M., Lacy, P. E., Shreffler, D. C. 1981. Murine pancreatic ß-cells express H–2K and H–2D but not Ia antigens. *Proc. Natl. Acad. Sci. USA* 78:1563
32. Baekkeskov, S., Kanatsuna, T., Klareskog, L., Nielsen, D. A., Peterson, P. A., Rubenstein, A. H., Steiner, D. F., Lernmark, A. 1981. Expression of major histocompatibility antigens on pancreatic islet cells. *Proc. Natl. Acad. Sci. USA* 78:6456
33. Faustman, D., Hauptfeld, V., Lacy, P., Davie, J. 1981. Prolongation of murine islet allograft survival by pretreament of islets with antibody directed to Ia determinants. *Proc. Natl. Acad. Sci. USA* 78:5156
34. Morrow, C. E., Sutherland, D. E. R., Steffes, M. W., Najarian, J. S., Bach, F. H. 1983. Lack of donor specific tolerance in mice with established anti–Ia treated islet allografts. *Transplantation*. In press
35. Morrow, C. E., Sutherland, D. E. R., Steffes, M. W., Kaufman, D., Najarian, J. S., Bach, F. H. 1983. Differences in susceptibility to rejection of mouse pancreatic islet allografts disparate for class I or class II major histocompatibility antigens. *J. Surg. Res.* 34:358
36. Bowen, K. M., Lafferty, K. J. 1980. Reversal of diabetes by allogeneic islet transplantation without immunosuppression. *Aust. J. Exp. Biol. Med. Sci.* 58:441
37. Donohoe, J. A., Andrus, L., Bowen, K. M., Simeonovic, C., Prowse, S. J., Lafferty, K. J. 1983. Cultured thyroid allografts induce a state of partial tolerance in adult recipient mice. *Transplantation* 35:62
38. Zitron, I. M., Ono, J., Lacy, P. E., Davie, J. M. 1981. Active suppression in the maintenance of pancreatic islet allografts. *Transplantation* 32:156
39. Faustman, D., Hauptfeld, V., Lacy, P., Davie, J. 1982. Demonstration of active tolerance in maintenance of established islet of Langerhans allografts. *Proc. Natl. Acad. Sci. USA* 79:4153
40. Faustman, D., Lacy, P., Davie, J., Hauptfeld, V. 1982. Prevention of allograft rejection by immunization with donor blood depleted of Ia–bearing cells. *Science* 217:157
41. Lafferty, K. J., Prowse, S. J., Agostino, M, Simeonovic, C. J. 1983. Effect of tissue culture on immunogenicity. Modulation of tissue immunogenicity. *Transplant. Proc.* 15:1366
42. Lau, H., Reemtsma, K., Hardy, M. A. 1983. Pancreatic islet allograft prolongation by donor-specific blood transfusions treated with ultraviolet irradiation. *Science* 221:754
43. Lance, E. M., Medawar, P. 1969. Quantitative studies on tissue transplanation immunity. IX. Induction of tolerance with antilymphocytic serum. *Proc. Roy. Soc. B.* 173:447
44. Wood, M. L., Monaco, A. P., Gozzo, J. J., Liegeois, A. 1971. Use of homozygous allogeneic bone marrow for induction of tolerance with antilymphocyte serum: dose and timing. *Transplant. Proc.* 3:676
45. Brent, L., Kilshaw, P. J. 1970. Prolongation of skin allograft survival with spleen extracts and antilymphocytic serum. *Nature* 227:898
46. Floersheim, G. L. 1969. A study of combined treatment with chemical immunosuppressants and antilymphocyte serum to prolong skin allograft survival. *Transplantation* 8:392
47. Wood, P., Horsburgh, T., Brent, L. 1981.

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Annual Reviews

Specific unresponsiveness to skin allografts in mice. VI. Graft survival in mice pretreated with blood. *Transplantation* 31:8
48. Opelz, G., Terasaki, P. I. 1980. Dominant effect of transfusions on kindney graft survival. *Transplantation* 29:153
49. Gebel, H. M., Yasunami, Y., Dieckgraefe, B., Davie, J. M., Lacy, P. E. 1983. Ia–bearing cells within isolated canine islets. *Transplantation* 36:346
50. Steinman, R. M., Nussenzweig, M. C. 1980. Dendritic cells: features and functions. *Immunol. Rev.* 53:127
51. Steinman, R. M., Witmer, M. D. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natl. Acad. Sci. USA* 75:5132
52. Nussenzweig, M. C., Steinman, R. M., Witmer, M. D., Gutchinov, B. 1982. A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA* 79:161
53. Steinman, R. M., Gutchinov, B., Witmer, M. D., Nussenzweig, M. C. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613
54. Hart, D. N. J., Fabre, J. W. 1981. Demonstration and characterization of Ia–positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J. Exp. Med.* 153:347
55. Simeonovic, C. J., Bowen, K. M., Kotlarski, I., Lafferty, K. J. 1980. Modulation of tissue immunogenicity by organ culture. *Transplantation* 30:174
56. Mullen, Y., Shintaku, I. P. 1982. Fetal pancreas allografts for reversal of diabetes in rats. II. Induction of lifeterm-specific unresponsiveness to pancreas allografts across nonmajor histocompatibility complex barriers. *Transplantation* 33:3
57. Simeonovic, C. J., Lafferty, K. J. 1982. The isolation and transplantation of foetal mouse proislets. *Aust. J. Exp. Biol. Med. Sci.* 60:383
58. Simeonovic, C. J., Lafferty, K. J. 1982. Immunogenicity of isolated foetal mouse proislets. *Aust. J. Exp. Biol. Med. Sci.* 60:391
59. Sollinger, H. W., Burkholder, P. M., Rasmus, W. R., Bach, F. H. 1977. Prolonged survival of xenografts after organ culture. *Surgery* 81:74
60. Lacy, P. E., Davie, J. M., Finke, E. H. 1980. Prolongation of islet xenograft survival without continuous immunosuppression. *Science* 209:283
61. Lacy, P. E., Finke, E. H., Janney, C. G., Davie, J. M. 1982. Prolongation of islet xenograft survival by *in vitro* culture of rat megaislets in 95% O_2. *Transplantation* 33:588
62. Lacy, P. E., Davie, J. M., Finke, E. H. 1981. Prolongation of islet xenograft survival (rat to mouse). *Diabetes* 30:285
63. Janney, C. G., Davie, J. M., Lacy, P. E., Finke, E. H. 1982. Characterization of lymphocytes from rejected and nonrejected islet xenografts. *Transplantation* 33:585
64. Lacy, P. E., Lacy, E. T., Finke, E. H., Yasunami, Y. 1982. An improved method for the isolation of islets from the beef pancreas. *Diabetes* 31:109
65. Long, J. A., Britt, L. D., Olack, B. J., Scharp, D. W. 1983. Autotransplantation of isolated canine pancreatic islet cells. *Transplant. Proc.* 15:1332
66. Yasunami, Y., Lacy, P. E., Finke, E. H. 1983. A new site for islet transplantation—a peritoneal-omental pouch. *Transplantation* 36:181

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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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COLLAGEN AUTOIMMUNE ARTHRITIS[1]

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INTRODUCTION

Chronic arthritis is one of the most challenging of modern medical problems. In spite of considerable research, the etiology of many forms of arthritis remains obscure. However, several recent discoveries may shed light on the pathogenic mechanisms involved and possibly on the cause. A fundamental discovery was the association between rheumatic disease and genes in the major histocompatibility complex. Particular immune responses may be critical either for the initiation of arthritis or for its perpetuation. We do not know what the immune responses are or whether their importance lies in their specificity or in their magnitude and duration. However, an animal model was recently described that may prove useful in answering these questions. This model provides an example of a specific immune response capable of inducing synovitis and joint destruction.

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DESCRIPTION OF THE MODEL

In 1977 Trentham, Townes & Kang reported that immunizing rats with purified native type II collagen resulted in the development of polyarthritis (1). Immunization via an identical protocol but with other types of collagen or denatured type II collagen was not effective. The arthritis became evident only after an interval of latency and was associated with high levels of both cellular and humoral immunity to collagen (2). Type II collagen emulsified with either incomplete or complete Freund's adjuvant was arthritogenic, and the addition of mycobacteria did not alter the incidence of arthritis. This observation implied that mycobacteria were not essential to the induction of arthritis by collagen and did not amplify the mechanisms involved. Trentham found type II collagen not only arthritogenic but also unusually immunogenic compared to the other interstitial collagens. When the immune responses of rats injected with type II collagen emulsified in either incomplete or complete Freund's adjuvant were compared, the inclusion of mycobacteria had not increased the resulting antibody titer or lymphocyte proliferative responses. In contrast, responses to injection of types I and III collagen were extremely low when these antigens were emulsified in incomplete Freund's adjuvant but were amplified to levels comparable to that of type II collagen by complete Freund's adjuvant. Schoen et al coupled collagen to spleen cells by using a water-soluble carbodiimide (3). They injected the resulting collagen-spleen cells subcutaneously into syngeneic recipients and measured delayed hypersensitivity seven days later as responsiveness to an intradermal challenge with soluble collagen in phosphate-buffered saline. Rats that received type II collagen–coupled cells responded to the challenge, whereas those receiving types I or III collagen did not. Like the arthritis, this response depended upon the collagen being in its native conformation. Denatured Type II collagen coupled to spleen cells did not sensitize the recipients.

Because of type II collagen's distinctly greater arthritogenic and potent immunogenic properties compared to other interstitial collagens, early experiments were directed toward determining if the arthritis it caused emanated from a specific anticollagen immune response or from a nonspecific immunomodulating effect. Another possibility considered was that the arthritis resulted from an immune response to, or an immunomodulating effect of, a contaminant in the collagen preparation. Collagen is isolated from complex tissues and purified by a series of precipitations from solutions of varying pH and ionic strengths. Many of the more powerful protein purification methods such as gel filtration are difficult to apply to native collagen because of its unusual physicochemical properties. The two most likely sources of potential contamination are bacterial products resulting from bacterial growth during the initial isolation of collagen or copurified proteoglycans from the tissue matrix. Bac-

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terial products have both arthritogenic and immunomodulating effects and proteoglycans resemble bacterial peptidoglycans, which have been associated with arthritogenicity in rats. Because of these concerns, the disease was referred to as collagen-induced arthritis rather than collagen autoimmune arthritis until the mechanism of its induction was clarified. A closely related problem was the relationship of collagen-induced arthritis to adjuvant-induced arthritis. If type II collagen had adjuvant properties, collagen-induced arthritis might be a form of adjuvant disease.

Several experiments provided indirect evidence that arthritogenicity was a specific and intrinsic property of type II collagen unrelated to adjuvanticity. When other types of collagen were identically prepared and administered, no arthritis developed (1). In addition the collagen had to be in its native conformation, since gentle thermal denaturation (45°C for 15 minutes) of the collagen abrogated the arthritic response. If a contaminating infectious agent or bacterial product caused this arthritis, the agent must have uniquely infected type II collagen preparations and been very sensitive to heat. Furthermore, type II collagens from several sources were efficient inducers of arthritis including chick sternal cartilage, bovine articular cartilage, and bovine vitreous (1,4). Since the noncollagenous components of vitreous and cartilage differ considerably, it seemed unlikely that a copurified noncollagenous substance was responsible for the arthritis. Finally, rigorous attempts to demonstrate an immune enhancing property of type II collagen failed (5). Thus, an arthritogenic type II collagen preparation had no classic adjuvant properties and was free of contaminating substances with adjuvanticity.

HISTOLOGIC CHANGES

The major histologic changes seen in animals with collagen-induced arthritis include synovial hyperplasia, infiltration of the subsynovial tissue with inflammatory cells (a mixture of polymorphonuclear leukocytes and mononuclear cells), exudation of cells into the joint space, marginal erosion, periostitis, and fraying or fragmentation of the cartilage surface (1,6). These lesions closely resemble those of adjuvant-induced arthritis, the principal difference being granulomas frequently found in the latter disease. They also resemble the lesions seen in humans with rheumatoid arthritis. However, periostitis is present in collagen-induced arthritis but not in rheumatoid arthritis, whereas vasculitis often develops in rheumatoid synovial tissue but not in collagen-induced arthritis.

Although arthritis is usually most severe in ankle joints, for technical reasons Caulfield et al analyzed in detail the knee joints of experimental animals with collagen-induced arthritis (7). Approximately 20% of immunized animals developed these lesions, suggesting that the knee joint is involved in about

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one half or less of animals with clinically evident arthritis in any joint. The earliest lesion seen was deposition of fibrin in the synovium on day 5 after immunization. By day 12, fibrin deposition was extensive, and synovial hyperplasia was present. By day 19, infiltration of the mononuclear and polymorphonuclear cells had begun. Other researchers have described cellular infiltrates in the ankles as early as day 10 (1). Among the interesting negative results presented by Caulfield et al was the failure to detect IgG or C3 in the synovium before day 10. In fact, only 5 of 13 animals sacrificed between days 14 and 20 had detectable IgG in joint tissue. The authors concluded that two stages of collagen-induced arthritis could be defined, an early stage with fibrin deposition and hyperplasia of the synovium and a later stage with cellular infiltration. Klareskog et al characterized the cellular infiltrates by using rabbit anti-Ia antibodies and monoclonal antibodies to cell-surface markers (8). Infiltrates in lesions of animals with early disease had an abundance of Ia-positive cells. Of these positive lymphocytes, the majority were of the "T helper" phenotype, based on their reaction with the monoclonal antibody W3/25, similar in abundance to the T helper cells in the characteristic infiltrates of rheumatoid arthritics. Only a minor fraction reacted with either OX8 antibody (to "supressor/cytotoxic" cells) or with anti-Ig. The authors concluded that functional T cells are required for the induction of collagen-induced arthritis and that this disease and rheumatoid arthritis may share some pathogenic mechanisms.

Both Caulfield et al and Klareskog et al concentrated on changes in synovial tissue (7,8). This focus is justified because the earliest lesions detected by routine light microscopy occur in the synovium, whereas the cartilage generally appears normal until the disease is quite advanced. A major problem is that the inciting antigen (type II collagen) has never been found in synovial tissue. If collagen-induced arthritis is an "autoimmune" mediated disease, then it would seem that the most important immunologic reactions would occur at the site of the autoantigen. Relatively few studies of changes in the cartilage during the course of disease are available. DeSimone et al noted a loss of proteoglycan, manifested by a loss of Safranin O positive staining material on the cartilage surface beginning at the time cellular infiltrates first appeared (9). Chondrocytes were obtained from the superficial zone of articular cartilage and subjected to transmission electron microscopy. During one month of disease, these chondrocytes lost ultrastructural detail and gradually degenerated. In agreement with Caulfield, DeSimone et al found electron dense material resembling fibrin deposited on the articular surface very early in the disease. They also noted that synoviocytes on the articular surface contained little rough endoplasmic reticulum but numerous mitochondria. Late in the disease, the proportion of degenerating chondrocytes increased, and dying cells appeared to "lie within moats of amorphous electron dense material."

Annual Reviews

Morphologic changes in the collagen itself, however, were not striking. Collagen content apparently increased, probably reflecting only a relatively larger loss of proteoglycans.

IMMUNOPATHOLOGY

Although considerable evidence indicates that collagen-induced arthritis is caused by an immune response to type II collagen, the mechanism by which arthritis develops is not yet fully understood. Among individual animals within a susceptible strain, the magnitude of immune responsiveness correlated with the presence of arthritis (2,10). In addition, arthritis was not apparent until high levels of both IgM and IgG anticollagen antibodies were detected in the animals' sera (11). Clague et al examined three rat strains for susceptibility to arthritis and found that the strain with the highest incidence of arthritis also had the highest overall antibody responses (12). When both antibody levels and cell-mediated immunity were measured, the mutual correlation was good; and both correlated positively, although less well, with the presence of arthritis (2). Arthritis was never seen without high levels of collagen immunity, but high levels of collagen immunity developed in the absence of arthritis. Thus collagen immunity appeared to be an essential but not by itself a sufficient stimulant for the development of arthritis.

In more direct studies of the immunopathology of disease, Trentham et al reported that arthritis could be passively transferred from immunized donors to syngeneic unimmunized recipients with cells but not with serum (13). These experiments suffered from several deficiencies, which made interpretation of their significance difficult. A large number of cells was necessary, with most successful transfers requiring $6-10 \times 10^8$ cells; the fewest required was 4×10^8. B cells were present in the transferred population. Cells from either arthritic or nonarthritic donors would transfer disease, although cells from arthritic donors were somewhat more efficient (40% successful compared to 28%). Neither cellular reactions nor antibodies to collagen were detected in recipients. Since arthritis after collagen injection has never been reported in the absence of measurable anticollagen reactivity, it is not clear if the transferred disease involves the same mechanism as the disease incited by active immunization. In addition, the question of whether arthritis can be transferred by T cells alone is unanswered. Thirty animals were given sera from arthritic donors, but none developed arthritis, although the antibody levels achieved by passive administration of serum were much lower than those of the actively immunized donors (mean titer of 5.7 as compared to 12 when measured by passive hemagglutination).

We have now successfully transferred this disease with an immunoglobulin-rich serum concentrate (14). The concentrate was obtained from acutely arthritic

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outbred Wistar rats after immunization with chick type II collagen in incomplete Freund's adjuvant. Recipients received approximately the amount of immunoglobulin contained in the sera of two donors. By a combination of ammonium sulfate precipitation, affinity chromatography, and gel filtration chromatography, we were able to isolate an IgG fraction of this serum that retained arthritogenic activity. IgM from an equivalent volume of serum did not transfer disease. Passively transferred arthritis was evident within 24–48 hr of antibody administration. Although the histopathology of the disease resembled that of arthritis after immunization with type II collagen, the passively transferred arthritis was transient.

Since type II collagen is not present in synovial tissue, it was necessary to be sure that the disease was not caused by circulating immune complexes deposited in the synovium. In fact, injection of preformed collagen immune complexes into joints can cause synovitis (15). The presence of circulating immune complexes would explain the prominent synovitis and the failure of some rats with high antibody levels to develop arthritis. Several experiments were undertaken: (*a*) Sera from acutely arthritic rats were assayed for immune complexes by radioactive Clq binding, but no complexes were detected. (*b*) Collagenase was added to serum concentrate in order to remove the collagenous component of the putative complexes, but this treatment did not alter the ability of the concentrate to transfer arthritis or the antibody level as measured by enzyme-linked immunoassay. However, adding native collagen did inhibit antibody binding; and the level was totally restored by collagenase digestion, proving that the collagenase was active and capable of removing collagen from complexes. (*c*) Rats were then immunized with radiolabeled collagen and their acutely arthritic joints analyzed for the isotope. Neither joints nor sera obtained at the onset of arthritis contained the label. For these reasons, it was unlikely that a circulating immune complex was involved.

We postulated that collagen arthritis is a true autoimmune disease initiated by binding of antibody to autologous collagen in the affected joints. In support of this hypothesis, we were able to absorb the arthritogenic antibody from serum with homologous type II collagen, demonstrating both the presence of antiself and its essential role in the passive transfer of disease (16). The question remaining was whether the transferred disease had the same pathogenesis as did disease caused by active immunization. Although this question has not been answered conclusively, considerable indirect evidence denotes that antibody plays a critical role in the development of arthritis after the injection of type II collagen. By immunofluorescence, we demonstrated both IgM and IgG on the surface of articular cartilage when arthritis first became apparent. These studies did not, however, prove that the antibody identified in the joints was specific for collagen. To determine the specificity of the antibodies present, we removed acutely arthritic joints, homogenized them, and washed them

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extensively with phosphate-buffered saline to remove nonspecific immunoglobulin. Specific antibodies were eluted with glycine-HCl, pH 2.8, and tested for their reactivity with collagen. When total Ig was quantitated, specific antitype II collagen antibodies proved to be concentrated 15-fold in the eluate compared with their concentration in sera obtained simultaneously from the same source.

Several other studies have underscored the importance of humoral mechanisms in initiation of arthritis. Morgan et al depleted complement in rats immunized with type II collagen by using cobra venom factor (17). Arthritis was delayed in onset until complement levels returned toward normal. This observation was confirmed by Kerwar et al, who later showed that complement was also critical to passively transferred arthritis (18). When rats depleted of complement received arthritogenic doses of anticollagen Ig, joint inflammation failed to develop, although antibody could be demonstrated on articular surfaces by immunofluorescence. Helfgott et al were able to suppress arthritis by treating Sprague-Dawley rats with anti-mu antiserum to prevent B cell maturation (19). These rats had reduced antibody responses to type II collagen injections and a reduced incidence of arthritis. The magnitude of the delayed hypersensitivity response to collagen as measured by radiometric ear assay was comparable to that of rats treated with an irrelevant antiserum.

These data should not be construed to exclude the involvement of cell-mediated reactions in the disease that develops after immunization. The role of T effector cells has largely been neglected, so there are insufficient data to assign them a specific role. Of course T helper cells must be involved in generation of the antibody response since type II collagen is a T-dependent antigen (20–22).

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EXTRAARTICULAR MANIFESTATIONS

Type II collagen is located in parts of the body other than joints. Therefore, it is reasonable to expect autoimmunity to type II collagen to have extraarticular manifestations. When collagen arthritis was first described, however, a general survey of internal organs failed to identify such lesions. Since then, at least two sites of collagen autoimmune tissue damage have been identified.

We found that 14% of Wistar outbred rats developed inflammatory nodules in external ear tissue after immunization with bovine type II collagen (23). The ear lesions became apparent much later than the arthritis and were independent of it. The mean time of onset was 50 days after immunization. On histologic examination, we saw marked destruction of the cartilage plate with focal areas of poorly organized regeneration near the center of the lesions. The lesions began with subperichondral infiltration of inflammatory cells and progressed centrifugally. The lesions were easily distinguishable from the ear

Annual Reviews

nodules of animals with adjuvant disease, which tended to be subcutaneous and not primarily chondritic. Immunofluorescence demonstrated deposition of IgG and C3 in early lesions. McCune et al described a similar lesion in Sprague-Dawley rats immunized with chick type II collagen, although the incidence was much lower (24). We found that inbred Wistar-Lewis rats were resistant to the development of these ear lesions in spite of being just as susceptible to collagen arthritis as simultaneously immunized outbred Wistar rats.

The histopathology of collagen-induced chondritis resembles that of relapsing polychondritis in humans. Relapsing polychondritis is a rheumatic disease of unknown etiology characterized by episodic inflammation of the external ear, nasal septum, and joints. It may be complicated by hearing loss and death from collapse of the trachea. Several reports have described antitype II collagen antibodies in the sera of patients with relapsing polychondritis (25–28).

Another site of collagen autoimmune tissue damage is the inner ear. We found that approximately 75% of Wistar rats immunized with bovine type II collagen had otospongiosus, vestibular dysfunction, and hearing loss (29,30). Immunofluorescent staining demonstrated type II collagen in the involved areas. Infection and other trivial explanations for these findings have not been entirely ruled out but are unlikely. Because some of the lesions resembled those of humans with Meniere's disease and others those of otosclerosis, we tested sera from patients with those diseases for antibodies to collagen. Some sera samples from patients with each disease contained antitype II collagen antibodies (31). In contrast, none of the patients had antibodies to types I or IV collagen.

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COLLAGEN ARTHRITIS IN MICE

Although early studies were confined to rats, Courtenay et al found that some mouse strains were also susceptible to collagen arthritis (32). This discovery permitted more detailed analysis of the genetic control of both susceptibility to arthritis and immune responsiveness to type II collagen. Additionally, comparison of the immune responses in the two species became possible to determine important similarities or differences. In both rats and mice, immunization with native type II collagen is essential for induction of arthritis; other types of collagen or denatured type II collagen are ineffective. In both species, arthritis is associated with high levels of collagen immunity (33), and disease can be transferred with a concentrate of immune sera (34).

There are also important differences. Under the conditions of immunization employed in mice, quantitatively equal antibody responses follow the injection of either native or denatured type II collagen (33). These data confirm that the difference in antibody concentration is not the cause of arthritis. In addition, the antibodies generated in response to denatured collagen cross-react

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extensively with native collagen but apparently do not induce arthritis. In contrast, anti-native chick type II collagen antibodies have limited cross-reactivity with denatured type II collagen and only 50% cross-reactivity with native bovine type II collagen. Cellular responsiveness as measured by lymphocyte proliferation in vitro also shows extensive cross-reactivity. Cells from mice immunized with either native or denatured chick type II collagen respond equally well to chick native or denatured type II collagen, bovine type II collagen, and rat type I collagen. No cells have been identified that react exclusively with native collagen. In contrast, studies in rats show type and conformation specificity of the proliferative response to collagen (2).

Collectively these observations suggest that a qualitative property of the immune response to native type II collagen may be important for the induction of arthritis. In particular, a subset of anti-native type II collagen antibodies may be important. This possibility is strengthened by the results of passively transferring of arthritis in mice (34). Disease induced by immunization is H–2 linked and associated with a high immune response to type II collagen. Several strains, however, have high immune responses to type II collagen but do not develop arthritis. These strains are susceptible to passively transferred disease. Several strains with independent H–2 haplotypes were administered serum concentrate from immunized susceptible mice, and individuals in each of the strains developed arthritis. Haplotypes of both high- and low-responder strains were used.

Based on these results, we postulate that injection of native type II collagen results in the production of diverse antibodies. Only a limited number of these are arthritogenic, and arthritis depends upon the presence of sufficient arthritogenic antibodies. To test that hypothesis, we have developed a series of monoclonal antibodies to type II collagen. Donor immune splenocytes were obtained from susceptible DBA/1 mice. These were fused with P3X63Ag8653 myeloma cells, and anticollagen antibody producing cell lines were isolated. At least one hybridoma has been identified that induces arthritis when carried as an ascites tumor in BALB/c × DBA/1 recipients (35). Too few cell lines are available at present to allow identification of the characteristics necessary for arthritogenicity; however, we intend to analyze a sufficient number to permit comparison of epitope specificity and isotype restriction of arthritogenic and nonarthritogenic antibodies. The simplest explanation for arthritogenicity at present seems to be that only a limited number of epitopes are exposed in vivo, and only antibodies reactive with those epitopes can initiate arthritis.

RELATIONSHIP TO ADJUVANT ARTHRITIS

Although type II collagen is an unusually potent immunogen, its arthritogenic effect does not appear to be related to adjuvanticity. Adjuvant arthritis, on the

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other hand, is intimately associated with immunization by adjuvant-active compounds. Although collagen arthritis occurs only in the presence of specific immunity to the immunizing type II collagen, adjuvant arthritis can be induced by immunization with compounds against which specific immune responses do not develop (38). These data suggest that events in the induction phase of these two diseases differ in significant aspects. Collagen arthritis is not a type of adjuvant arthritis. However, the converse possibility has not been entirely resolved. That is, the arthritis of adjuvant disease may be related in some way to collagen autoimmunity. Adjuvant disease is linked to the RT–1 major histocompatibility complex, and the haplotypes involved are also those that control susceptibility to collagen arthritis and the immune response to type II collagen (37).

Several investigators have reported immune reactions to collagen in rats with adjuvant arthritis. Steffen & Wick found delayed hypersensitivity reactions (38). Trentham et al recorded cellular reactivity to type II collagen using the radioactive ear assay and specific anticollagen antibodies (39). Holoshitz et al noted that T-cell lines from rats immunized with mycobacteria cross-reacted with type II collagen (40). Such T-cell lines also induced arthritis in unimmunized recipients.

Naturally, the mere presence of immune reactions to collagen in adjuvant arthritis does not prove their involvement in its pathogenesis. One approach to establishing a causal relationship has been to alter the host's immune responsiveness to type II collagen and then administer arthritogenic doses of complete adjuvant. This procedure is somewhat analogous to preventing experimental autoimmune encephalomylitis by preadministering basic protein in incomplete Freund's adjuvant (resulting in immunity to basic protein but not disease) prior to injecting encephalitogenic doses in complete Freund's adjuvant (41). In studies of arthritis, Lewis rats were given an alum flocculate of bovine type II collagen followed by an arthritogenic dose of *Mycobacteria butyricum* in oil (42). This immunization regimen resulted in low levels of anticollagen antibodies, and the pretreatment with collagen reduced the severity of the arthritis that developed. The effect could be duplicated by preadministration of anti–type II collagen antibodies followed by immunization with mycobacteria. Trentham & Dynesius-Trentham injected Lewis rats with syngeneic spleen or red blood cells to which chick type II collagen had been coupled by using carbodiimide (43). Previous studies had shown that this regimen suppressed collagen arthritis and the immune response to type II collagen (44). The animals were subsequently injected with N,N-diotadecyl-N′,N′-bis(hydroxyethyl) propanediamine (CP–20961), a synthetic compound with adjuvant activity and the ability to induce arthritis in rats. Rats pretreated with collagen-coupled cells had attenuated disease. In contrast to mycobacteria-induced adjuvant disease, arthritis induced in rats by CP–20961 in the absence of pretreatment

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did not involve demonstrable anticollagen reactivity. However, rats pretreated with ovalbumin coupled to red blood cells and subsequently given CP–20961 did have positive responses to type II collagen in the radiometric ear assay. Why pretreatment with ovalbumin induced this response is not clear. None of the animals had anticollagen antibodies regardless of pretreatment; therefore, it is unlikely that the mechanism of disease prevention was the same as in the studies of Welles & Battisto (42). Conceivably, the pretreatment regimen in both studies led to nonspecific suppression of the immune response or altered phlogistic factors affecting the development of arthritis.

Two additional studies have addressed the question of whether alteration of responsiveness to collagen or mycobacteria could affect arthritis. The conclusions reached were different from those of either Welles & Battisto (42) or Trentham & Dynesius-Trentham (43). Cremer et al found that intravenous injection of type II collagen induced profound immunologic unresponsiveness to type II collagen and resistance to collagen arthritis but did not protect rats from arthritis when they were subsequently given arthritogenic injections of mycobacteria (45). Iizuka & Chang administered mycobacteria to newborn rats, inducing refractoriness to challenge with arthritogenic mycobacterial preparations without affecting their ability to develop arthritis in response to type II collagen (46). The latter authors also confirmed that type II collagen has no adjuvant properties by injecting EL–4 cells in an emulsion containing type II collagen. At low doses of collagen, no alteration of either cellular or antibody reactivity to the EL–4 cells was noted. At high collagen doses the response to EL–4 was suppressed.

In summary, some rats with adjuvant arthritis also have collagen autoimmunity, but the evidence that this phenomenon is of pathogenic importance is limited. We believe the balance of evidence suggests that adjuvant arthritis and collagen arthritis have basically different pathogenic mechanisms.

GENETIC CONTROL

Two variables are important in the genetic control of collagen arthritis. First is the immune response to collagen. Second is the susceptibility to arthritis. In initial studies, native collagen seemed to have T-independent antigenic properties (47), but subsequent research conclusively demonstrated T dependence (20–22). Both responsiveness (the magnitude of the immune reaction to collagen) and susceptibility (to the development of arthritis) after immunization are controlled by genes within or linked to the major histocompatibility loci in rats and mice.

Although the structures of interstitial collagens are quite similar, Hahn et al found antibody responses in rats and rabbits to be essentially type-specific (48). Nowack et al showed that the antibody responses of mice to bovine types

Annual Reviews

I and II collagen were controlled by genes in the H–2 complex (49). High responders to these two types of collagen were separable according to H–2 haplotype. It is of interest that rats and mice tend to produce antibodies reactive with epitopes in the body of the molecule and dependent upon the latter's conformation (50). These epitopes have been referred to as helical determinants.

Much less information is available on the specificity of delayed hypersensitivity reactions to collagen. Adelmann et al showed that, after sensitization of guinea pigs with type I collagen, both native and denatured collagen elicited equally good skin-test responses (51). Beard et al produced a similar reaction by injecting guinea pigs with type II collagen (52). Rosenwasser et al immunized mice with denatured type II collagen, after which their T cells proliferated when cultured with native collagen, denatured collagen, and even synthetic nonhelical collagen-like peptides (53). This response was under the control of H–2 linked Ir genes. The common denominator in each of the above-mentioned studies is that delayed hypersensitivity reactions depend neither upon the conformation of collagen nor even upon the type of collagen for either induction of immunity or elicitation of a response. The authors suggested that T cells from mice may react with only two major determinants.

From these data, the T-cell response to collagen seems much more restricted than the B-cell response in the sense that fewer distinct epitopes are recognized. Also T cells do not recognize conformation-dependent epitopes. Since collagen arthritis is highly dependent upon immunization with native collagen, differences in T-cell specificity probably do not account for susceptibility to arthritis. There are two caveats to this statement, however. First, all the data were obtained either in living animals or from bulk cultures, and small but significant T-cell subsets may not have been detected. Second, the mice used to obtain these data are of the H–2^b phenotype and respond to type II collagen but are not susceptible to arthritis.

In rats, responsiveness to bovine type II collagen and arthritis is linked to the RT1 major histocompatibility locus. $RTH1^u$, $RT1^l$ and $RT1^a$ type rats develop strongly reactive skin tests and arthritis after immunization with type II collagen (54,55). Of these, susceptible rats also develop high antibody levels; moreover, some rats with high antibody levels are not susceptible to arthritis.

Wooley et al found responsiveness to type II collagen and susceptibility to arthritis linked to the H–2 locus in mice (56). As with rats, all susceptible strains were also high responders for production of antibody, but some strains with high responses were resistant to arthritis. This situation is complicated, however, because both responsiveness and susceptibility vary depending on the species from which the immunizing type II collagen originated (57). H–2^q mice are highly susceptible to arthritis after immunization with chick type II collagen but only slightly or moderately susceptible to arthritis produced

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by bovine or rat type II collagen. H−2^r mice, which readily develop arthritis after immunization with bovine type II collagen, are resistant to arthritis from chick or rat type II collagen injection. Both strains develop intermediate or high antibody levels in response to type II collagen from any of the three species. Murine type II collagen is difficult to obtain in sufficient quantity to permit extensive testing, and the degree of cross-reactivity between murine collagen and sera from the various strains and collagen species tested in these experiments is unknown.

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COLLAGEN AUTOIMMUNE ARTHRITIS IN HUMANS

Considerable effort has been devoted to ascertaining the presence and specificity of collagen autoimmunity in human diseases, particularly rheumatic diseases. Because collagen autoimmunity occurs in individuals with rheumatoid arthritis, collagen-induced arthritis of rodents has been suggested as an appropriate model for the study of rheumatoid arthritis. The relationship between these diseases was reviewed recently (58,59). Support for a relationship depends primarily upon the similarity of the histopathologic changes, the presence of collagen autoimmunity in both, and their mutual association with major histocompatibility genes controlling collagen autoimmunity. Since there are no histopathologic changes pathognomonic of rheumatoid arthritis, morphologic evidence of similarity must be interpreted with caution. This makes the finding of collagen autoimmunity in rheumatoid arthritis a crucial one.

Steffen reported in 1963 that antibodies to collagen were present in sera from patients with rheumatoid arthritis and proposed that collagen autoimmunity may be involved in the pathogenesis of the disease (60). This hypothesis was attractive because it explained both the systemic nature of rheumatoid arthritis (collagen was known to be widely distributed in the body) and its chronicity. Over the ensuing 15 years, however, the hypothesis fell into disfavor. Results from assays for anticollagen antibodies in human sera proved capricious. Consequently many investigators reached one of two disparate conclusions. Some, who were unable to confirm significant antibody levels in any patient's serum, suggested that most or all of the activity could be accounted for by anomalous reactions (61–63). Others found anticollagen antibodies in patients with unrelated diseases (64,65) and stronger reactivity with denatured collagen than native (66), suggesting that, even if present, the antibodies were secondary to inflammation and of no pathogenic significance.

Several factors led to a resurgence of interest in the possible role of collagen autoimmunity as a cause of arthritis. One was the discovery that collagen exists in at least five major distinct forms and one of these, type II collagen, is limited in its bodily distribution primarily to hyaline cartilage. A reaction specific for type II collagen might thus explain the tendency of rheumatoid

Annual Reviews

arthritis to involve diarthrodial joints. Also, either cross-reactions or specific reactions with other collagens might account for some of the extraarticular manifestations of disease. Andriopoulos et al reported that sera from patients with rheumatoid arthritis contained antibodies reactive with each of the three interstitial collagens (67). The prevalence of antibodies was about the same (about 70%) for each type of collagen, but titers were generally higher against type II collagen, ranging up to 1:512. No correlation was apparent with stage of disease, rheumatoid factor, or antinuclear antibodies. Using selected sera, this group found that reactions to one type of collagen could not be inhibited by other types of collagen, thus demonstrating considerable specificity (68).

We have tested sera from rheumatoid arthritis patients for antibodies not only to types I, II, and III collagen but also to types IV and V (69). Human collagens were used in both native and denatured form, and control sera were obtained from patients with other chronic arthritic diseases. Although there was a high prevalence of antibodies to all of these collagens in sera from rheumatoid arthritis patients, many patients with other types of arthritis also had detectable antibodies. Most of the reactivity was directed against denatured collagen, and there was substantial cross-reactivity of the antibodies with all of the collagens used. A few sera from rheumatoid arthritis patients had relatively high levels of antibodies specific for native type II collagen. These data suggest that antibodies to denatured collagen may frequently accompany chronic inflammatory arthritis regardless of its etiology but that some patients with rheumatoid arthritis have qualitatively different antibodies. The significance of any of these antibodies is unknown.

The situation is further complicated by a growing conviction among many investigators that rheumatoid arthritis is mediated primarily by delayed hypersensitivity reactions. This stance evolved primarily because investigation of rheumatoid factors proved disappointing in explaining the pathogenesis of arthritis and because large numbers of T cells were found in synovial tissues and fluids of rheumatoid arthritics (8,70). Synovial tissue appeared to be driven by infiltrating T cells to progressively invade and destroy the joint cartilage. Trentham et al found cellular reactions specific for types II and III collagen in approximately 75% of patients with rheumatoid arthritis, but no response to type I collagen or denatured collagen (71). We tested patients with rheumatoid arthritis for cellular reactions to collagen using a different assay system and found reactivity with types I, II, and III collagen (72). The strongest reactivity was with denatured collagen. Occasional patients with inflammatory arthritis other than rheumatoid arthritis also had reactivity, but far less prevalently. Smolen et al confirmed that patients with rheumatoid arthritis additionally had cellular reactivity to denatured collagen (73). These studies were extended by Solinger et al, who found that cellular reactions to collagen in humans are linked to the major histocompatibility locus (74). High responses were associated with HLA-DR4, a haplotype found in 75% of patients with rheumatoid arthritis (75,76).

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INDUCTION OF UNRESPONSIVENESS

Since collagen autoimmunity can cause arthritis, it is reasonable to expect that collagen-induced, antigen-specific suppression of the immune response would abrogate arthritis. Staines et al tested that possibility by pretreating rats with intravenous collagen, sera from collagen immunized donors, and a combination of both (77). Pig collagen (but not rat collagen) was used for pretreatment and challenge, after which antibodies were measured at intervals to both pig and rat collagen. All three pretreatment regimens were effective in reducing the incidence and severity of arthritis and levels of antibody to collagen. The injection of both antiserum and collagen on consecutive days was more effective than either injection alone. The authors commented that pretreatment with anticollagen antiserum tended to yield greater suppression of rat collagen specific antibodies than of pig specific antibodies.

A somewhat different tactic was employed by Schoen et al, who injected rats with chick type II collagen coupled to spleen cells (44). A series of four intravenous injections reduced anticollagen IgG antibody titers in response to subsequent immunization by about 50%. The incidence of arthritis was also reduced by about 60%. An unusual and unexpected finding was that neither hemagglutinating antibodies nor cellular reactions to collagen were affected.

Cremer et al induced unresponsiveness to collagen in rats by injecting soluble type II collagen of either bovine or chick origin (45). A single intravenous injection of 1 mg collagen reduced the incidence of arthritis by 70–90% in various groups. Treatment was effective when given as early as 32 days before immunization or as late as 7 days afterward. IgM, IgG, and delayed hypersensitivity responses were all reduced in treated animals. Injection of either bovine or chick collagen prevented arthritis in response to immunization with bovine collagen.

Trentham has suggested that induction of antigen specific unresponsiveness may be the best way to test whether collagen autoimmunity is involved in human arthritis (59). This may be difficult if the precise reaction involved is not known, since that reaction could not be measured before and after treatment. However, the ability of one species of collagen to suppress arthritis induced by collagen of other species offers the hope that unresponsiveness may depend upon a less restricted property of type II collagen than does disease induction. In addition, the response to collagen in HLA–DR4 positive human subjects may be due to absence of a suppressor cell found in other subjects (78).

SUMMARY

The evidence is now fairly conclusive that collagen-induced arthritis in rodents is mediated by antitype II collagen autoimmunity. Arthritis is probably initiated

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by binding of antibodies to the surface of intact articular cartilage. Many o: the major manifestations of arthritis, including synovial proliferation, pannu: formation, marginal erosion of bone, and destruction of cartilage, can b duplicated by injection of isolated antitype II collagen antibodies. It is no known whether delayed hypersensitivity reactions to collagen can provok similar lesions in the absence of antibody, but circumstantial evidence suggest: they do not. Also clear is that not all anticollagen antibodies are capable o inducing arthritis. The minimal requirements for arthritogenic potential ar currently under investigation but probably include the ability to bind nativ autologous type II collagen. Also IgM antibodies alone are either ineffectiv or are required in relatively higher concentrations than IgG for induction o arthritis.

Autoimmunity to collagen is found in many spontaneous and induced rheu matic diseases other than collagen-induced arthritis. There is at present, how ever, no direct evidence that this autoimmunity actually contributes to th arthritic process. Nevertheless, the human disease most often associated wit collagen autoimmunity is rheumatoid arthritis. In many respects the immun reactions detected in humans with rheumatoid arthritis parallel those of arthriti: in rodents. That is, responsiveness is under the control of genes within o linked to the major histocompatibility locus. High responders are limited t only a few haplotypes. Cell-mediated reactions are most vigorous in respons to denatured collagen and probably have limited specificity for the type o collagen recognized. Antibodies may be separated into at least two groups one with broad specificity for denatured collagen and a second highly specifi for conformation-dependent determinants on native type II collagen. The latte antibodies are of most interest to researchers because they may be like thos that induce arthritis in rodents. There is also ample evidence that antibodie are deposited in the joints of rheumatoid arthritis patients, although the spec ificity of these antibodies is unknown (79–81).

Generally, collagen-induced arthritis is a model of antibody-initiate autoimmunity arthritis. Specifically, it is a model of type II collagen autoim mune arthritis. In consideration of its extraarticular manifestations, it ma justifiably be referred to as type II collagen autoimmune disease. Whether i can be applied to the study of human arthritis as either a general or specifi model, and to which human diseases it has relevance, remain unknown.

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Literature Cited

1. Trentham, D. E., Townes, A. S., Kang, A. H. 1977. Autoimmunity to type II collagen: An experimental model of arthri tis. *J. Exp. Med.* 146:857

Annual Reviews

Annu. Rev. Immunol. 1984.2:199-218. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

2. Trentham, D. E., Townes, A. S., Kang, A. H., David, J. R. 1978. Humoral and cellular sensitivity to collagen in type II collagen–induced arthritis in rats. *J. Clin. Invest.* 61:89
3. Schoen, R. T., Mahlman, H., Trentham, D. E., Perry, L., Greene, M. I., David, J. R. 1982. Autoimmunity induced by type II collagen–coupled spleen cells. *J. Immunol.* 128:717
4. Stuart, J. M., Cremer, M. A., Dixit, S. N., Kang, A. H., Townes, A. S. 1979. Collagen-induced arthritis in rats: Comparison of vitreous and cartilage-derived collagens. *Arth. Rheum.* 22:347
5. Cremer, M. A., Stuart, J. M., Townes, A. S., Kang, A. H. 1980. Collagen-induced polyarthritis in rats: A study of native type II collagen for adjuvant activity. *J. Immunol.* 124:2912
6. Terato, K., Hashida, R., Miyamoto, K., Morimoto, T., Kato, Y., Kobayashi, S., Tajima, T., Otake, S., Hori, H., Nagai, Y. 1982. Histological, immunological and biochemical studies on type II collagen–induced arthritis in rats. *Biomed. Res.* 3:495
7. Caulfield, J. P., Hein, A., Dynesius-Trentham, R., Trentham, D. E. 1982. Morphologic demonstration of two stages in the development of type II collagen–induced arthritis. *Lab. Invest.* 46:321
8. Klareskog, L., Forsum, U., Scheynius, A., Kabelitz, D., Wigzell, H. 1982. Evidence in support of a self-perpetuating HLA–DR–dependent delayed-type cell reaction in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* 79:3632
9. DeSimone, D. P., Parsons, D. B., Johnson, K. E., Jacobs, R. P. 1983. Type II collagen induced arthritis, a morphologic and biochemical study of articular cartilage. *Arth. Rheum.* 26:1245
10. Morgan, K., Clague, R. B., Shaw, M. J., Holt, P. J. L. 1980. Native type II collagen–induced arthritis in the rat. I. Incidence and humoral response to collagen. *Ann. Rheum. Dis.* 39:285
11. Stuart, J. M., Cremer, M. A., Kang, A. H., Townes, A. S. 1979. Collagen–induced arthritis in rats: Evaluation of early immunologic events. *Arth. Rheum.* 22:1344
12. Clague, R. B., Morgan, K., Shaw, M. J., Holt, P. J. L. 1980. Native type II collagen–induced arthritis in the rat. 2. Relationship between the humoral immune response to native type II collagen and arthritis. *J. Rheumatol.* 7:775
13. Trentham, D. E., Dynesius, R. A., David, J. R. 1978. Passive transfer by cells of type II collagen–induced arthritis in rats. *J. Clin. Invest.* 62:359
14. Stuart, J. M., Cremer, M. A., Townes, A. S., Kang, A. H. 1982. Type II collagen–induced arthritis in rats: Passive transfer with serum and evidence that IgG anticollagen antibodies can cause arthritis. *J. Exp. Med.* 155:1
15. Steffen, C., Kovac, W., Endler, T. A., Menzel, J., Smolen, J. 1977. Induction of acute and chronic arthritis by intraarticular injection of preformed collagen-anticollagen complexes. *Immunology* 32:161
16. Stuart, J. M., Tomoda, K., Townes, A. S., Yoo, T. J., Kang, A. H. 1983. Serum transfer of collagen–induced arthritis. II. Identification and localization of autoantibody to type II collagen in donor and recipient rats. *Arth. Rheum.* 26:221
17. Morgan, K., Clague, R. B., Shaw, M. J., Firth, S.A., Twose, T. M., Holt, P. J. L. 1981. Native type II collagen–induced arthritis in the rat: The effect of complement depletion by cobra venom factor. *Arth. Rheum.* 24:1356
18. Kerwar, S. S., Englert, M. E., McReynolds, R. A., Landes, M. J., Lloyd, J. M., Oronsky, A. L., Wilson, F. J. 1983. Type II collagen–induced arthritis. Studies with purified anticollagen immunoglobulin. *Arth. Rheum.* 26:1120
19. Helfgott, S. M., Bazin, H., Trentham, D. E. 1983. Effect of anti-mu antisera on collagen arthritis: Evidence that antibody is required for the induction of disease. *Arth. Rheum.* 26 (Suppl.): S14 (Abstr.)
20. Hedrick, S. M., Watson, J. D. 1980. Genetic control of the immune response to collagen. I. Quantitative determination of response levels by multiple I–region genes. *J. Immunogenet.* 7:271
21. Hedrick, S. M., Watson, J. 1979. Genetic control of the immune response to collagen. II. Antibody responses produced in fetal liver restored radiation chimeras and thymus reconstituted F_1 hybrid nude mice. *J. Exp. Med.* 150:616
22. Hedrick, S. M., Watson, J. D. 1980. Genetic control of the immune response to collagen. III. Coordinate restriction of cellular cooperation and antigen responsiveness by thymus-directed maturation. *J. Immunol.* 125:1782
23. Cremer, M. A., Pitcock, J. A., Stuart, J. M., Kang, A. H., Townes, A. S. 1981. Auricular chondritis in rats: An experimental model of relapsing polychondritis induced with type II collagen. *J. Exp. Med.* 154:535
24. McCune, W. J., Schiller, A. L., Dynesius-Trentham, R. A., Trentham, D. E. 1982. Type II collagen–induced auricular chondritis. *Arth. Rheum.* 25:266

Annual Reviews

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25. Rogers, P. H., Boden, G., Tourtellotte, C. D. 1973. Relapsing polychondritis with insulin resistance and antibodies to cartilage. *Am. J. Med.* 55:243
26. Foidart, J.-M., Abe, S., Martin, G. R., Zizic, T. M., Barnett, E. V., Lawley, T. J., Katz, S. I. 1978. Antibodies to type II collagen in relapsing polychondritis. *N. Eng. J. Med.* 299:1203
27. Ebringer, R., Rook, G., Swana, G. T., Bottazzo, G. F., Doniach, D. 1981. Autoantibodies to cartilage and type II collagen in relapsing polychondritis and other rheumatic diseases. *Ann. Rheum. Dis.* 40:473
28. Rogers, P. H., Boden, G., Tourtellotte, C. D. 1973. Relapsing polychondritis with insulin resistance and antibodies to cartilage. *Am. J. Med.* 55:243
29. Yoo, T. J., Tomoda, K., Stuart, J. M., Kang, A. H., Townes, A. S. 1983. Type II collagen-induced autoimmune otospongiosis. A preliminary report. *Ann. Otol. Rhinol. Laryngol.* 92:103
30. Yoo, T. J., Tomoda, K., Stuart, J. M., Cremer, M. A., Townes, A. S., Kang, A. H. 1983. Type II collagen-induced autoimmune sensorineural hearing loss and vestibular dysfunction in rats. *Ann. Otol. Rhinol. Laryngol.* 92:267
31. Yoo, T. J., Stuart, J. M., Kang, A. H., Townes, A. S., Tomoda, K. 1982. Type II collagen autoimmunity in otosclerosis and Meniniere's disease. *Science* 217:1153
32. Courtenay, J. S., Dallman, M. J., Dayan, A. D., Martin, A. B., Mosedale, B. 1980. Immunization against heterologous type II collagen induces arthritis in mice. *Nature* 283:666
33. Stuart, J. M., Townes, A. S., Kang, A. H. 1982. Nature and specificity of the immune response to collagen in type II collagen-induced arthritis in mice. *J. Clin. Invest.* 69:673
34. Stuart, J. M., Dixon, F. J. 1983. Serum transfer of collagen-induced arthritis in mice. *J. Exp. Med.* 158:378
35. Stuart, J. M., Dixon, F. J. 1983. Identification and characterization of a monoclonal antibody to type II collagen capable of inducing arthritis in mice. *Arth. Rheum.* 26 (Suppl.): S14 (Abstr.)
36. Chang, Y. H., Pearson, C. M., Chedid, L. 1981. Adjuvant polyarthritis. V. Induction by N–acetylmuramyl–L–alanyl–D–isoglutamine, the smallest peptide subunit of bacterial peptidoglycan. *J. Exp. Med.* 153:1021
37. Battisto, J. R., Smith, R. N., Beckman, K., Sternlicth, M., Welles, W. L. 1982. Susceptibility to adjuvant arthritis in DA and F_{344} rats: A dominant trait controlled by an autosomal gene locus linked to the major histocompatibility complex. *Arth. Rheum.* 25:1194
38. Steffen, C., Wick, G. 1971. Delayed hypersensitivity reactions to collagen in rats with adjuvant-induced arthritis. *Z. Immunitätsforsch.* 141:169
39. Trentham, D. E., McCune, W. J., Susman, P., David, J. R. 1980. Autoimmunity to collagen in adjuvant arthritis of rats. *J. Clin. Invest.* 66:1109
40. Holoshitz, J., Naparstek, Y., Ben-nun, A., Cohen, I. R. 1983. Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science* 219:56
41. Lisak, R. P., Zweiman, B., Dzida, L., Rosenblum, F., Rorke, L. B., Barger, G. 1980. *In vitro* response to basic protein in experimental allergic encephalomyelitis: Effect of pretreatment with basic protein in incomplete adjuvant. *Cell Immunol.* 52:443
42. Welles, W. L., Battisto, J. R. 1981. Suppression of adjuvant arthritis by antibodies specific for collagen type II. *Immunol. Commun.* 10:673
43. Trentham, D. E., Dynesius-Trentham, R. A. 1983. Attenuation of an adjuvant arthritis by type II collagen. *J. Immunol.* 130:2889
44. Schoen, R. T., Greene, M. I., Trentham, D. E. 1982. Antigen-specific suppression of type II collagen–induced arthritis by collagen–coupled spleen cells. *J. Immunol.* 128:717
45. Cremer, M. A., Hernandez, A. D., Townes, A. S., Stuart, J. M., Kang, A. H. 1983. Collagen–induced arthritis in rats, antigen-specific suppression of arthritis and immunity by intravenously injected native type II collagen. *J. Immunol.* In press
46. Iizuka, Y., Chang, Y.-H. 1982. Adjuvant polyarthritis. VII. The role of type II collagen in pathogenesis. *Arth. Rheum.* 25:1325
47. Mozes, S., Schmitt-Vern, A. M., Fuchs, S. 1975. The effect of the thymus-independent antigens, collagen and synthetic collagen–like peptides on the requirement of cell cooperation in the immune response to thymus-dependent antigens. *Eur. J. Immunol.* 5:549
48. Hahn, E., Timpl, R., Miller, E. J. 1974. The production of specific antibodies to native collagens with the chain compositions $[\alpha\ 1(I)]_3$ and $[\alpha\ 1(II)]_2\ \alpha\ 2$. *J. Immunol.* 13:421
49. Nowack, H., Hahn, E., Timpl, R. 1974. Specificity of the antibody response in inbred mice to bovine type I and type II collagen. *Immunology* 29:621

Annual Reviews

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50. Timpl, R., Wick, G., Gay, S. 1977. Antibodies to distinct types of collagens and procollagens and their application in immunohistology. *J. Immunol. Meth.* 18:165
51. Adelmann, B. C., Kiranne, J. A., Glynn, L. E. 1972. The structural basis of cell-mediated immunological reactions of collagen: Characteristics of cutaneous delayed hypersensitivity reactions in specifically sensitized guinea pigs. *Immunology* 23:723
52. Beard, H. K., Ueda, M., Faulk, W. P., Glynn, L. E. 1978. Cell-mediated and humoral immunity to chick type II collagen and its cyanogen bromide peptides in guinea pigs. *Immunology* 34:323
53. Rosenwasser, L. F., Bhatnagar, R. S., Stobo, J. D. 1980. Genetic control of the murine T lymphocyte proliferative response to collagen: Analysis of the molecular and cellular contributions to immunogenicity. *J. Immunol.* 124:2854
54. Griffiths, M. M., DeWitt, C. W. 1981. Immunogenetic control of experimental collagen-induced arthritis in rats. II. ECIA susceptibility and immune response to type II collagen (calf) are linked to RTI. *J. Immunogenet.* 8:463
55. Griffiths, M. M., Eichwald, E. J., Martin, J. H., Smith, C. B., DeWitt, C. W. 1981. Immunogenetic control of experimental type II collagen-induced arthritis. I. Susceptibility and resistance among inbred strains of rats. *Arth. Rheum.* 24:781
56. Wooley, P. H., Luthra, H. W., Stuart, J. M., David, C. S. 1981. Type II collagen-induced arthritis in mice. I. Major histocompatibility complex (I region) linkage and antibody correlates. *J. Exp. Med.* 154:688
57. Wooley, P. H., Dillon, A. M., Luthra, H. S., Stuart, J. M., David, C. S. 1983. Genetic control of type II collagen-induced arthritis in mice. Factors influencing disease susceptibility and evidence for multiple MHC-associated gene control. *Trans. Proc.* 15:180
58. Stuart, J. M., Townes, A. S., Kang, A. H. 1982. The role of collagen autoimmunity in animal models and human diseases. *J. Invest. Dermatol.* 79:121
59. Trentham, D. E. 1982. Collagen arthritis as a relevant model for rheumatoid arthritis. Evidence pro and con. *Arth. Rheum.* 25:911
60. Steffen, C., Timpl, R. 1963. Antigenicity of collagen and its application in the serologic investigation of rheumatoid arthritis sera. *Int. Arch. Allergy Appl. Immunol.* 22:333
61. Beard, H. K., Lea, D. J., Ryvar, R. 1979. Anomolous reactions in the hemagglutination assay for anti-collagen antibodies: Studies on patients with rheumatoid arthritis or chronic low back pain. *J. Immunol. Meth.* 31:119
62. Conochie, L. B., Scott, J. E., Faulk, W. P. 1975. A passive agglutination method using collagen-coated tanned sheep erythrocytes to demonstrate collagen-glycosaminoglycan interaction. *J. Immunol. Meth.* 7:393
63. Adelmann, B., Schoning, B. 1980. Binding of native and denatured collagen to immunoglobulins and cold insoluble globulin in serum of patients undergoing orthopedic surgery. *Klin. Wochenschr.* 58:625
64. Michaeli, D., Fudenberg, H. H. 1974. Antibodies to collagen in patients with emphysema. *Clin. Immunol. Immunopathol.* 3:187
65. Suou, T., Hirayama, C. 1980. Antibodies to denatured bovine collagens in sera of patients with liver disease. *Clin. Exp. Immunol.* 39:119
66. Michaeli, D., Fudenberg, H. H. 1974. The incidence and antigenic specificity of antibodies against denatured human collagen in rheumatoid arthritis. *Clin. Immunol. Immunopathol.* 2:153
67. Andriopoulos, N. A., Mestecky, J., Miller, E. J., Bradley, E. L. 1976. Antibodies to native and denatured collagen in sera of patients with rheumatoid arthritis. *Arth. Rheum.* 19:613
68. Andriopoulos, N. A., Mestecky, J., Wright, G. P., Miller, E. J. 1976. Characterization of antibodies to the native human collagens and to their component α chains in the sera and synovial fluids of patients with rheumatoid arthritis. *Immunochemistry* 13:709
69. Stuart, J. M., Huffstutter, E. H., Townes, A. S., Kang, A. H. 1983. Incidence and specificity of antibodies to type I, II, III, IV, and V collagen in rheumatoid arthritis and other rheumatic diseases as measured by ^{125}I-radioimmunoassay. *Arth. Rheum.* 26:1157
70. Froland, S. S., Abrahamsen, T. G. 1979. Lymphocyte populations in blood, synovial fluid and synovial tissue in rheumatoid arthritis. In *Immunopathogenesis of Rheumatoid Arthritis*, ed. G. S. Panayi, P. M. Johnson. Chertsey, Surrey: Redbooks. 25 pp.
71. Trentham, D. E., Dynesius, R. A., Rocklin, R. E., David, J. R. 1978. Cellular sensitivity to collagen in rheumatoid arthritis. *N. Eng. J. Med.* 299:327
72. Stuart, J. M., Postlethwaite, A. E., Townes, A. S., Kang, A. H. 1980. Cell-mediated immunity to collagen and col-

Annual Reviews

lagen α chains in rheumatoid arthritis and other rheumatic diseases. *Am. J. Med.* 69:13

73. Smolen, J. S., Menzel, E. J., Scherak, O., Kojer, M., Kolarz, G., Steffen, C., Mayr, W. R. 1980. Lymphocyte transformation to denatured type I collagen and B lymphocyte alloantigens in rheumatoid arthritis. *Arth. Rheum.* 23:424
74. Solinger, A. M., Bhatnagar, R., Stobo, J. D. 1981. Cellular, molecular, and genetic characteristics of T cell reactivity to collagen in man. *Proc. Natl. Acad. Sci. USA* 78:3877
75. McMichael, A. J., Sasazuki, T., McDevitt, H. O., Payne, R. O. 1977. Increased frequency of HLA–Cw3 and HLA–Dw4 in rheumatoid arthritis. *Arth. Rheum.* 20:1037
76. Stastny, P., Fink, C. W. 1979. Different HLA–D associations in adult and juvenile rheumatoid arthritis. *J. Clin. Invest.* 63:124
77. Staines, N. A., Hardingham, T., Smith, M., Henderson, B. 1981. Collagen–induced arthritis in the rat: Modification of immune and arthritic responses by free collagen and immune anti-collagen antiserum. *Immunology* 44:737
78. Solinger, A. M., Stobo, J. D. 1982. Immune response gene control of collagen reactivity in man: Collagen unresponsiveness in HLA–DR4 negative nonresponders is due to the presence of T–dependent suppressive influences. *J. Immunol.* 129:1916
79. Cooke, T. D., Hurd, E. R., Jasin, H. E., Bienenstock, J., Ziff, M. 1975. Identification of immunoglobulins and complement in rheumatoid articular collagenous tissues. *Arth. Rheum.* 18:541
80. Derek, T., Cooke, V., Richer, S. 1975. Localization of antigen-antibody complexes in intraarticular collagenous tissues. *Ann. NY Acad. Sci.* 256:10
81. Ohno, O., Cooke, T. D. 1978. Electron microscopic morphology of immunoglobulin aggregates and their interactions in rheumatoid articular collagenous tissues. *Arth. Rheum.* 21:516

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION:

Exploring Obscure Relationships

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INTRODUCTION

The ease of induction of specific immune tolerance in neonatal animals has been amply demonstrated since the initial experiments of Billingham et al (1). The notion of neonatal tolerance fits well with the inactivation of self-recognizing clones of immunocompetent cells suggested by the clonal selection theory of Burnet (2). In the neonatal rodent, tolerance has been induced to a large variety of foreign substances, including alloantigens (1, 3–8). However, the duration of the "window" of tolerance is short, and few mice can be tolerized to transplantation antigens more than 72 hr after birth (8).

It is interesting that several investigators have described a nonspecific suppressor cell of in vitro alloreactivity (mixed leukocyte reaction, MLR) in the neonatal spleen during the "window" of tolerance (9–15). The relationship of the suppressor cells to the cellular basis of transplantation tolerance has remained obscure. Most research efforts have attempted to elucidate the mechanism of the maintenance of neonatally induced tolerance to allogeneic tissues. Both clonal deletion and active suppression by antigen-specific suppressor T cells appear to contribute to the maintenance of the tolerant state (16–18). Cellular mechanisms that contribute to the induction rather than the maintenance of tolerance are less well understood. However, recent work suggests that the naturally occurring suppressor cells in the neonatal spleen are intimately involved in the induction of tolerance to allogeneic tissues, since these

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Annual Reviews

suppressor cells inhibit the generation of antigen-specific cytolytic cells and enhance the generation of antigen-specific suppressor cells (19). Our understanding of the nature of the nonspecific suppressor cells has been aided by the ability to propagate these cells in vitro and to develop cloned cell lines.

Although the nonspecific suppressor cells may contribute to the ease of tolerization in the neonate, the markedly reduced proportions of mature T and B lymphocytes and macrophages in the neonatal lymphoid tissues also play important roles (16,20). Within a few weeks after birth, the peripheral lymphoid tissues of the mouse develop the characteristics of the adult, including the loss of the nonspecific suppressor cells and the acquisition of the full complement of mature T and B cells and macrophages (21–25). Associated with these changes are increased immune responsiveness and decreased susceptibility to tolerization. However, this process can be reversed in the adult by treatment with high-dose, fractionated lymphoid irradiation (total lymphoid irradiation, TLI) (26). For several weeks after TLI, the lymphoid tissues again contain nonspecific suppressor cells, in association with a marked reduction of the proportion of mature T and B cells (25,27). During this period the susceptibility to tolerization increases, which allows for the permanent acceptance of allogeneic organ grafts. However, as in the neonate, this "window" of tolerance persists for several days, and the normal adult immune system recovers thereafter (28). The subsequent sections of this article review the similarities in the lymphoid tissue cell composition and function in the neonate and in the adult treated with TLI during the "window" of tolerance, as well as similarities in the cloned "natural" suppressor cell lines that can be obtained from these tissues.

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INDUCTION OF TOLERANCE IN NEONATAL MICE

Billingham et al (1) first demonstrated actively acquired neonatal tolerance in the laboratory. These investigators injected dissociated lymphoid cell suspensions from allogeneic adult mice intravenously into neonatal mice within one to two days after birth. Depending upon the strain combination and source of cells used, graft-vs-host disease (GVHD) could be avoided and the neonates developed into healthy adults (29). The latter adults were chimeras, and donor cells persisted in the recipient lymphoid tissues. The chimeras were specifically tolerant of the donor tissues. Donor-strain skin grafts transplanted to the flank were permanently accepted, but skin grafts from other strains were rapidly rejected (1,29). With the introduction of congenic strains of mice that differ only at the Class I or Class II major histocompatibility genetic loci, it has been clearly demonstrated that neonatal tolerance can be achieved to both antigenic groups alone or in combination (30).

Annual Reviews

The ease of tolerization in the neonatal rodent has been extended to a wide variety of antigens, including heterologous serum proteins, heterologous red blood cells, and bacterial proteins (3–8, 31). Although Dresser (32) showed that normal adult mice can be tolerized to foreign proteins, the foreign substances must be manipulated in some way (i.e. deaggregation) in order to tolerize the recipient. In contrast, tolerance in the neonate is reproducibly achieved in the absence of these manipulations.

MAINTENANCE OF NEONATAL TOLERANCE: CLONAL DELETION AND ACTIVE SUPPRESSION

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The cellular basis of the maintenance of neonatal transplantation tolerance in the chimeric adult has been the subject of extensive investigation during the past 20 years. Two major mechanisms have been postulated: clonal deletion and active suppression. Initial proponents of clonal deletion argued against active suppression because they could not demonstrate suppressor cells or humoral factors in tolerant adults (39). However, these arguments may have been based on the use of assay systems not sufficiently sensitive to identify suppressor cells or factors. In addition, lack of reactive clones in the adult may be the result of specific inactivation by suppressor cells. Thus, the presence of clonal deletion neither denies the presence of suppressor cells nor provides evidence for direct inactivation of clones by antigen. Indeed, assays of clonal deletion or inactivation must first separate suppressor cells from the putative reactive cells. Such assay systems have only recently become available with the development of cloning technology that allows for the examination of the reactivity of individual cytolytic T–cell precursors.

Using sensitive suppressor-cell assays and cloning technology, several laboratories have generated data that support both clonal deletion and active suppression as mechanisms of the maintenance of tolerance. Roser and his colleagues (33–35) showed that neonatally tolerized rats have specific suppressor T cells circulating in the thoracic duct lymph during adulthood. The latter cells were identified in adoptive transfer experiments. Sublethally irradiated adult recipients syngeneic with the chimeric host received host thoracic duct cells and donor-type skin grafts along with third-party skin grafts. The survival of donor-type skin grafts was considerably prolonged as compared to that of the third-party grafts. Evidence supporting the contribution of suppressor cells in the maintenance of neonatal tolerance has also been reported by Holan et al (36), Rieger & Hilgert (37), and Streilein & Gruchalla (30).

Evidence for early clonal deletion in neonates tolerized to histocompatibility antigens was reported by Nossal & Pike (38). These investigators used limiting dilution techniques to examine the reactivity of cytolytic T-cell precursors at

Annual Reviews

the clonal level in the thymus and spleen of neonatal CBA mice injected with (CBA × BALB/c)F_1 hybrid cells on the day of birth. It is of interest that clonal reactivity to F_1 cells was lost in the thymus by the fifth day of life and lost in the spleen by the eighth to tenth day of life. These experiments did not determine whether active suppression developed simultaneously or whether suppressor cells are involved in the mechanism of clonal deletion.

NATURALLY OCCURRING SUPPRESSOR CELLS IN THE NEONATAL SPLEEN

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Several laboratories have reported that dissociated spleen cells from neonatal mice within a few days after birth inhibit a wide variety of in vitro and in vivo immune responses (9–15,19,40–42). The nature of these antigen-nonspecific suppressor cells has been controversial. T lymphocytes (9–12), non-T lymphocytes (25,40), and promonocytes (20,41,42) have been postulated to mediate the suppressive activity. It is possible that more than one type of suppressor cell is present and that the different readout systems used to assay for suppression identify different types of suppressor cells.

Most of the studies have investigated nonspecific suppressor cells of alloreactivity. The neonatal splenic suppressor cells inhibit both cellular proliferation and the generation of cytolytic T cells by responder cells in the mixed leukocyte reaction (MLR) (9–15,19,40). These suppressor cells have also been shown to inhibit GVHD in vivo (9,10,13). Co-transfer of neonatal spleen cells with adult allogeneic bone marrow into lethally irradiated adult mice prevents observable signs of GVHD in the recipients and allows for permanent chimerism.

Until recently, neonatal suppressor cell surface markers had been studied using negative selection procedures. Some investigators concluded that the suppressor cells of the MLR are members of the T-cell lineage, since treatment of neonatal spleen cells with anti-Thy-1 antibodies and complement eliminates the suppressive activity (9–12). Others were unable to eliminate suppressive activity using this procedure, and concluded that the MLR suppressors are non-T cells (25,40). However, there has been general agreement that the cells are not mature macrophages, since they do not adhere firmly to plastic or glass and are not phagocytic (9–15,19,40–42).

Oseroff et al (25) used the panning procedure of Wysocki & Sato (43) to select neonatal splenic suppressor cells both positively and negatively according to their surface markers. This study had the advantage of quantitating the cells bearing specific surface markers in the separated fractions used to inhibit the MLR. In addition, monoclonal antibodies were used to identify surface antigens. The experimental data showed that the suppressor cells were confined to the "null" cell fraction, since neither positively selected T cells nor B cells

Annual Reviews

carried suppressive activity (25). The "null" cells did not bear mature macrophage surface markers identified by monoclonal antibodies, and the suppressive activity was not blocked by an inhibitor of prostaglandin synthesis, indomethacin.

Oseroff et al (25) also showed that the kinetics of the reduction in the suppressive activity of neonatal spleen cells is temporally related to the reduction in the proportion of "null" cells from about 80% of spleen cells during the first few days after birth to approximately 10% one month after birth (Figure 1). The neonatal suppressor cells have characteristics in common with the natural killer (NK) cells (44–47): Both are large granular lymphocytes found in the "null" cell population, both lack antigen specificity, and both carry out their effector function without antigenic stimulation. However, the neonatal suppressors appear to lack natural killer activity as measured by the lysis of YAC-1 cells (25). In view of the characteristics shared with the NK

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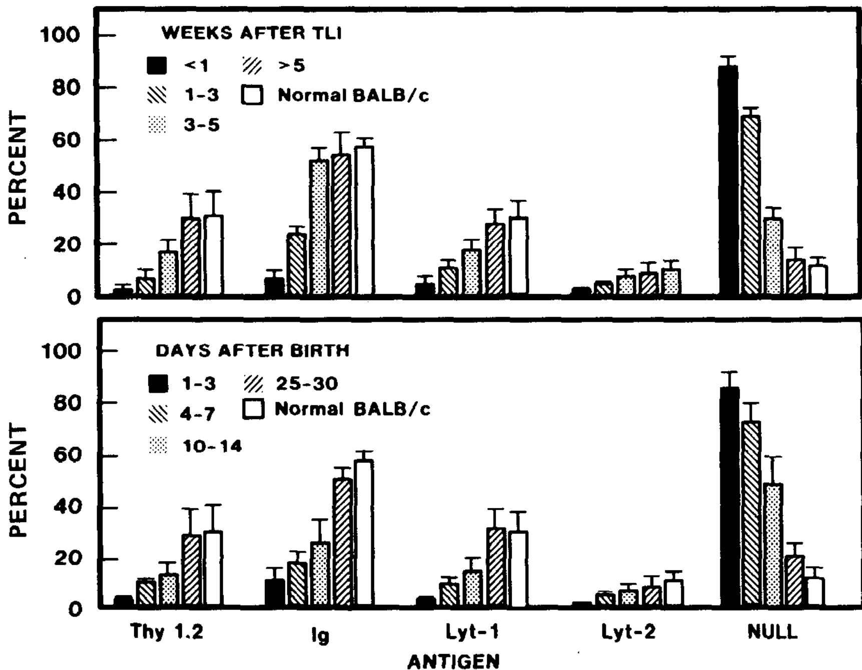

Figure 1 Time course of the immunofluorescent staining for lymphocyte markers of TLI-treated (upper panel) and neonatal (lower panel) spleen cells. Each bar represents the mean of 4–8 animals or groups of pups in separate experiments at the indicated time point (except for 1–3 days after birth, where n = 3). The error bar indicates the SEM. Percent null cells was calculated as 100 minus (percent Thy 1.2 + percent Ig) for each experiment.

Annual Reviews

cells, the neonatal suppressors have been called "natural" suppressor (NS) cells.

The latter cells appear to be different from cells in the neonatal spleen that suppress the in vitro antibody response to sheep red blood cells and the expression of Ia antigens on the surface of macrophages (20,21). The MLR suppressor cells are not inhibited by indomethacin and do not differentiate into mature macrophages after short-term tissue culture (25). In contrast, the suppressor cells of antibody synthesis are inhibited by indomethacin, and differentiate into classical macrophages in vitro (21). Neonatal spleen cells that suppress the expression of surface Ia antigens appear to be similar to the antibody suppressors, since suppressive activity in both instances appears to be mediated by prostaglandins (20). Thus, the neonatal spleen appears to contain at least two populations of suppressor cells, "null" lymphocytes and macrophage precursors. Only the former cells appear to block alloreactivity.

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EASE OF TOLERANCE INDUCTION IN ADULT MICE GIVEN TOTAL LYMPHOID IRRADIATION (TLI)

Fractionated irradiation targeted to the lymphoid tissues has been used in humans to treat Hodgkins' disease for about 20 years (48). Shielding of the marrow, lungs, and other vital nonlymphoid tissue with lead permits the cumulative dose to exceed 4000 rads without frequent severe side effects (48). Similar fractionation and shielding techniques can be used to deliver 3400 rads (17 fractions of 200 rads each) to the lymph nodes, spleen, and thymus of adult mice without substantial morbidity and mortality (26). The immune systems of the treated mice have many similarities to those of the neonatal mice, including the ease of tolerization and the high proportion of "null" cells (Figure 1). Thus, allogeneic bone marrow injected intravenously into mice within one week after TLI results in permanent chimerism without the development of GVHD (49–52). The resultant chimeras are specifically tolerant of the tissues of the marrow-donor strain and will permanently accept donor-type skin grafts and promptly reject third-party grafts (49–52).

As in the case of the neonate, the ease of tolerance induction for alloantigens is short-lived. Although about 90% of irradiated mice will accept allogeneic marrow grafts within one to two days after the completion of irradiation, only about 50% will accept grafts transplanted seven days after irradiation. By day 21, none of the recipients will accept marrow grafts (28). The tolerogenic effects of the radiotherapy are related to the total dose of radiation, the dose per fraction, and the amount of lymphoid tissue enclosed within the radiation ports (28,53). A cumulative dose of at least 3000 rads is required to achieve tolerance in a high proportion of marrow recipients (28,53). Although the major lymph nodes above and below the diaphragm as well as the spleen must

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be irradiated in order to prepare mice for successful marrow transplantation, irradiation of the thymus is not essential (28). Rats given TLI have also been successfully tolerized to alloantigens, and permanently accept skin and heart transplants following marrow transplantation (54).

The ability to tolerize adult mice to heterologous serum proteins continues for at least 100 days after TLI (55). Thus, the "window" of tolerance for these proteins is considerably longer than for allogeneic marrow grafts. The induction of tolerance to bovine serum albumin (BSA) or to bovine gamma globulin (BGG) after TLI can be accomplished without chemical or physical modification of the proteins, such as deaggregation. Two intraperitoneal injections of either protein in aqueous solution results in a tenfold reduction in the humoral antibody response to the appropriate protein or hapten conjugated to the protein when injected in complete Freund's adjuvant one month later (55).

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ROLE OF ANTIGEN-SPECIFIC SUPPRESSOR CELLS IN THE MAINTENANCE OF TOLERANCE AFTER TLI

Allogeneic bone marrow chimeras prepared with TLI are specifically unresponsive to the tissues of both the recipient and donor strains as judged by cell proliferation and the generation of cytolytic cells in the MLR (49,54,56). In addition, purified donor cells obtained from the spleens of the chimeras are unable to mount a lethal GVHD after injection into irradiated recipient-strain mice (57). However, the transfer of the same cells to irradiated third party–strain mice results in the death of most animals by 30 days (57). The specific unresponsiveness of the donor-type cells to recipient alloantigens could be explained by clonal deletion and/or active suppression.

In order to identify antigen-specific donor-type suppressor cells in tolerant chimeras, purified donor cells from the spleens of mice bearing allogeneic skin grafts for at least 100 days were tested for their ability to inhibit the MLR of normal donor-type spleen cells against recipient-type and third-party stimulator cells (56). Addition of donor-type chimeric cells to the MLR cultures markedly inhibited the generation of cytolytic cells when recipient-type cells were used as stimulators, but failed to inhibit cultures when third-party cells were used as stimulators (see Table 1) (56). Thus, active suppression may play an important role in the maintenance of donor-vs-host tolerance in these chimeras and in the prevention of GVHD. Identification of recipient-type suppressor cells in these chimeras was not undertaken owing to the low percentage of recipient cells in the chimeric lymphoid tissues (56).

TLI-treated mice rendered tolerant to BSA were also examined for the presence of antigen-specific suppressor cells (55). The spleen cells of the tolerant animals were co-transferred with primed T cells (BSA) and primed B cells [dinitrophenyl (DNP) hapten] to irradiated recipient mice (55). The reconsti-

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Table 1 Suppression of the generation of cytolytic cells in the MLR by the addition of spleen cells from (C57BL/Ka→BALB/c) chimeras prepared with TLI

MLR					
Responder cells[a]	Stimulator cells[b]	Co-cultured cells[c]	CML assay tumor target cells	Lytic units/ culture	Percentage of suppression
C57(H−2^b)	BALB(H−2^d)	C57 (normal)[d]	P815(H−2^d)	346.2	57.4
C57	BALB	C57 (chimeric)[e]	P815	147.5	
C57(H−2^b)	C3H(H−2^k)	C57 (normal)	BW5147(H−2^k)	128.6	−9.6
C57	C3H	C57 (chimeric)	BW5147	140.9	

[a]3×10^5 normal C57 spleen cells

[b]3×10^5 normal BALB or C3H spleen cells were given 3000 rads in vitro.

[c]6×10^5 spleen cells were given 1500 rads in vitro.

[d]A mixture of normal C57 and BALB spleen cells in the same ratio as that found in the chimera was treated with anti-H−2^d and complement. Residual cells were purified normal C57 cells.

[e]Spleen cells from a chimera were treated with anti-H−2^d antiserum and complement. Residual cells were purified chimeric C57 cells.

tuted mice were challenged with the conjugate DNP–BSA in saline, and the serum anti-DNP response was measured thereafter. A combination of primed T and B cells restored a vigorous anti-DNP response, but co-transfer of the tolerant cells inhibited the adoptive antibody response by about 90% (55). However, no inhibition was observed when the tolerant cells were co-transferred with T cells primed to BGG and B cells primed to DNP (55). Thus, the suppressor cells found in the tolerant animal were antigen-specific. These cells appear to be members of the T-cell lineage, since treatment of tolerant spleen cells with anti-Thy-1 antibodies and complement before co-transfer eliminates the suppressive activity (55).

NATURALLY OCCURRING SUPPRESSOR CELLS IN THE SPLEEN OF ADULT MICE GIVEN TLI

Spleen cells obtained from TLI mice within a short time after the completion of radiotherapy can inhibit the proliferation of responder cells and generation of cytolytic cells in the MLR (27,58). As in the case of neonatal suppressor cells, inhibition is independent of the mouse strain used as the source of responder or stimulator cells (27,58). Thus, the neonatal and TLI suppressor cells are antigen-nonspecific and are not restricted by the H-2 type of the responder cell. In both cases, the suppressor cells are found transiently and can no longer be detected by about one month after birth or radiotherapy. The presence of the suppressor cells is linked temporally to the ease of induction of tolerance to alloantigens (27,28). Interestingly, Waer et al (59) have shown that those TLI-irradiation regimens that allow for the successful engraftment

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of allogeneic bone marrow are associated with the presence of nonspecific suppressor cells in the spleen. These investigators have also shown that suppressor cells of the MLR are also present in the spleen of mice given whole-body irradiation in addition to TLI (59). These cells are first identified several days after irradiation, and they do not persist for nearly as long as those observed in the spleen of neonates or TLI-treated mice (59).

The spleen cells obtained from TLI-treated mice are also able to inhibit GVHD in vivo (27). Allogeneic bone marrow cells from C57BL/Ka (H–2^b) mice ordinarily produce lethal GVHD in whole-body irradiated BALB/c (H–2^d) recipients. However, co-transfer of spleen cells from TLI-treated adult BALB/c mice along with C57BL/Ka marrow cells into irradiated BALB/c recipients blocks the development of GVHD. Nevertheless, the repopulation of the recipient lymphoid tissues with C57BL/Ka cells is unimpeded (27).

The nonspecific suppressor cells found in the spleen of TLI-treated mice were initially thought to be T cells, since three rounds of treatment with anti-Thy-1 antibodies and complement substantially reduced the capacity of the spleen cells to inhibit the MLR (27,60). However, subsequent studies using the "panning" procedure to select the suppressor cells both positively and negatively showed that the suppressive activity was confined to cells that bore neither mature T cell (Thy-1) nor B cell (immunoglobulin) surface markers as judged by adherence to antibody-coated plates and by immunofluorescent staining (25). Using similar selection procedures, the suppressor cells were found to lack surface markers (identified by anti-MAC-1 and F4/80 monoclonal antibodies) and physical characteristics (adherence to glass and plastic) associated with mature macrophages (25). Thus, the suppressor cells may have small amounts of Thy-1 antigen on the surface, detected by sensitive cytotoxicity assays, but they are members of the "null" cell population as judged by conventional immunofluorescence staining techniques (25). In view of the "null" surface phenotype, the antigen nonspecific suppressive activity, the lack of antigenic stimulation required to induce suppression, and apparent absence of natural killer activity, these cells as well as the neonatal suppressor cell have been called natural suppressor (NS) cells (25). The suppressive activity of NS cells from both neonatal and TLI-treated animals was not inhibited by indomethacin. Thus, the NS cells appear to differ from the promonocyte suppressor cell (21) found in the neonatal spleen. During in vitro culture for up to six weeks, the NS cells failed to differentiate into mature macrophages, but maintained their suppressive activity (25). Recent work by Slavin and his colleagues (61) also indicates that suppressor cells of the MLR are confined to the non-T cell population of spleens from TLI-treated mice.

Non-specific suppressor cells of antibody formation are also found in the TLI-treated spleens. However, the characteristics of the latter cells appear to be different from those of the MLR suppressor. The suppressor cells of the in

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vitro T-cell independent antibody response to trinitrophenylated *Brucella abortus* (TNP–BA) bear neither T- nor B-cell markers, but approximately half the cells bear the MAC-1 surface antigen (62). Thus, at least a considerable portion of these cells may be members of the macrophage lineage. On the other hand, nonspecific suppressor cells of the adoptive in vivo T-cell dependent antibody response to DNP–BSA appear to be immature T cells (55,60). The latter cells bear both the Thy-1 and the TL surface antigens ordinarily found only on thymocytes (60).

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NATURALLY OCCURRING SUPPRESSOR CELLS IN THE BONE MARROW OF NORMAL ADULT MICE

Mitchell and his colleagues (63,64) originally described a "natural suppressor" cell in the bone marrow of normal adult mice that inhibited the generation of cytolytic cells in the MLR. These suppressors did not carry Thy-1 surface antigen and were described as non-T cells (64). Salvin and his colleagues (61) also have shown that marrow cells that suppress MLR proliferation have non-T cell surface characteristics similar to those of MLR suppressor cells in the TLI-treated spleen. The marrow also contains suppressor cells of the in vitro antibody response to TNP–BA (62). However, the latter cells appear to differ from the MLR suppressors present in the TLI-treated spleen, since about half of the marrow cells bear the MAC-1 antigen, which is not expressed on the MLR suppressors. Mitchell and his colleagues also suggested that the bone marrow may contain two populations of suppressors—a macrophage suppressor inhibited by indomethacin, and a nonmacrophage suppressor not affected by indomethacin (64). It is interesting that all tissues in which adult suppressor cells have been identified [adult bone marrow (61–64), neonatal spleen (9–15), and spleens of adult mice treated with TLI (27,58), BCG (63,64), cyclophosphamide (65), and Sr^{91} (66)] are sites of intense hematopoiesis in the rodent. Similar suppressor cells have not been observed in the spleen of the normal adult mouse, even when the purified "null" cell population has been assayed (25). Thus, the appearance of these cells in the adult appears to be limited to the bone marrow, unless a stimulus for intense hematopoiesis is given to the peripheral lymphoid tissues.

IN VITRO CULTURE AND CLONING OF NATURAL SUPPRESSOR (NS) CELLS FROM THE SPLEENS OF NEONATAL AND TLI-TREATED MICE: SIMILAR CHARACTERISTICS

Spleen cells obtained from mice within one week after TLI continue to proliferate in vitro in the presence of tissue culture medium conditioned with

Annual Reviews

supernatants from rat spleen cells stimulated with concanavalin A (25). Although a heterogeneous population of cells grows out initially, a homogeneous population of large granular lymphocytes that are weakly adherent to plastic can be selected for continued culture (25). The latter cells during the first six weeks of culture continue to show suppressive activity in the MLR, and bear the "null" surface phenotype (25). However, after a few months in culture, almost all cells express large quantitites of Thy-1 antigen on their surface as judged by immunofluorescent staining. The latter cells have been cloned by limiting dilution, and have been maintained in conditioned medium for more than one year as stable cell lines (B. Hertel-Wulff, S. Okada, A. Oseroff, S. Strober, submitted). Table 2 lists the surface characteristics of the cloned cells, and Figure 2 shows their suppressive activity as compared to T-cell lines of BALB/c origin. It is of interest that the cloned suppressor cells inhibit the MLR by about 50% at a responder-to-suppressor cell ratio of about 50:1. Freshly obtained spleen cells from TLI-treated mice lose their suppressive activity at a ratio of about 4:1. The surface phenotype of the cloned cells most closely resembles that reported for cloned natural killer (NK) lines (67), since both are Thy-1^+, Lyt-1^-, Lyt-2^-, Ig^- and asialo-GM^+. However, the NS cells in contrast to the NK cells have no lytic activity on YAC-1 target cells (67).

Interestingly, cloned NS cells grown from the spleen of neonatal mice using similar culture techniques have the same surface phenotype as shown in Table 1, and suppress the MLR with slightly greater potency (R. Schwadron, S. Strober, manuscript in preparation). Cloned NS cells from the neonatal spleen also lack killing activity on YAC-1 cells. It is of interest that both the NS and NK cells are members of the "null" cell population in vivo but acquire large quantities of surface Thy-1 antigen after long-term culture in conditioned

Table 2 Surface markers on cloned "natural" suppressor cell lines

Marker	Source: Spleen from TLI-treated adult BALB/c mouse	Source: Spleen from neonatal BALB/c mouse
Thy 1.2	+	+
Lyt 1.2	−	−
Lyt 2.2	−	−
Ig	−	−
Mac−1	−	−
F4/80	−	−
Ia	−	−
H−2K,D	+	+
Asialo GM1	+	+

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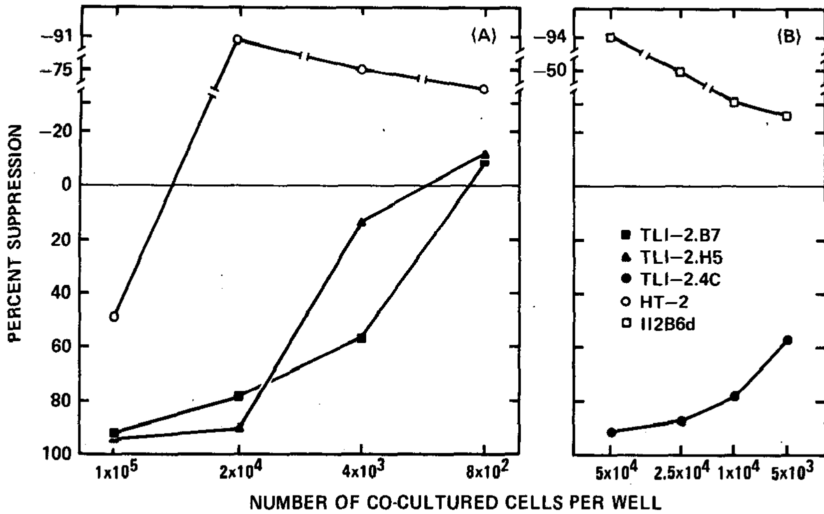

Figure 2 Suppression of the MLR after the addition of cloned "natural" suppressor (NS) cells obtained from the spleens of TLI-treated adult BALB/c mice. [3H]-Thymidine uptake was measured after pulsing five-day cultures with 5×10^5 normal BALB/c spleen responder cells and 5×10^5 normal C57BL/Ka stimulator cells (given 3300 rads in vitro): ■ - ■ TLI - 2.B7 (a cloned NS cell line); ▲ - ▲ TLI-2.H5 (a cloned NS cell line), ● - ● TLI - 2.4C (a cloned NS cell line); ○ - ○ HT-2 (a cloned BALB/c T cell line) (69); □ - □ II2B6d (a cloned BALB/c T cell line) (70). All co-cultured cells were irradiated in vitro (3300 rads) before addition to the MLR. Percent suppression is compared to control MLR without co-cultured cells. Control culture in Figures 2A and 2B gave 138,137 ± 8,651 cpm and 45,000 ± 962, respectively, using the mean of triplicate values ± standard error. Background counts were 5,242 ± 675 cpm and 3,239 ± 548 respectively. A representative experiment is shown.

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medium (67). Thus, the NK and NS cells may be members of the same lineage but mediate different regulatory and/or effector functions.

RELATIONSHIP AMONG NATURAL SUPPRESSOR (NS) CELLS, ANTIGEN-SPECIFIC SUPPRESSOR CELLS, AND TRANSPLANTATION TOLERANCE

The experimental work reviewed thus far indicates that neonatal or adult TLI-treated allogeneic bone marrow chimeras first develop antigen-nonspecific suppressor cells of alloreactivity and then develop antigen-specific suppressor cells. The nonspecific suppressor cells are found in the spleen before the infusion of bone marrow cells, and the specific suppressors are found several months after the marrow infusion when the animal is specifically tolerant to donor-strain tissues. The disappearance of nonspecific suppressor cells during

Annual Reviews

the first few weeks after birth or radiotherapy does not depend upon the infusion of marrow cells; it appears to be related to the repopulation of the peripheral lymphoid tissues with mature T and B cells (25).

The relationship between the nonspecific suppressor cells from both TLI-treated and neonatal mice and the subsequent generation of antigen-specific suppressor cells has been studied recently in an in vitro model system (19,58). Spleen cells obtained from both neonates and TLI-treated mice a few days after birth or radiotherapy, respectively, were added to the MLR; the generation of antigen-specific cytolytic and suppressor cells was measured five to six days later. Both the neonatal and TLI suppressor cells and the stimulator cells were irradiated in vitro before culture such that neither could proliferate during the culture period. Control cultures were also set up with identical adult responder and stimulator cells but without the addition of neonatal or TLI suppressor cells.

Control cultures with BALB/c ($H-2^d$) responder and C57BL/Ka ($H-2^b$) stimulator cells generated a vigorous cytolytic cell response by six days as judged by a Cr^{51} release assay on $H-2^b$ target cells (19,58). The killing of the target cells was antigen-specific, since no cytotoxicity was observed with C3H/He ($H-2^K$) target cells. Assays for antigen-specific suppressor cells were carried out on day five of the MLR culture. In order to assay for suppressive activity, cells from five-day MLR cultures were added to fresh MLR cultures containing normal BALB/c responder cells and either C57BL/Ka (syngeneic to first stimulator) or C3HHe (allogeneic to first stimulator) stimulator cells. Cells from the five-day MLR cultures markedly inhibited both the proliferation of responder cells and the generation of cytolytic cells in the fresh MLR cultures. However, suppression was antigen nonspecific, since fresh MLR cultures with C57BL/Ka or C3H/He stimulator cells were equally inhibited. Thus, the control MLR cultures generated both antigen-nonspecific suppressor cells and antigen-specific cytolytic cells after five to six days.

As expected, MLR cultures containing normal BALB/c responder cells, C57BL/Ka stimulator cells, and BALB/c neonatal or TLI-treated spleen cells were inhibited with regard to both responder-cell proliferation and the generation of cytolytic cells (19,58). Despite the scarcity of antigen-specific cytolytic cells in the six-day MLR cultures, cells from the same cultures markedly suppressed both proliferation and the generation of cytolytic cells of fresh MLR cultures (19,58). Unexpectedly, the suppression was antigen specific, since suppression was observed in fresh cultures with BALB/c responder and C57BL/Ka stimulator cells but not with BALB/c responder and C3H/He stimulator cells (19,58). Thus, the addition of neonatal or TLI-treated spleen cells to MLR cultures resulted in at least three regulatory changes: (*a*) a reduction in the generation of antigen-specific cytolytic cells; (*b*) a marked reduction in the generation of antigen-nonspecific suppressor cells; and (*c*) the appearance

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of highly active antigen-specific suppressor cells. The latter cells may be generated in two-party MLR cultures (responder and stimulator cells only), but would be "masked" by the nonspecific suppressor cells. Figure 3 summarizes these regulatory changes in diagrammatic form. Control five-day cultures containing BALB/c responder, BALB/c stimulator, and BALB/c neonatal or TLI-treated spleen cells showed little or no suppressive activity when assayed in fresh MLR cultures.

Thus, the natural suppressor (NS) cells in the neonatal and TLI-treated spleen are able to block the cytolytic arm of the alloreactive immune response but do not block the antigen-specific suppressive arm. This provides an expla-

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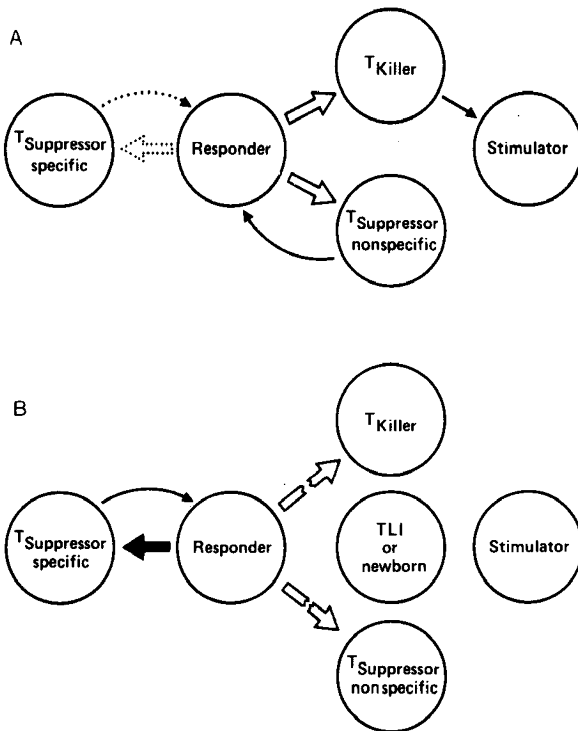

Figure 3 Regulatory Cell Model. In the two-party MLR culture containing normal responder cells and normal stimulator cells (irradiated in vitro) (A), the responder cells generate cytolytic cells, antigen-nonspecific suppressor cells, and perhaps antigen-specific suppressor cells masked by antigen-nonspecific suppressor cells. After the addition of spleen cells from adult mice given TLI or from newborn mice, the generation of cytolytic cells and nonspecific suppressor cells is blocked. However, the generation of antigen-specific suppressor cells (B) proceeds without impairment. Thus, continued alloantigen stimulation produces a pool of antigen-specific suppressor cells that continue to block the generation of cytolytic cells. The latter pool may play an important role in maintaining tolerance in vivo even after the nonspecific suppressor cells found after TLI or in newborn mice disappear.

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nation for the in vivo transition of nonspecific to specific suppressor cells in neonatal or TLI-treated mice tolerized with allogeneic marrow infusions (33–35,56). As shown in Figure 4, the small number of immunocompetent host cells responding to the allogeneic marrow cells will generate preferentially antigen-specific suppressor cells and few cytolytic cells. Eventually a large pool of host-type antigen-specific suppressor cells will accumulate and contribute to the maintenance of host-vs-graft tolerance. The latter cells will persist subsequent to the disappearance of the NS cells during the repopulation of the host lymphoid tissues with mature lymphocytes. In addition, the small number of immunocompetent donor cells contained in the marrow infusion will also generate preferentially antigen-specific suppressor cells in the presence of NS cells. A persistent pool of these donor-type suppressor cells would contribute to the maintenance of donor-vs-host tolerance. Recent studies have clearly demonstrated the specificity of host tolerance in purified donor-type cells from TLI-chimeras (57), and the presence of donor-type antigen-specific suppressor cells in this cell population (56).

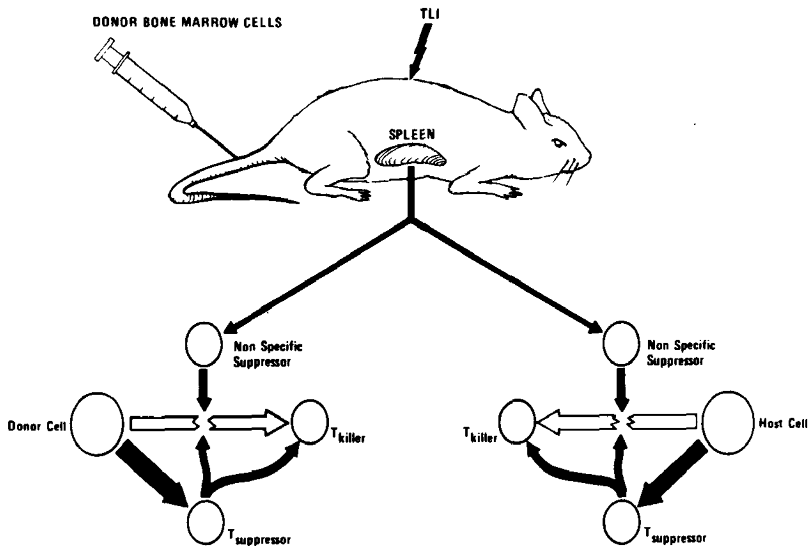

Figure 4 Model for the mechanism of mutual tolerance after allogeneic bone marrow transplantation. Nonspecific suppressor cells found in the spleens of adult mice given TLI or of newborn mice before marrow transplantation block the generation of cytolytic T cells but allow for the development of a pool of antigen-specific suppressor T cells after marrow transplantation. The latter cells derived from the host subsequently maintain tolerance in long-term chimeras by blocking the generation of cytolytic T cells directed toward donor cells even after the nonspecific suppressor cells disappear. Donor-derived, antigen-specific suppressor cells prevent graft-vs-host disease by blocking the generation of cytolytic cells directed toward host cells. Broken arrow shows site of action of both nonspecific and specific suppressor cells.

Annual Reviews

The regulatory effects of NS cells can explain both the increased susceptibility of host-vs-graft and graft-vs-host tolerance during the "window" of tolerance of both neonatal and TLI-treated mice. However, the role of the NS cells must be viewed in the context of several regulatory mechanisms that promote tolerance in the early phases of population of the peripheral lymphoid tissues during this "window" of tolerance. It is clear that immature B cells and T cells per se are more easily tolerized than mature cells (16), so that clonal inactivation or deletion may play an important or predominant role in tolerization during these periods. For example, Zan-Bar et al (68) have shown that purified B cells from TLI-treated mice are easily tolerized as compared to those from unirradiated adults. The NS cell may be part of a "fail-safe" mechanism in which the few clones that escape central inactivation may be effectively inhibited in the periphery. On the other hand, the NS cells may also play a role in the mechanism of central clonal inactivation. Finally, the immaturity of nonlymphocytic antigen presenting cells may also contribute to the ease of tolerance induction, since immature macrophages from the peritoneal cavity of the neonate appear to be deficient in their capacity to present antigens to T cells (20).

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SUMMARY

Although several laboratories have shown that the appearance of naturally occurring suppressor cells in the spleens of neonatal or irradiated mice is temporally related to the ease of induction of tolerance, the characteristics of these cells and their regulatory functions have been poorly understood until recently. The experimental data reviewed herein suggests that these cells are related to NK cells with regard to surface phenotype but differ with regard to function. The natural suppressor (NS) cells appear only briefly during the early maturation of the lymphoid tissues but can be induced in adults by manipulation of the lymphoid tissues with certain treatment regimens such as total lymphoid irradiation (TLI). In addition, the NS cells can be propagated and cloned in long-term tissue culture, thereby allowing a more detailed investigation of their properties. The cells have the unique feature of inhibiting the antigen-specific cytolytic arm of alloreactive immune responses but leaving intact the antigen-specific suppressive arm. In this way, alloreactions in the regulatory milieu of NS cells generate large numbers of antigen-specific suppressor cells that can maintain tolerance in vivo. Thus the NS cells may play an important role in the development of host-vs-graft and graft-vs-host tolerance in allogeneic bone marrow chimeras during the "window" of tolerance in which neonate and TLI-treated mice accept the infused allogeneic cells.

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Literature Cited

1. Billingham, R. E., Brent, L., Medawar, P. B. 1953. Actively acquired tolerance of foreign cells. *Nature* 172:603
2. Burnet, F. M. 1959. *Clonal Selection Theory of Acquired Immunity.* Cambridge: Cambridge Univ. Press
3. Gowans, J. C., Gesner, B. M., McGregor, D. D. 1961. *The Immunological Activity of Lymphocytes in Biological Activity of the Leucocyte.* CIBA Found. Study Group No. 10, p. 32. London: Churchill
4. Smith, R. T., Bridges, R. A. 1958. Immunological unresponsiveness in rabbits produced by neonatal injection of defined antigens. *J. Exp. Med.* 108:227
5. Pike, B. L., Kay, T. W., Nossal, G. J. V. 1980. Relative sensitivity of fetal and newborn mice to induction of hapten-specific B cell tolerance. *J. Exp. Med.* 152:1407
6. Shellam, G. R., Nossal, G. J. V. 1968. Mechanisms of induction of immunological tolerance. IV. The effects of ultra-low doses of flagellin. *Immunology* 14:273
7. Smith, R. T. 1961. Immunological tolerance of non-living antigens. *Adv. Immunol.* 1:67
8. Brent, L., Brooks, C. G., Medawar, P. B., Simpson, E. 1976. Transplantation tolerance. *Br. Med. Bull.* 32:101
9. Argyris, B. F. 1978. Suppressor activity in the spleen of neonatal mice. *Cell. Immunol.* 36:354
10. Argyris, B. F. 1982. Nature of neonatal suppressors in the mouse. *Cell. Immunol.* 66:352
11. Murgita, R. A., Hooper, D. C., Stegagno, M., Delovitch, T. L., Wigzell, H. 1981. Characterization of murine newborn inhibitory T lymphocytes: functional and phenotypic comparison with an adult T cell subset activated *in vitro* by alpha–fetoprotein. *Eur. J. Immunol.* 11:957
12. Mosier, D. E., Mathieson, B. J., Campbell, P. S. 1977. Ly phenotype and mechanism of action of mouse neonatal suppressor T cells. *J. Exp. Med.* 146:59
13. Skowron-Cendrzak, A., Ptak, W., 1976. Suppression of local graft-versus-host reactions by mouse fetal and newborn spleen cells. *Eur. J. Immunol.* 6:451
14. Ptak, W., Skowron-Cendrzak, A. 1977. Fetal suppressor cells. Their influences on the cell-mediated immune responses. *Transplantation* 24:45
15. Basset, M., Coons, T. A., Wallis, W., Goldberg, E. H., Williams, R. C. Jr., 1977. Suppression of stimulation in the mixed leukocyte culture by newborn splenic lymphocytes in the mouse *J. Immunol.* 119:1855
16. Nossal, G. J. V. 1983. Cellular mechanisms of immunological tolerance. *Ann. Rev. Immunol.* 1:33
17. Roser, B., Dorsch, S. 1979. The cellular basis of transplantation tolerance in the rat. *Immunol. Rev.* 46:55
18. Steinmuller, D. 1978. Suppressor cells and transplantation tolerance. *Transplantation* 26:2
19. Okada, S., Strober, S. 1982. Spleen cells from adult mice given total lymphoid irradiation (TLI) or from newborn mice have similar regulatory effects in the mixed leukocyte reaction (MLR). II. Generation of antigen-specific suppressor cells in the MLR after the addition of spleen cells from newborn mice. *J. Immunol.* 129:1892
20. Snyder, D. S., Lu, C. Y., Unanue, E. R. 1982. Control of macrophage Ia expression in neonatal mice—role of a splenic suppressor cell. *J. Immunol.* 128:1458
21. Piguet, P. F., Irle, C., Vassalli, P. 1981. Immunosuppressor cells from newborn mouse spleen are macrophages differentiating *in vitro* from monoblastic precursors. *Eur. J. Immunol.* 11:56
22. Haaijman, J. J., Micklem, H. S., Ledbetter, J. A., Dangel, J. L., Herzenberg, L. A. 1981. T cell ontogeny: organ location of maturing populations (defined by surface antigen markers) is simlar in neonates and adults. *J. Exp. Med.* 153:605
23. Adler, W. H., Takiguchi, T., Marsh, B., Smith, R. T. 1970. Cellular recognition by mouse lymphocytes *in vitro.* II. Specific stimulation by histocompatibility antigens in mixed cell culture. *J. Immunol.* 105:984
24. Hayakawa, K., Hardy, R. R., Parks, D. R., Herzenberg, L. A. 1981. The "ly-1 B" cell subpopulation in normal immunodefective and autoimmune mice. *J. Exp. Med.* 157:202
25. Oseroff, A., Okada, S., Strober, S. 1984. Natural suppressor (NS) cells found in the

Annual Reviews

spleen of neonatal mice and adult mice given total lymphoid irradiation (TLI) express the null surface phenotype. *J. Immunol.* 132:101
26. Strober, S., Slavin, S., Gottlieb, M., Zan-Bar, I., King, D. P., Hoppe, R. T., Fuks, Z., Grumet, F. C., Kaplan, H. S. 1979. Allograft tolerance after total lymphoid irradiation (TLI). *Immunol. Rev.* 46:88
27. King, D. P., Strober, S., Kaplan, H. S. 1981. Suppression of the mixed leukocyte response and graft-vs.-host disease by spleen cells following total lymphoid irradiation (TLI). *J. Immunol.* 126:1140
28. Gottlieb, M., Strober, S., Kaplan, H. S. 1979. Allogeneic marrow transplantation after total lymphoid irradiation (TLI): effect of dose/fraction, thymic irradiation, delayed marrow infusion, and presensitization. *J. Immunol.* 123:379
29. Billingham, R. E., Silvers, W. K. 1961. Quantitative studies on the ability of cells of different organs to induce tolerance of skin homografts and cause runt disease in neonatal mice. *J. Exp. Zool.* 146:113
30. Streilein, J. W., Gruchalla, R. S. 1981. Analysis of neonatally induced tolerance of H-2 antigens. *Immunogenetics* 12:161
31. Gowans, J. L., McGregor, D. D. 1963. The origin of antibody forming cells. *Immunopathology,* 3rd Int. Symp. Basel: Schwabe & Co., p. 89
32. Dresser, D. W. 1962. Specific inhibition of antibody production. I. Protein overloading paralysis. *Immunology* 5:161
33. Dorsch, S., Roser, B. 1982. Suppressor cells in transplantation tolerance. II. Identification and probable mode of action of chimeric suppressor T cells. *Transplantation* 33:525
34. Dorsch, S., Roser, B. 1975. T cells mediate transplantation tolerance. *Nature* 258:233
35. Dorsch, S., Roser, B. 1977. Recirculating, suppressor T cells in transplantation tolerance. *J. Exp. Med.* 145:1144
36. Holan, V., Chutna, J., Hasek, M. 1977. Specific suppressor cells in rats with neonatally induced transplantation tolerance. *Folia Biol. (Praha)* 23:66
37. Rieger, M., Hilgert, I. 1977. The involvement of a suppressor mechanism in neonatally induced allograft tolerance in mice. *J. Immunogenet.* 4:61
38. Nossal, G. J. V., Pike, B. L. 1981. Functional clonal deletion in immunological tolerance to major histocompatibility complex antigens. *Proc. Natl. Acad. Sci. USA* 78:3844
39. Brent, L., Brooks, C., Lubling, N., Thomas, A. V. 1972. Attempts to demonstrate an in vivo role for serum blocking factors in tolerant mice. *Transplantation* 14:382
40. Rodrigues, G., Andersson, G., Wigzell, H., Peck, A. B. 1979. Non-T cell nature of the naturally occurring, spleen-associated suppressor cells present in the newborn mouse. *Eur. J. Immunol.* 9:737
41. Piguet, P. F., Irle, C., Vassalli, P. 1981. Immunosuppressor cells from newborn mouse spleen are macrophages differentiating *in vitro* from monoblastic precursors. *Eur. J. Immunol.* 11:56
42. Peeler, K., Wigzell, H., Peck, A. B. 1983. Isolation and identification of the naturally occurring, newborn spleen-associated suppressor cells. *Scand. J. Immunol.* 17:443
43. Wysocki, L. J., Sato, V. L. 1978. "Panning" for lymphocytes: a method for cell selection. *Proc. Natl. Acad. Sci. USA* 75:2844
44. Kiessling, R., Hochman, P. S., Haller, O., Shearer, G. M., Wigzell, H., Cudkowicz, G. 1972. Evidence for a similar or common mechanism for natural killer activity and resistance to hematopoietic grafts. *Eur. J. Immunol.* 7:655
45. Herberman, R. B., Nunn, M. E., Holden, T. T. 1978. Low density of Thy-1 antigen on mouse effector cells mediating natural cytotoxicity against tumor cells. *J. Immunol.* 121:304
46. Cudkowicz, G., Hochman, P. S. 1979. Do natural killer cells engage in regulated reactions against self to ensure homeostasis? *Immunol. Rev.* 49:13
47. Herberman, R. B., Dyen, J. Y., Kay, H. D., Ortaldo, J. R., Riccardi, C., Bonnard, G. D., Holden, H. T., Fagnani, R., Santoni, A., Puccetti, P. 1979. Natural killer cells: characteristics and regulation of activity. *Immunol. Rev.* 44:33
48. Kaplan, H. S. 1972. *Hodgkin Disease.* Cambridge: Harvard Univ. Press. p. 283
49. Slavin, S., Strober, S., Fuks, Z., Kaplan, H. S. 1977. Induction of specific tissue transplantation tolerance by using fractionated total lymphoid irradiation in adult mice: long-term survival of allogeneic bone marrow and skin grafts. *J. Exp. Med.* 146:43
50. Slavin, S., Fuks, Z., Kaplan, H. S., Strober, S. 1978. Transplantation of allogeneic bone marrow without graft-versus-host disease using total lymphoid irradiation. *J. Exp. Med.* 147:963
51. Strober, S., Slavin, S., Gottlieb, M., Zan-Bar, I., King, D. P., et al. 1979. Allograft tolerance after total lymphoid irradiation (TLI). *Immunol. Rev.* 46:87
52. Strober, S., Gottlieb, M., Slavin, S., King, D. P., Hoppe, R. T., Fuks, Z., Bieber,

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C. P., Kaplan, H. S. 1980. Immunosuppression and tolerance after total lymphoid irradiation (TLI). *Transplant. Proc.* 12:477

53. Waer, M., Ang, K. K., van der Schueren, E., Vandeputte, M. 1984. Influence of radiation field and fractionation schedule of total lymphoid irradiation (TLI) on the induction of suppressor cells and stable chimerism after bone marrow transplantation in mice. *J. Immunol.* In press
54. Slavin, S., Reitz, B., Bieber, C. P., Kaplan, H. S., Strober, S. 1978. Transplantation tolerance in adult rats using total lymphoid irradiation (TLI): Permanent survival of skin, heart, and marrow allografts. *J. Exp. Med.* 147:700
55. Zan-Bar, I., Slavin, S., Strober, S. 1978. Induction and mechanism of tolerance to bovine serum albumin in mice given total lymphoid irradiation (TLI). *J. Immunol.* 121:1402
56. Okada, S., Palathumpat, V., Strober, S. 1983. Identification of donor-derived antigen-specific suppressor cells in murine bone marrow chimeras prepared with total-lymphoid irradiation. *Transplantation* 36:417
57. Gottlieb, M., Strober, S., Kaplan, H. S. 1980. Cellular basis of graft versus host tolerance in chimeras prepared with total lymphoid irradiation. *J. Exp. Med.* 152:736
58. Okada, S., Strober, S. 1982. Spleen cells from adult mice given total lymphoid irradiation (TLI) or from newborn mice have similar regulatory effects in the mixed leukocyte reaction (MLR). I. Generation of antigen-specific suppressor cells in the MLR after the addition of spleen cells from adult mice given TLI. *J. Exp. Med.* 156:522
59. Waer, M., Ang, K. K., van der Schueren, E., Vandeputte, M. 1984. Allogeneic bone marrow transplantation in mice after total lymphoid irradiation: influence of breeding conditions and strain of recipient mice. *J. Immunol.* In press
60. King, D. P., Strober, S. 1981. Immunoregulatory changes induced by total lymphoid irradiation (TLI). II. Development of TL^+ and TL^- suppressor T cells which differ in their regulatory function. *J. Exp. Med.* 154:13
61. Weigenberg, M., Morecki, S., Weiss, L., Fuks, Z., Slavin, S. 1984. Suppression of cell mediated immune responses following total lymphoid irradiation (TLI). I. Characterization of suppressor cells of the mixed leukocyte reaction. *J. Immunol.* In press
62. May, R. D., Slavin, S., Vitetta, E. S. 1984. The partial characterization of suppressor cells in the spleens of mice conditioned with fractionated total lymphoid irradiation (TLI). *J. Immunol.* 131:1108
63. Bennett, J. A., Rao, V. S., Mitchell, M. S. 1978. Systemic bacillus Calmette-Guérin (BCG) activates natural suppressor cells. *Proc. Natl. Acad. Sci. USA* 75:5142
64. Bennett, J. A., Mitchell, M. S. 1980. Systemic administration of BCG activates natural suppressor cells in the bone marrow and stimulates their migration into the spleen. In *Neoplasm and Immunity: Experimental and Clinical,* ed. R. G. Crispen, p. 397. Amsterdam: Elsevier North Holland
65. McIntosh, K. R., Segre, M., Segre, D. 1982. Characterization of cyclophosphamide-induced suppressor cells. *Immunopharmacology* 4:279
66. Merluzzi, V. J., Levy, E. M., Kumar, V., Bennett, M., Cooperband, S. R. 1978. In vitro activation of suppressor cells from spleens of mice treated with radioactive strontium. *J. Immunol.* 121:505
67. Dennert, G., Yogeeswaron, G., Yamagata, S. 1981. Cloned cell lines with natural killer activity. Specificity, function, and cell surface markers. *J. Exp. Med.* 153:545
68. Zan-Bar, I. 1983. Modulation of B and T cell subsets in mice treated with fractionated total lymphoid irradiation. II. Tolerance susceptibility of B cell subsets. *Eur. J. Immunol.* 13:236
69. Watson, J. 1979. Continuous proliferation of murine antigen specific helper T lymphocytes in culture. *J. Exp. Med.* 150:1510
70. Jones, B., Janeway Jr., C. A. 1981. Cooperative interaction of B lymphocytes with antigen-specific helper T lymphocytes is MHC restricted. *Nature* 292:547

Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES

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INTRODUCTION

Over the last two decades a great deal of information has accumulated on the structure and function of the immunoglobulin molecules. Structural studies of the myeloma proteins defined the variable and constant regions. Analysis of the variable regions showed the existence of hypervariable regions (1) and began to give insight into the nature of the interaction between the antibody molecule and its specific antigen. The existence of both variable and constant regions on the same molecule gave rise to the concept "two genes—one polypeptide chain" (2), which challenged then-accepted genetic ideas.

With the advent of modern molecular biologic techniques it became clear that the immunoglobulin (Ig) molecule, both heavy and light chain, was encoded by multiple DNA segments. In order to generate a gene encoding a functional Ig molecule, somatic rearrangements of distinct DNA segments must take place. For a complete light-chain gene a V region must be brought next to a J segment to create an active transcription unit. For a heavy-chain gene, V, D, and J segments must be assembled next to a constant-region gene; the initial constant-region gene utilized is the μ gene. In addition, during immune response maturation heavy-chain class switching can occur, whereupon VDJ is recom-

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bined next to a different constant-region gene to change the class but not the specificity of the molecule being synthesized.

In addition to somatic rearrangement, the expression of immunoglobulin (Ig) genes must be regulated during B-lymphocyte differentiation. Transcription of unrearranged V_κ gene segments is not detected prior to rearrangement (3). Rearrangement is necessary but not sufficient for expression (4–7). When B lymphocytes develop into antibody-secreting plasma cells the rate of transcription of the rearranged immunoglobulin genes increases greatly so that in some plasma cells immunoglobulin transcripts may constitute 10% of the mRNA population (8). DNA sequence studies of genes give little insight into the mechanisms of their control. However, if one can modify genes in vitro and transfect them into cells of the appropriate phenotype, it is possible to begin to define the regions of the Ig genes that are important for the regulation of their expression and to identify the basis for differential immunoglobulin gene expression at different stages of lymphocyte differentiation.

The study of the structure and function of the immunoglobulin molecule has been of great interest because of the ability of the immunoglobulin molecule to react with a diverse family of ligands, because different immunoglobulin molecules contain different effector functions, and because of the biologic importance of antibody molecules. The use of myelomas, and more recently hybridoma proteins, has permitted the study of homogeneous populations of antibodies. However, in these cases one is limited to the study of a protein the animal happens to produce. DNA-mediated transfection and immunoglobulin gene expression provide an important new tool for the study of immunoglobulin molecules. By using this technique it will be possible to study the function of novel chain combinations and novel chain structures created in vitro and then expressed following gene transfection. Additionally, in vitro site-specific mutagenesis techniques can be used to construct specific mutations in immunoglobulin genes that can be expressed after transfection. Because sufficient quantities of immunoglobulin are produced in the transformants, quantities of protein necessary for detailed analyses can be obtained.

VECTORS, SELECTIVE TECHNIQUES, AND TRANSIENT VS STABLE EXPRESSION

When transfecting cells two basic approaches can be used. Transient expression can be assayed, or stable transformants can be selected.

To study transient expression the foreign DNA is introduced into the recipient cells; then, following an appropriate interval (usually 48–72 hr), the cell population is harvested and expression is assayed. This method allows an answer to be obtained rapidly; and, since no selection of the recipient population is required, no selectable marker is necessary in the transfecting vector.

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In most cases, expression in a transient assay is monitored by S1 analysis of the mRNA produced (9). This provides the most sensitive assay available, and sensitivity is often required for transient assays where little material is available. To increase the sensitivity of the transient assay, vectors are used that replicate in the recipient cells and so increase the gene copy number. For COS cells (10), a monkey kidney cell line that contains an integrated and expressed copy of SV40 T antigen, vectors containing an SV40 *ori* are suitable. These vectors, however, do not replicate in mouse cells. To overcome this shortcoming, vectors containing a polyoma early region can be used in mouse cells (11–15). These replicate to high copy number (50,000–400,000 copies per cell) and so provide gene amplification in the transient assay. Using these vectors, sufficient expression is obtained to permit visualization of Ig proteins on SDS-polyacrylamide gels following radiolabeling and immunoprecipitation (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished).

As an alternative to transient expression assays, expression by stable transformants can be studied. The disadvantage of these experiments is that they are much more time consuming than those for transient expression. Two to four weeks are required to accumulate enough cells for analysis. Such experiments have the advantage of providing a cloned population of cells for long-term analysis. In addition, sufficient expression from a single or low copy number of transfected genes can be obtained.

In transient expression 30–80% of the treated cells will express the transfected genes. However only 10^{-3} to 10^{-6} cells will go on to become stably transformed. Therefore, selective techniques are required that permit the isolation of the rare stably transformed cells from among the many nontransformed cells.

The initial selective technique developed used the expression of the thymidine kinase gene from *Herpes simplex* as a selectable marker (16, 17). Cells that were deficient in endogenous thymidine kinase were transfected with vectors containing the viral enzyme. Only recipient cells that expressed viral thymidine kinase could survive selection in HAT medium (hypoxanthine/aminopterin/thymidine) (18, 19). This proved to be an effective selective technique, but it could be applied only to recipient cell populations deficient in endogenous thymidine kinase. Since it is not a trivial matter to put a drug marker into a cell type, one was limited in the number of potential recipients using these vectors.

To circumvent this problem vectors with dominant selectable markers have been developed. Dominant-acting genetic markers produce a selectable change in the phenotype of normal cells. When a dominant selectable marker is used, the recipient cell population need not be drug marked. Therefore, with a dominant marker any cell can be used as a recipient.

One dominant selection utilizes expression of the bacterial xanthine-guanine

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phosphoribosyltransferase gene (*gpt*) (14, 15). The enzyme encoded by this gene, 5-phospho-α-D-ribose-1 diphosphate:xanthine phosphoribosyltransferase (XGPRT), differs from the analogous mammalian enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPT) in that it can efficiently use xanthine as a substrate in nucleotide synthesis while the mammalian enzyme cannot; mammalian cells do not convert xanthine to xanthylic acid or to guanylic acid at a significant rate. Therefore, in the presence of mycophenolic acid (which inhibits the conversion of IMP to XMP) and xanthine, only cells that express bacterial XGPT survive (Figure 1). Growth in mycophenolic acid + xanthine provides a dominant selection system for unmarked cell lines. The bacterial XGPRT can also be used to complement the enzymatic deficiency in HGPT-deficient cell lines; expression of the XGPRT permits HPGT-deficient cells to survive HAT selection.

A second selectable system relies on acquisition of resistance to the aminoglycoside antibiotic G418 (20). The structure of G418 resembles gentamycin, neomycin, and kanamycin; but, unlike these related compounds, G418 interferes with the function of 80S ribosomes and blocks protein synthesis in eukaryotic cells. Bacterial phosphotransferases inactivate this class of antibiotic (21). Therefore when the phosphotransferase from Tn5 (designated *neo*) is included in a mammalian transcription unit, it confers resistance to G418 when introduced into eukaryotic cells (22).

Any number of different vectors are available for gene expression. The most commonly used vectors to date in lymphoid cells have been the pSV2 vectors (14, 15, 22). The prototype vector with its essential features is shown in Figure 2. These vectors contain the pBR322 *ori* and ß-lactamase gene. Thus they can be propagated and selected as plasmids in *E. coli*. This facilitates in vitro

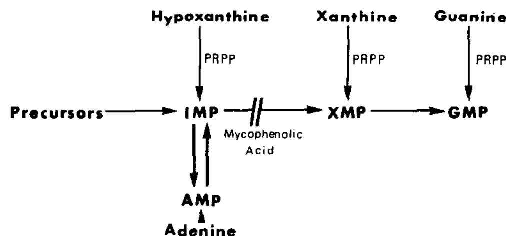

Figure 1 Pathway of purine nucleotide synthesis. Salvage of free purines occurs by condensation with phosphoribosyl pyrophosphate (PRPP). Adenine phosphoribosyl transferase (APRT) accounts for the formation of AMP from adenine and hypoxanthine phosphoribosyltransferase (HPRT) converts hypoxamine to IMP (inosinic acid). No mammalian enzyme is known that can convert xanthine to XMP (xanthylic acid). Mycophenolic acid, an inhibitor of IMP dihydrogenase (19a) prevents the formation of XMP and thereore of GMP. Since normal mammalian cells do not convert xanthine to XMP, they cannot grow in medium containing mycophenolic acid supplemented with xanthine. However, cells that express the bacterial XGPRT gene can grow under these conditions.

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manipulation of these vectors and easily enables one to obtain the quantities of DNA required for transfection. In addition these vectors contain a mammalian transcription unit with a protein coding sequence joined at its 5′ end to a segment containing the SV40 early region promoter and at its 3′ end to an SV40 segment containing an intervening sequence and polyadenylation signal. This transcription unit is used to direct the transcription of the selectable marker. The presence of the SV40 early promoter permits the expression of the bacterial gene in mammalian cells. Restriction endonuclease sites (Pst1, BamH1, and EcoR1) are available in regions of extraneous DNA into which can be inserted the additional genes of interest. These vectors need not be propagated as viruses; therefore, there is no theoretical limit on the size of the genes that can be inserted, and genes larger than 25 kb have been used with ease (23).

Many modifications of the different vector systems exist, depending on the assay to be used. As mentioned earlier, the most significant modification entails including the polyoma early region in the vector so that it can replicate and be amplified in mouse cells.

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METHODS OF TRANSFECTING CELLS

Three basic techniques have been used to introduce DNA into recipient lymphoid cells: $CaPO_4$ precipitation, treatment with DEAE-dextran, and protoplast fusion.

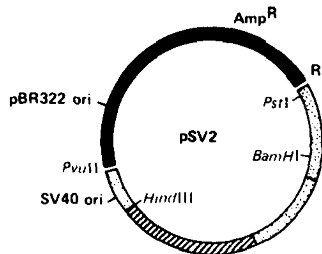

Figure 2 Structure of the pSV2 vectors. pBR322 DNA is represented by a solid black line and contains the plasmid origin of replication and ß-lactamase gene. The hatched segments represent a gene that provides a selectable marker in eukaryotic cells. The two commonly used genes are *gpt* and *neo*. The stippled segments are derived from simian virus 40 (SV40). The Pvu II to Hind III segment contains the SV40 origin of DNA replication (*ori*) and early promoter. The SV40 sequences immediately downstream of the selectable marker contain a splice and polyadenylation site. The early promoter, splice, and polyadenylation sites constitute a transcription unit that permits the expression of a bacterial gene in a eukaryotic cell. The EcoRI, Pst I and BamHI sites are located in SV40 sequences that are not necessary for vector function and thus provide convenient sites into which other genes can be inserted. [Diagram adapted from (14)]

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$CaPO_4$ precipitation has been used to introduce DNA into lymphoid cells for stable transfection experiments (24, 25) or to study transient expression (26–28). The basic method used is that of Graham & van der Eb (29) with the modification of Chu & Sharp (30) for suspension cells. Using $CaPO_4$ sufficient expression is achieved for either enzyme analysis assay (28) or S1 analysis of the recipients (26, 27). Stable transfectants can be recovered at a frequency of approximately 10^{-5} to 5×10^{-6} from myeloma cells treated with $CaPO_4$ precipitated DNA (25).

Treatment with DEAE-dextran has also been used to introduce DNA into lymphoid cells (11–13, 27, 31) and in many cases is less toxic to the cells than $CaPO_4$. Using this method 60–70% of treated myeloma cells were found to express antigens encoded by the transfecting DNA 48 hr after treatment (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished), and sufficient mRNA was produced to permit S1 analysis of the recipient cells (11, 12, 27).

The most efficient way of introducing DNA into lymphoid cells may be by protoplast fusion. In this method, *E. coli* bearing the plasmids of interest are treated with lysozyme to remove the bacterial cell wall (32). The resulting spheroplasts are then fused to the myeloma cells using methods similar to those used to create somatic cell hybrids. This method has been used to study both transient expression (13) and stably transformed cells (23, 25, 33–35). Using this method, transfection frequencies ranging from 10^{-4} (23, 33) to as high as 10^{-3} (34) have been observed.

Gene transfection into a wide variety of lymphomas, hybridomas, and myelomas has now been reported. These include the mouse myelomas J558 (25, 34, 35), MPC-11 (12), and X63-Ag8 (31, 27); the rat myeloma Y3 (25); hybridomas 27–44 (25), SP/20Ag14, and SP6 and their mutants (23, 33); and the lymphomas BW5147 (13,25), 18–81 (24), and 70Z (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished). Under the proper conditions and with the appropriate vector, all of these cell lines appear to be potential recipients. Including Ig sequences in the vector often increases the ability to transfect lymphoid cells. When a polyoma vector is used, transient expression of T antigen in 70Z and 18–81 is observed only when the heavy-chain gene is contained within the vector (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished). Including light chain in the pSV2*gpt* vector increased the transfection frequency of the J558 myeloma 10- to 100-fold (25). However, comparable transfection frequencies were observed whether or not the kappa light chain was included in the pSV2-*neo* vector (33). The exact mechanisms by which the Ig sequences increase the transfection frequency have not yet been defined. However, evidence is accumulating that Ig genes contain lymphoid-specific enhancer regions (see below for discussion) and that these facilitate the expression of the selectable markers.

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EXPRESSION OF IMMUNOGLOBULIN MOLECULES

When the MOPC-41 kappa light chain was inserted into the pSV2-*gpt* vector between the EcoR1 and BamH1 sites, stable transfectants of the A-MuLV transformed cell line 81–A2 were recovered that synthesized detectable quantities of kappa light chain. The kappa light chain was oriented in the pSV2-*gpt* vector so that its direction of transcription was opposite to that of the *gpt* gene from the SV40 promoter (24). The kappa specific mRNA was found to be of three sizes in the transfectants: 1.2 kb, 1.9 kb, and 2.6 kb. The 1.2 kb transcript represented the correctly processed mRNA while the 1.9 and 2.6 kb transcripts were incorrectly processed and still contained the kappa intervening sequence. The transfected kappa chain was able to assemble with the resident γ_{2b} heavy chain so that H_2L_2 molecules were observed in the cytoplasm. LPS stimulation increased the level of synthesis of the transfected kappa chain synthesis about 5-fold, just as it does in the parental Abelson line, so that the final level of kappa synthesis was about $1/15$ of that observed in the MPC-11 myeloma. Thus the gene sequences responsive to LPS stimulation are contained within the transfected gene fragment. However, for the transfected light chain, LPS stimulation effects preferential increase in the quantity of the aberrant 1.9-kb transcript.

The S107A kappa light-chain gene was also capable of transfection and expression in stable transformants when included in the pSV2-*gpt* vector (25). The light chain was oriented in the vector such that its direction of transcription was opposite to that of the *gpt* gene from the SV40 promoter; however, in subsequent experiments the orientation of the light-chain gene was reversed and the same level of kappa chain expression was observed (S. L. Morrison, V. T. Oi, unpublished observation). Thus the transcription from the SV40 promoter appears to neither facilitate nor interfere with kappa chain expression. Two-dimensional gel analysis of the kappa chain produced by the cells transfected with the S107A light chain showed it to be indistinguishable from that produced by the S107 myeloma. In addition, when the S107A kappa light chain was produced in the absence of heavy chain, it was not secreted, just as it is not secreted in the parental myeloma in the absence of heavy-chain synthesis. However, in the presence of heavy chain it can assemble and be secreted as part of an H_2L_2 molecule.

The light chain from a TNP-specific hybridoma was capable of expression when ligated into the pSV2-*neo* vector and transfected into a recipient hybridoma cell line (33). Expression occurred whether the light chain was in the same or opposite orientation from the SV40 promoter. As noted by previous investigators, variability of expression occurs among different independent transformants; however, in this case expression of light chain seemed consis-

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tently better when the light chain was oriented such that its transcription was in the opposite direction from transcription from the SV40 promoter. The recipient cell line originally synthesized antibodies specific for TNP but had lost the ability to synthesize the TNP-specific light chain. Transfection with the TNP-specific light chain restored the ability of the Ig to bind antigen and the secretion of TNP-specific IgM. The hemagglutination titer of anti-TNP antibody in some transformants was comparable to that of the parental anti-TNP hybridoma.

Transfection of the S107A light chain into a heavy-chain producing cell line isolated from an anti-PC hybridoma was also able to restore the ability of this cell line to bind antigen (C. Desmeyard, S. L. Morrison, M. D. Scharff, unpublished observation). In these transfectants there was also an increase in cytoplasmic heavy chain as judged by immunofluorescence; presumably this occurred because the light chain interacted with the cytoplasmic heavy chain to protect it from degradation.

It is also possible to get efficient expression of heavy chain following gene transfection. When the γ_{2b} heavy chain from the MOPC-141 myeloma was included in the pSV2-*gpt* vector and used to transfect the J558L cell line, stable transfectants synthesizing approximately 20% of the amount of heavy chains synthesized by the parental myeloma were isolated (34). The heavy mRNA was 1.7 kb in length and of a discrete size; the 1.7 kb size is that expected for a secreted γ_{2b} protein. The γ_{2b} gene included in the expression vector did not include the membrane-specific exon and so could not code for mIg. Transcripts 1.7 kb in size were also observed when this γ_{2b} gene was used to transfect mouse L cells. In the J558L myeloma, which synthesizes lambda light chain, assembly between the γ_{2b} and the lambda light chain occurred such that an anti-γ_{2b} antiserum immunoprecipitated both chains.

By transferring a heavy-chain gene from an anti-TNP-specific myeloma into a light chain–producing variant of that myeloma, it was possible to express a pentameric IgM molecule with anti-TNP activity (23). Cotransfer of specific light chain and heavy chain into a nonproducing myeloma also resulted in the production of pentameric, antigen-binding antibody. Analysis of a transformant obtained using the heavy chain into a light chain–producing myeloma showed that it synthesized about 25% as much protein as the parental myeloma. A transformant obtained after cotransfer of heavy plus light chain synthesized about 10% of the normal amount of IgM. The heavy-chain gene used in these experiments contained the membrane exon, and two mRNA species—a 2.7-kb species presumably with the membrane exon, and a 2.4-kb species coding for the secreted protein—were synthesized in the transformants. However, no membrane-specific protein could be detected either by immunofluorescence or by biosynthetically labeling with radioactive amino acids and immunopre-

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cipitation. These results suggest either that the 2.7-kb mRNA is an aberrant transcript, that it is not translated, or that its product is rapidly degraded.

Using a transient expression assay, R. J. Deans and co-workers observed the production of both secreted and membrane alpha chain in NS-1, a light chain–producing myeloma. The rearranged alpha-chain gene from the myeloma M603 was inserted into a polyoma vector. The alpha-chain gene was oriented such that it was expressed either using its own promoter or using the late polyoma transcription signals. No difference in expression was observed with these two different promoters. Immunofluorescence revealed both cytoplasmic and surface IgA, and RNA dot blots showed the presence of both membrane and secretory forms of the mRNA. NS-1 clones transfected with alpha gene synthesized 3–8% of their protein as Ig while BW5147, a thymoma, and 3T3 cells synthesized only 0.3% and 0.8%, respectively, of their protein as Ig. NS-1 transfectants secreted approximately 25% as much IgA in a 72-hr period as did a myeloma. It is however difficult to relate these figures to normal alpha synthesis since the myeloma contains only one active chromosomal gene while the polyoma transfectants contain 50,000–400,000 extrachromosomal copies of the gene per cell.

With the demonstration that it is possible to produce both heavy and light chains efficiently by gene transfection, it is now possible to begin to produce novel Ig molecules using this technique. To this end the variable region from the heavy chain of an anti-*p*-azophenylarsonate-specific hybridoma has been fused to the kappa constant-region gene (J. Sharon, M. L. Gefter, T. Manser, S. L. Morrison, V. T. Oi, M. Ptashne, unpublished). The chimeric gene is expressed as a kappa-sized (approximately 1.2 kb) mRNA that hybridized both to a V_H-specific and to a C_κ-specific probe. The mRNA is translated into a chimeric protein of approximately 25,000 daltons that reacts with both anti-kappa- and anti-$Id_{(heavy)}$-specific antisera and is secreted. This demonstrates that the splice donor from the V_H can be successfully spliced to the C_κ acceptor and generate a mRNA still in reading frame. The chimeric light chain is synthesized in only about 10% of the quantity of the endogenous lambda chain produced by the recipient myeloma, but it still constitutes about 1% of the cell protein. In vitro reassociation experiments show that the V_HC_κ chimeric protein can associate with the light chain from a myeloma of the same specificity to generate a heterodimer that reacts with antigen.

In similar experiments the V_H from the S107 heavy chain has been fused to C_κ (V. T. Oi and S. L. Morrison, unpublished). This chimeric gene has been co-transfected with the S107A light-chain gene into the hamster lymphoma GD-36. Once again the chimeric gene is expressed as a protein and the V_HC_κ and light-chain proteins are found in the secretions of the lymphoma.

Gene transfection can also be used to study regulation of DNA rearrange-

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ment and other events that occur during lymphocyte differentiation. K. Black well & F. W. Alt (unpublished) constructed a plasmid with a murine heavy chain D segment (DQ52) separated from a part of the J_H cluster containing JH_3 and JH_4 by a *Herpes* thymidine kinase gene. This plasmid was co-trans fected with pSV2-*neo* into a *tk*$^-$ derivative of the Abelson line 38B9, which rearranges its own heavy-chain genes in culture. Stable transformants were then selected for loss of expression of the *tk* gene. Surviving cells were found to have completed rearrangements of either JH_3 or JH_4. One rearrangement a D-J_3 joint, has been cloned and sequenced. The D and JH_3 segments were found to have recombined in the appropriate regions. These experiments show that the D–J joining is not a function of the chromosomal location of the Ig genes.

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TISSUE SPECIFICITY OF IMMUNOGLOBULIN EXPRESSION

Igs are the products of cells of the B lymphoid series and are not observed to be synthesized in other cell types. At least part of this preferential synthesis is a result of Ig genes' being rearranged only in B lymphocytes. Now, using gene transfection, it is becoming apparent that regulation in addition to rear rangement is responsible for specificity of expression.

The first experiment to demonstrate clearly that nonlymphoid cells do not efficiently synthesize Ig proteins from the Ig promoter was that of Falkner & Zachau (36). When monkey cells (CV1) were transfected with plasmids con taining light-chain genes, light-chain-specific mRNA was seen only if the kappa-chain gene was placed near (within 31 bp) the SV40 early promoter When 0.8 or 3.0 kb of mouse DNA were interspersed, such that the Ig pro moter region should be intact and capable of functioning, transcripts contain ing kappa-specific sequences were heterogeneously large. Kappa-specific translation products could be observed in CV1 cells containing kappa mRNA When mouse L cells were used as a recipient, no kappa-specific mRNA or protein could be detected.

Similar results have been obtained with the lambda light-chain gene (26) In order to achieve efficient lambda-chain expression in HeLa cells the SV40 enhancer element has to be included 150 base pairs upstream of the cap site of the lambda chain. In this case the lambda transcripts had the same 5'-end as authentic mRNA. If the lambda-chain promoter was separated from the SV40 enhancer sequences by more than 1 kb pair of spacer DNA, then lambda chain transcripts were not correctly initiated in HeLa, CV1, or 3T6 cells.

The tissue specificity of expression is not limited to cells of the nonlymphoid series. In one case a myeloma recipient from a different species (mouse gen

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into rat myeloma) was unable to express the transfected Ig gene efficiently (25). In addition, thymoma (BW5147) cells do not efficiently synthesize transfected Ig genes. In a stable transfection assay, L chain synthesis was not detected in BW5147 (25). However, on further examination thymoma transfectants were found to synthesize kappa light chains, but the quantity was less than 5% of that synthesized by a myeloma recipient (S. L. Brown, S. L. Morrison, unpublished).

To compare transcripts initiated from the Ig promoter with those initiated from a different promoter in the same cell lines, Stafford & Queen (12) constructed a vector containing pSV2-*neo*, the MOPC-41 kappa chain, and the polyoma T antigen and *ori* to permit replication in mouse cells. Since light chain and *neo* are on the same vector they serve as internal standards for each other. Mouse 3T3, L, and MPC-11 myeloma cells were transfected with these vectors and RNA prepared and quantitated 48 hr after transfection. All three recipients synthesized the same quantity of *neo*-specific mRNA. Kappa-specific transcripts were seen only in MPC-11. These results suggest that nonlymphoid cells fail to transcribe the transfected immunoglobulin gene.

Rearranged kappa-chain genes introduced into the mouse genome by microinjection of nuclei also show tissue specificity of expression (37). Spleens of transgenic mice were found to have large quantities of mRNA originating from the injected rearranged gene. Up to 50% of the total kappa mRNA in spleens from positive mice were found to bear the V region of the injected gene while less than 4% of the total kappa mRNA from normal littermates was positive. Spleens of transgenic mice also contained 1.5–2 times as much mRNA as normal controls, so the level of expression of the injected gene is relatively high. Examination of livers from injected mice showed no evidence of kappa expression. Therefore, even though hepatocytes contain the rearranged kappa gene, they cannot express it.

EXPRESSION OF NON-Ig IMMUNE RELATED MOLECULES

With the ability to transfect and express genes in lymphoid cells, one can also study the expression and function of non-Ig, immune related molecules. To this end $A\beta^k$ in the pSV2-*gpt* vector was introduced into an Ia^+ B lymphoma (38). Of the transfectants analyzed, one had several copies of the gene while the other had only one. The level of $A\beta^k$ expression in the transfectants was similar to that of $I\text{-}A^k$ in B-cell lines; the transfectant with several gene copies appeared to make slightly more than the transfectant with a single copy. The transfected genes are expressed so that they can be recognized by the appropriate monoclonal antibodies and can stimulate a MLR response. The trans-

Annual Reviews

fected gene functions in the antigen-dependent activation of an I-A^k restricted T-cell clone. This methodology clearly can be used for the further detailed analysis of the structure and function of I-A molecules.

CONTROL REGIONS IN Ig GENES

Heavy Chains

Although much has recently been learned about the structure of the Ig genes, little is known about their regulation and control. Analysis of transfected genes provides one way to investigate Ig regulation. One can begin to find answers to such questions as why Ig V regions are transcribed only following rearrangement, how Ig synthesis is amplified as a B lymphocyte matures to a plasma cell, and how the cell makes a choice of membrane vs secreted forms of the mRNA and protein.

Regulation of heavy-chain synthesis has been studied in myeloma cells transfected with heavy-chain genes or gene fragments. J558 cells transfected with a γ_{2b} heavy-chain gene effectively synthesized that protein (34). However if the 1-kb Xba I fragment with the internal EcoR1 was deleted from the IVS heavy chain, synthesis decreased to 5% of that seen using an intact heavy-chain gene (see Figure 3). Most of the intervening sequence can be deleted with no influence on heavy-chain synthesis as long as sequences within the 1-kb Xba I fragment are present. These necessary sequences can be inserted in either orientation at the Xba I site 5′ of the heavy-chain gene or within the IVS. When Δ1 was assayed it was found to be active in expression; Δ2 was found to be inactive in Ig expression (Figure 3). Fine structure analysis has

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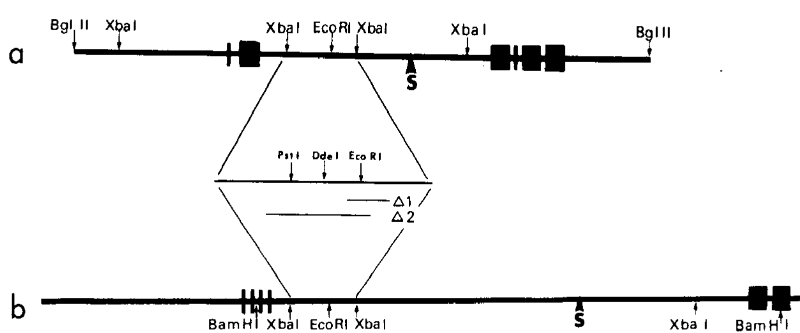

Figure 3 Schematic representation of the heavy-chain gene fragments used for assay of enhancer activity. *a:* The functionally rearranged γ_{2b} gene from the MOPC-141 myeloma (34). Solid boxes represent exons. The 1-kb Xba I fragment with enhancer activity is shown on a larger scale. Sequences deleted from the Xba I fragment are indicated by a solid line. *b:* The μ heavy-chain locus. Banerji et al (31) assayed gene fragments from an unrearranged μ gene. Mercola et al (28) assayed fragments from a rearranged μ gene. However, the fragments were from 3′ of the VDJ joining and so were not affected by gene rearrangement.

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shown that most active sequences are contained in a segment lying between the Pst I and Dde I sites within the Xba I fragment. These sequences are effective not only in enhancing transcription of the Ig promoter, but also permit transcription of an enhancerless SV40 promoter. The effect of the sequence appears to be lymphoid-cell specific. When heavy-chain expression is studied in L cells the presence or absence of the 1-kb Xba fragment does not affect the level of heavy-chain synthesis. In addition, this sequence does not permit the expression of an enhancerless SV40 promoter in L cells.

A slightly different approach was used by Banerji (31) to identify enhancing sequences within the heavy-chain IVS. In this case the three Xba I fragments from an unrearranged μ chain gene were tested for their ability to increase the synthesis of T antigen in a vector lacking the SV40 enhancer sequence. The 1-kb Xba I fragment was found to enhance T-antigen expression in myeloma but not HeLa cells. It functioned only in *cis*. This same gene fragment could enhance the expression of β-globin gene transcripts in myeloma cells when located 500 bp upstream or 2500 bp downstream of the β-globin promoter. The Ig enhancer does not function in 3T6, mink lung cells, or human HeLa cells but does function in all myeloma lines tested. Fine structure mapping indicates that sequences important for enhancing are distributed over most of the EcoRI to Pst I fragment of the 1-kb Xba I piece.

Transcriptional enhancer elements have also been detected in the heavy-chain IVS when assayed in a nonlymphoid system (28). In this case an enhancerless SV40 promoter was used to direct transcription of the bacterial chloramphenicol acetyltransferase (CAT) gene. The enhancer activity of various gene fragments was quantified by measuring CAT activity in transfected COS cells. COS cells are an SV40-transformed monkey cell line that constitutively express T antigen (10). The 7.6-kb J_H-C_μ BamHI fragment containing the heavy-chain intervening sequence was capable of enhancing CAT transcription when cloned 3′ to the gene in the "antisense" orientation, but not in the opposite orientation. The Ig sequence had about 60% of the activity of the SV40 enhancer and did not function by increasing gene copy number. When the IVS was divided at the EcoR1 site, both sequences 5′ and 3′ of the EcoR1 site had enhancer activity, the 3′ piece having greater activity. Using this same assay system it is not possible to demonstrate enhancer activity of the J_H-C_μ sequence in CV1 cells, the nontransformed parent line to COS cells. In addition it is unclear how this enhancement in a nonlymphoid system relates to the lymphoid-specific enhancement described above.

Light Chain

Experiments have also identified sequences within the kappa-chain IVS that are necessary for efficient expression of kappa light chain. Queen & Baltimore (11) studied regulation of kappa expression in a transient expression assay. A

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vector was constructed with the SV40 origin (minus enhancer) to permit replication in COS cells, and the polyoma early region to permit replication in mouse cells. The kappa-chain gene was inserted so that a SV40 splice and poly A site were 3′ to the gene, so that truncated kappa transcripts could be correctly polyadenylated and processed. Two kappa genes were studied: an intact MOPC41 gene, and an altered MOPC41 gene terminating at the Hind III site within the major intervening sequence (see Figure 4). When these vectors were used to transfect COS cells, both vectors gave rise to the same transcripts in approximately equal quantities. The transcripts originated about 20 bp before the AUG codon used to initiate translation. When the mouse myeloma MPC-11 was used for transfection, the vector with the intact kappa-chain gene gave rise to transcripts originating 20–30 bp before the AUG. When the truncated kappa chain was used, no transcripts originating at the correct 5′ end were observed. Sequences within the kappa-chain gene 3′ of the Hind III site therefore appear necessary for its expression in lymphoid cells.

Picard & Schaffner (unpublished observations) searched for enhancing sequences within the kappa IVS by seeking to identify gene fragments that would enhance expression from a β-globin promoter in a mouse myeloma. When the Hind III-BamHI piece that includes the constant region from kappa chain was included in the vector, correctly initiated β-globin transcripts were seen. Recombinants with λ gene fragments yielded correct β-globin transcripts in neither a kappa nor a lambda myeloma cell line. Fine structure analysis indicated that the entire kappa-chain enhancing activity was contained on a 473 bp Alu I fragment which included a DNase-hypersensitive site (39). The kappa-chain enhancer appears lymphocyte specific because when it was included in the vector, correct β-globin transcripts were not observed in 3T6 cells. In the assay system used, the κ gene enhancer was only about 5% as active as the heavy-chain enhancer, and LPS treatment did not potentiate its activity.

The experiments of Sharon et al (unpublished) demonstrate that the light-chain enhancer can function to enhance transcription from the heavy-chain

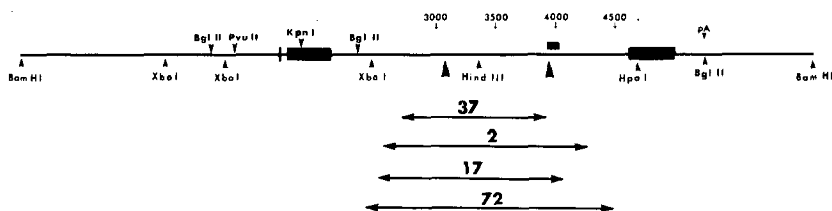

Figure 4 Partial restriction map of the rearranged kappa-chain gene from S107A. The extent of a series of Bal 31 deletions is indicated by solid lines under the gene. Hypersensitive sites in the IVS are indicated by arrowheads (39,40), while the highly conserved sequence is identified by a solid line above the gene (41).

Annual Reviews

promoter. In the construction lacking the Xba I fragment but containing the Hind-Bam piece from light chain, the V_H was effectively expressed. Adding the Xba I piece into the vector did not increase the level of transcription. Thus in this system the kappa-chain enhancer was fully active and its activity could not be further increased by the heavy-chain enhancer.

To investigate the extent of the regulatory sequences within the kappa IVS, we constructed a series of Bal31 deletions centering around the Hind III site (S. L. Morrison, V. T. Oi, unpublished). Attention was focused on identifying a region within the IVS necessary for kappa-chain synthesis. The Bal31 deletions were assayed both in a hamster lymphoma (GD36) and mouse myeloma (J558L). In both cell lines deletion 37 was found to be functional in directing kappa light chain synthesis while Δ2, Δ17, and Δ72 were decreased in the level of light-chain synthesis. The deletions were reassorted about the Hind III site and either the 5′ or 3′ side of the deletion assayed for its influence on L-chain expression. When assayed in GD36 the 3′ side of Δ2, Δ17, and Δ72 did not direct light-chain synthesis while the 3′ side of 37 and all 5′ deletions were effective in directing L-chain synthesis. A sequence lying 3′ of the terminus of Δ37 and 5′ of the terminus of Δ72 was thus implicated as being important for light-chain synthesis. The region implicated contains the DNase-hypersensitive site seen in the kappa chain of the lymphoma 7OZ (39), one of the DNase-hypersensitive sites seen in a mouse myeloma (40), and the region of sequence that is highly conserved between mouse, rabbit, and human (41). This region therefore appears to contain enhancer-like sequences necessary for expression in a lymphoma.

When the deletions were assayed in a mouse myeloma a different result was obtained. In the myeloma Δ2, Δ17, and Δ72 were deficient in their synthesis of Ig. However, when the 3′ and 5′ sides of the deletions were assayed, both were found to be active in directing light-chain synthesis. Therefore, in the myeloma cell lines two regions, one 5′ to the Hind III site, the second 3′ to the Hind III were shown to have enhancing activity. If both regions are deleted the kappa gene cannot be expressed following transfection. However, presence of either the 5′ or the 3′ sequence is adequate to permit kappa production. In fact, in contrast to studies of lymphoma, DNase sensitivity studies by Chung et al (40) have identified two DNase-sensitive regions in the IVS of kappa genes in myeloma cell lines, one 5′ of the Hind III site and the second 3′ of the Hind III site. It must now be shown that it is this second region that enhances the expression of kappa light chains in transfected J558 cells.

CONCLUSIONS AND PROSPECTS

Although gene transfection has only been used for a short time to study Ig synthesis, it has already proven itself a valuable tool.

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Transfection of Ig genes has been used to identify the first cellular enhancer elements and to localize them to the intronic sequences of Ig H- and L-chain genes. Furthermore, these enhancer-like sequences have been demonstrated to function only in B-cell lines; these results have led to the concept that cellular enhancer elements may provide the basis for tissue-specific or promoter-specific regulation of genes. A corollary to this is that genes coordinately expressed during differentiation may be controlled by recognition of shared sets of enhancer sequences. Experiments in the future must be designed to identify the molecular basis of enhancer function.

In addition to furthering our knowledge of Ig gene controlling elements, gene transfection also makes possible advances in our understanding of Ig structure and function. Transfected genes are expressed in sufficient quantity to permit the isolation of protein for detailed studies. Assembly between either a resident Ig chain and a transfected Ig chain or between two simultaneously transfected genes occurs, and the assembled molecules are inserted into membranes and/or secreted. Thus it is possible to generate cell lines making novel combinations of heavy and light chain. In addition, chimeric genes encoding molecules with reassorted variable and constant regions can be made. These Igs will provide valuable new reagents for immunochemical analyses. Using this methodology, we are on the threshold of a new understanding of antibody structure and function, and of the cell biology of Ig biosynthesis.

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Literature Cited

1. Wu, T. T., Kabat, E. A. 1970. An analysis of sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. *J. Exp. Med.* 132:211–50
2. Dreyer, W. J., Bennett, J. C. 1965. The molecular basis of antibody formation. A paradox. *Proc. Natl. Acad. Sci. USA* 54:865–69
3. Mather, E. L., Perry, R. P. 1981. Transcriptional regulation of immunoglobulin V genes. *Nucl. Acids Res.* 9:6855–67
4. Perry, R. P., Kelley, D. E. 1979. Immunoglobulin messenger RNAs in murine cell lines that have characteristics of immature B lymphocytes. *Cell* 18:1333–39
5. Paige, C. J., Kincade, P. W., Ralph, P. 1978. Murine B cell leukemia line with inducible surface immunoglobulin expression. *J. Immunol.* 12:641–47
6. Parslow, T. G., Granner, D. K. 1982. Chromatin changes accompany immunoglobulin κ gene activation: a potential control region within the gene. *Nature* 299:449–51
7. Alt, F. W., Rosenberg, N., Casanova, R. J., Thomas, E., Baltimore, D. 1982. Immunoglobulin heavy chain class

Annual Reviews

switching and inducible expression in an Abelsone murine leukaemia virus transformed cell line. *Nature* 296:325–31
8. Schibler, U., Marcu, K. B., Perry, R. P. 1978. The synthesis and processing of the messenger RNAs specifying heavy and light chain immunoglobulins in MPC-11 cells. *Cell* 15:1495–1509
9. Berk, A. J., Sharp, P. A. 1978. Spliced early mRNAs of simian virus 40. *Proc. Natl. Acad. Sci. USA* 75:1274–79
10. Gluzman, Y. 1981. SV40 transformed simian cells support the replication of early SV40 mutants. *Cell* 23:175–82
11. Queen, C., Baltimore, D. 1983. Immunoglobulin gene transcription is activated by downstream sequence elements. *Cell* 33:741–48
12. Stafford, J., Queen, C. 1983. Cell-type specific expression of a transfected immunoglobulin gene. *Nature* 306:77–79
13. Deleted in proof
14. Mulligan, R. C., Berg, P., 1980. Expression of a bacterial gene in mammalian cells. *Science* 209:1422–27
15. Mulligan, R. C., Berg, P. 1981. Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase. *Proc. Natl. Acad. Sci. USA* 78:2072–76
16. Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y. C., Axel, R. 1977. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. *Cell* 11:223–32
17. Pellicer, A., Wigler, M., Axel, R., Silverstein, S. 1978. The transfer of stable integration of the HSV thymidine kinase gene into mouse cells. *Cell* 14:133–41
18. Littlefield, J. W. 1964. Selection of hybrids from matings of fibroblasts *in vitro* and their presumed recombinants. *Science* 145:709–10
19. Szybalski, W., Szybalski, E. H., Ragni, G. 1962. Genetic studies with human cell lines. *Natl. Cancer Inst. Monogr. No.:* 7:75–88
19a. Franklin, T. J., Cook, J. M. 1969. The inhibition of nucleic acid synthesis by mycophenolic acid. *Biochem. J.* 113:515–24
20. Davies, J., Jiminez, A. 1980. A new selective agent for eukaryotic cloning vectors. *Am. J. Trop. Med. Hyg.* 29 (5):1089–92 (Suppl.)
21. Davies, J., Smith, D. I. 1978. Plasmid determined resistance to antimicrobial agents. *Ann. Rev. Microbiol.* 32:469–518
22. Southern, P. J., Berg, P. 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Molec. Appl. Genet.* 1:327–41
23. Ochi, A., Hawley, R. G., Hawley, T., Shulman, M. J., Traunecker, A., Kohler, G., Hozumi, N. 1983. Functional immunoglobulin M production after transfection of cloned immunoglobulin heavy and light chain genes in lymphoid cells. *Proc. Natl. Acad. Sci. USA* 80:6351–55
24. Rice, D., Baltimore, D. 1982. Regulated expression of an immunoglobulin κ gene introduced into a mouse lymphoid cell line. *Proc. Natl. Acad. Sci. USA* 79:7862–65
25. Oi, V. T., Morrison, S. L., Herzenberg, L. A., Berg, P. 1983. Immunoglobulin gene expression in transformed lymphoid cells. *Proc. Natl. Acad. Sci. USA* 80:825–29
26. Picard, D., Schaffner, W. 1983. Correct transcription of a cloned mouse immunoglobulin gene *in vivo*. *Proc. Natl. Acad. Sci. USA* 80:417–21
27. Deleted in proof
28. Mercola, M., Wang, X.-F., Olsen, J., Calame, K. 1983. Transcriptional enhancer elements in the mouse immunoglobulin heavy chain locus. *Science* 221:663–65
29. Graham, F. L., van der Eb, A. J. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456–67
30. Chu, G., Sharp, P. A. 1981. SV40 DNA transfection of cells in suspension: analysis of the efficiency of transcription and translation of T-antigen. *Gene* 13:197–202
31. Banerji, J., Olsen, L., Schaffner, W. 1983. A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* 33:729–40
32. Sandri-Goldin, R. M., Goldin, A. L., Levine, M., Glorioso, J. C. 1981. High-frequency transfer of cloned herpes simplex virus type 1 sequences to mammalian cells by protoplast fusion. *Molec. Cell. Biol.* 1:743–52
33. Ochi, A., Hawley, R. G., Shulman, M. J., Hozumi, N. 1983. Transfer of a cloned immunoglobulin light-chain gene to mutant hybridoma cells restores specific antibody production. *Nature* 302:340–42
34. Gillies, S. D., Morrison, S. L., Oi, V. T., Tonegawa, S. 1983. A tissue specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* 33:717–28

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Annual Reviews

35. Deleted in proof
36. Falkner, F. G., Zachau, H. G. 1982. Expression of mouse immunoglobulin genes in monkey cells. *Nature* 298:286–88
37. Brinster, R. L., Ritchie, K. A., Hammer, R. E., O'Brien, R. L., Arp, B., Storb, U. 1984. Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice. *Nature*. In press.
38. Germain, R. N., Norcross, M. A., Margulies, D. N. 1983. Functional expression of a transfected murine class II MHC gene. *Nature* 306:190–94
39. Parslow, T. G., Granner, D. K. 1983. Structure of
inside the imn
evidence for
Nucl. Acids R
40. Chung, S.-y.
1983. DNase
chromatin of i
genes. *Proc.*
80:2427–31
41. Emorine, L.,
P., Max, E.
sequence in
intron: possib
304:447–49

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:239-256. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359

ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395

IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429

COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461

C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS[1]

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INTRODUCTION

Leukocyte accumulation is vital for many immunologically mediated functions as well as for wound healing. Over a century ago it was recognized that phagocytes accumulated at sites of intrusion by foreign substances. These observations led Metchnikoff to propose that chemical signals emanating from foci of invading materials attracted leukocytes, which were important components of host defense against microbial diseases (1). Although the directed migration of leukocytes was appreciated in the 19th century, the quantification of leukocyte chemotaxis in vitro did not become possible until 1962. At this time, a method was developed for the analysis of leukocyte migration through microporous filters across which a chemical gradient could be established (2). In the initial studies using this technique it was shown that immune complexes,

[1]*Abbreviations*—ADP: adenosine-5[1]-diphosphate; AMP: adenosine-5[1]-monophosphate; ATP: adenosine-5[1]-triphosphate; cAMP: cyclic adenosine-5[1]-monophosphate; CCF: crystal induced chemotactic factor; cGMP: cyclic guanosine-5[1]-monophosphate; C5a: cleavage product of 5th component of complement; 5,12 diHETE: 5(5),12(R)-dihydroxy-6,8-*trans*-10,14-ciseicosatetraenoic acid; EHNA: erythro-9-[2-hydroxy-3-nonyl]adenine; fMet-Leu-Phe: formyl-methionyl-leucyl-phenylalanine; fNle-Leu-Phe-Nle-Tyr-Lys: formyl norleucyl-leucyl-phenylalanyl-norleucyl-tyrosinyl-lysine; GMP: guanosine-5[1]-monophosphate; gly-his-gly: glycyl-histidyl-glycine; GTP: guanosine-5[1]-triphosphate; GTPγS: guanosine-5[1]-0-3-thio-triphosphate; K_D: equilibrium dissociation constant; LTB_4: leukotriene B_4; LDCF: lymphocyte-derived chemotactic factor; PI: phosphatidylinositol; PMN: polymorphonuclear leukocyte; p[NH]ppA: adenylyl-imidodiphosphate; p[NH]ppG: guanylyl-imido-diphosphate; TPCK: tosylphenylalanylchloromethylketone.

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when added to fresh but not heat-inactivated serum, led to production of chemotactic activity. The ability to quantify leukocyte chemotactic responsiveness in vitro allowed the characterization of a number of substances, generated during immunological reactions, that lead to the accumulation of inflammatory cells.

The first chemoattractant to be identified and characterized was C5a, a product derived from the 5th component of complement (3–7). This chemotactic peptide also exhibits classical anaphylatoxic activity as well in that it contracts smooth muscle and degranulates mast cells (3, 8). C5a is the major source of chemotactic activity produced during the activation of complement in serum (9) or by the action of proteases on C5 (10-11). Direct cleavage of C5 by tissue hydrolases could provide an important source of chemoattractants at sites of nonspecific tissue damage or at areas of wound healing. C5a has been identified at sites of experimental inflammation in vivo (12), as well as in the synovial effusions of patients with inflammatory arthritis (13). Human C5a has been purified to homogeneity, and its primary amino acid sequence has been determined (14-16). Stimulation of lymphocytes by antigens or mitogens leads to the production of a chemoattractant termed lymphocyte-derived chemotactic factor (LDCF) (17). This protein is synthesized de novo by stimulated lymphocytes and has an apparent molecular weight of 12,500. LDCF has been isolated from sites of delayed inflammatory reactions in vivo (18). Other biologically relevant chemoattractants include a cell-derived chemotactic factor produced by polymorphonuclear leukocytes (PMNs) that have ingested crystalline material such as monosodium urate or calcium pyrophosphate dihydrate (19-20). Mast cells contain eosinophilotactic tetrapeptides that are released upon the exposure of these cells to specific antigen (21). Products of arachidonic acid lipoxygenation have also been shown to be chemoattractants (22-23). Leukotriene B_4 (5, 12-dihydroxyeicosatetraenoic acid) is the most potent chemoattractant of the arachidonic acid metabolites (24-25). Platelet factor 4 and platelet-derived growth factor have recently been reported to be chemotactic for leukocytes (26-27).

The interaction of chemoattractants with leukocytes initiates a series of coordinated biochemical and cellular events. These include alterations in ion fluxes (28-29) and transmembrane potential (30), change in cell shape from round to triangular (morphological polarization) (31-32), secretion of lysosomal enzymes (33), production of superoxide anions (34), and enhanced locomotion (35). Chemoattractants, therefore, are not only responsible for the accumulation of leukocytes at sites of inflammation but also can stimulate the cells to secrete lysosomal enzymes and to produce toxic oxygen radicals. These latter activities can result in the tissue destruction associated with inflammation. Interestingly, the biological responses of leukocytes to chemotactic factors can be divided into two groups—those related to cellular motility, and

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those related to the cytotoxic or microbicidal capability of leukocytes (i.e. superoxide anion production and lysosomal enzyme secretion). The two types of responses initiated by chemoattractants appear to be regulated differently and can be modified divergently by pharmacological agents (36-38). Low doses of chemoattractants simulate chemotaxis-related functions, whereas the cytotoxic properties of leukocytes are not stimulated unless at least ten-fold higher concentrations of chemoattractants are provided.

In this review we focus upon the characteristics and regulation of chemotactic-factor receptors on leukocytes. Leukocytes contain chemoattractant receptors on their plasma membranes. The affinity of the oligopeptide chemoattractant receptor is heterogeneous and dynamically regulated both by guanine nucleotides and prior agonist exposure (39-41). Transduction of chemoattractant-related signals requires transmethylation reactions mediated by S-adenosylmethionine and leads to the activation of phospholipases that liberate arachidonate from membrane phospholipids (42-45). Recent evidence suggests that protein kinase C is also involved in the transduction of some chemoattractant-induced signals (46).

LEUKOCYTE CHEMOATTRACTANT RECEPTORS

In the following discussion we highlight aspects of various types of chemoattractant receptors found on leukocytes. A summary of the characteristics of these receptors is shown in Table 1.

Table 1 Characteristics of chemoattractant receptors on leukocytes

Agonist	K_D (nM)	Receptors/Cell($\times 10^{-3}$)	Reference
N-formylated oligopeptides:			
fMet-Leu-Phe			
human PMNs	10.0–22.3	55	39, 51, 79
human monocytes	30.2	84	57
guinea-pig macrophages	11.0	10	55, 74, 104
fNle-Leu-Phe			
rabbit PMNs	1.5	100	52,63
fNle-Leu-Phe-Nle-Tyr-Lys			
human PMNs	1.0	120	53
human monocytes	1.7–2.7	10–18	56
C5a			
human PMNs	3-7	100-300	90, 91
Leukotriene B_4			
human PMNs	10.8-200	26-386	92, 93
Crystal-induced chemotactic factor			
human PMNs	446	600	19

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N-Formylated Oligopeptide Receptor

CHARACTERIZATION OF AGONIST BINDING The contention that leukocytes recognized chemoattractants via receptors was suggested by the strict structure-function relationships of synthetic N-formylated oligopeptides for initiating chemotactic and secretory activities by PMNs (47-48). These oligopeptides, which have been isolated from bacterial culture supernatants (49), and their recognition by leukocytes may provide a simple system for identifying microbial agents that initiate protein synthesis with N-formylmethionine (50). The availability of well-defined, potent chemoattractants spurred us to develop fMet-Leu-[^{3}H]Phe as a radioligand (51) to determine if leukocytes contained chemoattractant receptors. FMet-Leu-Phe has an ED_{50} of approximately 1 nM for inducing chemotaxis in human PMNs. Initial studies defining the presence of specific N-formylated peptide receptors was done by measuring the direct binding of the radiolabeled peptide to human PMNs (51). The fMet-Leu-[^{3}H]Phe used had biological activity identical to the unlabeled compound and a high specific activity (~ 46 Ci/mmol). The binding of fMet-Leu-[^{3}H]Phe to PMNs was saturable, and the number of binding sites per cell determined subsequently ranged from 40,000–60,000. The average equilibrium dissociation constant (K_D) for the interaction of the labeled peptide with human PMNs was 10–14 nM. The binding was rapid, and dissociation studies performed in the presence of excess unlabeled fMet-Leu-Phe indicated that a substantial portion of the peptide was bound to the exterior cell surface. The relative potencies of a series of N-formylated peptides for producing a chemotactic response were reflected in the specificity of their binding to the fMet-Leu-[^{3}H]Phe receptor. The order of potencies of the peptides for producing inhibition of fMet-leu-[^{3}H]Phe binding and for producing a chemotactic response were fMet-Leu-Phe > fMet-Met-Met > fMet-Phe > fMet-Leu >> fMet. There was excellent correlation ($r = 0.999$) between the doses of these compounds that produced half-maximal chemotactic responses and half-maximal inhibition of binding. N-formyl peptide receptors have been described on rabbit inflammatory peritoneal PMNs using a similar ligand, fNle-Leu-[^{3}H]Phe (52). In these cells that have already responded to chemoattractants in vivo, the affinity of the receptor is ten-fold higher than on human PMNs, and the protease inhibitor TPCK enhances binding. Another agonist used to identify N-formylated peptide receptors was described by Niedel et al (53). Using fNle-Leu-Phe-Nle-[^{125}I]-Tyr-Lys, Niedel et al estimated 120,000 receptors per human PMN with a K_D of 1 nM. Binding of this ligand was enhanced six-fold by divalent cations and had a sharp pH optimum at 6.75 with nonsaturating concentrations of the peptide. This ligand, unlike the other formylated peptides, is nondissociable after a 30-min incubation at 24°C. Interpretation of these data using methods that depend upon application of the mass action law

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is therefore not possible. Dissociability could be demonstrated as early as 2 min following incubation at 37°C, but endocytosis occurred following longer incubation times at this temperature. Further studies showed substantial receptor-mediated endocytosis of the ligand by the PMNs (54).

Specific receptors for N-formylated peptides have also been detected on the surface of mononuclear phagocytes. Our laboratory showed that guinea-pig peritoneal macrophages bind fMet-Leu-[^{3}H]Phe with a K_D of 11 nM and contain approximately 10,000 binding sites per cell (55). There was no quantitative difference in either the number or affinity of receptors on resident peritoneal macrophages as compared to those of macrophages elicited with an inflammatory stimulus. Weinberg et al (56) have demonstrated the presence of receptors for N-formylated peptides on adherent human peripheral blood monocytes using fNle-Leu-Phe-Nle-[^{125}I]-Tyr-Lys. The K_D for this interaction of peptide with receptors at 4°C was reported to be 1.7–2.7 nM, and the number of receptors per cell ranged from 10,000 to 18,000. The association of the hexapeptide to the cells at higher temperatures was not reversible. Direct visualization of rhodamine-labeled hexapeptide under video intensification showed rapid aggregation and internalization of the peptide at 37°C. Recent studies using fMet-Leu-Phe synthesized with ^{35}S (specific activity 498 Ci/mmol) have demonstrated the presence of N-formylated peptide receptors on nonadherent human monocytes (57). In experiments using this material, normal human monocytes were found to have 84,000 $\pm$ 11,200 receptors/cell with a K_D of 30.2 $\pm$ 5.6 nM.

Major advances have been made in the characterization of other cellular receptors, such as the adrenergic (58) and acetylcholine (59) receptors, primarily because of the availability of techniques to study these molecules in isolated membrane systems. Since accurate quantitative and qualitative measurement of receptor function in intact cells is precluded by ongoing cellular metabolic events, we developed methods to measure receptors in cellular membrane preparations. Precise quantification of binding parameters was effected by subjecting the experimental data to a computer modeling technique, based on the law of mass action, which provides analysis of the binding of multiple ligands to multiple classes of receptors. The computer program, termed SCTFIT, allows the determination of the affinity constants and concentration of each class of receptors (60-61). Computer analysis of binding of fMet-Leu-[^{3}H]Phe to viable cells showed a single class of receptor sites with no statistical improvement for a two-site fit (38). The average K_D and number of sites per PMN obtained from 25 experiments were 22.3$\pm$2.4 nM and 55,700$\pm$4,800, respectively (Figure 1A). Unlike those from intact cells, data derived from the binding of fMet-Leu-[^{3}H]Phe to PMN membranes fit significantly ($p < 0.001$) better to a model employing two classes of receptor sites (39) (Figure 1B). The K_Ds of these sites were 0.53 $\pm$ 0.01nM and 24.4 $\pm$ 1.2nM. The

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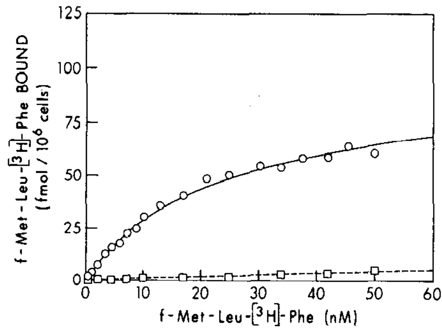
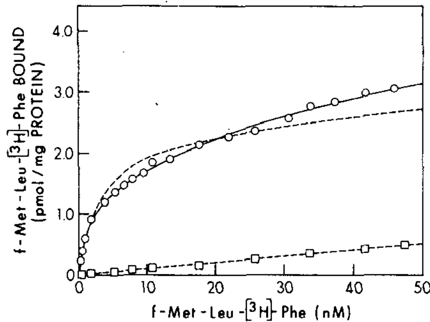


Figure 1 A: FML[^{3}H]P binding isotherms of intact PMNs. (○)—total ligand bound; (□)—nonspecific binding (defined as the amount of radioligand bound in the presence of 1000-fold excess of unlabeled ligand). The solid line represents the computer-fitted line for the total binding; the dotted line represents the nonspecific component. The K_D calculated from 25 experiments was 22.3 ± 2.4 nM. The number of sites per cell was calculated to be 55,760 ± 4,800 [from (39)].



B: FML[^{3}H]P binding isotherms of PMN membrane preparations. The solid line shows a two-site fit to the data while the upper dotted line shows a one-site fit to the same data. The two-site fit was the significantly better fit with $p < 0.001$. The lower dotted line represents nonspecific binding. The results for 20 such experiments yielded the following K_Ds: $K_H = 0.53 \pm 0.01$ nM; $K_L = 24.4 \pm 1.2$ nM. (○) total binding; (□) nonspecific binding [from (39)].

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concentration of high-affinity receptors in different membrane preparations varied 10–30%. Dissociation kinetics of fMet-Leu-[^{3}H]Phe in membrane preparations in the presence and absence of excess cold ligand were identical. These experiments suggested that the two classes of receptors with different affinities could be explained best by a model employing heterogeneity of binding sites rather than negative cooperativity involving a single class of receptors. In contrast to intact viable PMNs, formalin permeabilized whole PMNs also demonstrated heterogeneity of binding sites with K_Ds of $K_H = 0.55 \pm 0.3$nM and $K_L = 18.6 \pm 3.1$nM, results in good agreement with those found in isolated membranes (39). Based on these findings, it appears that an ongoing metabolic process(es) in viable PMNs allows receptor interconversion

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and thus permits detection of only a single average affinity state of the chemoattractant receptor.

Experiments by Seligmann et al (62) have also demonstrated nonlinear Scatchard analysis of fMet-Leu-[^{3}H]Phe binding to PMN membranes. Hill coefficients for these isotherms were 0.69 ± 0.07 for plasma membranes and 0.64 ± 0.06 for intact cells. Since dissociation kinetics were not performed, these authors felt that the data are compatible with either heterogeneity of binding sites or negative cooperativity. Further support of the complex nature of N-formylated peptide binding to leukocytes was also provided by Mackin et al (63), who found that fMet-Leu-[^{3}H]Phe bound to two populations of receptors on rabbit PMN membranes with K_Ds of 0.5 ± 0.25 nM and 48 ± 6.0 nM. The percentage of high-affinity receptors in these studies was only 1–5% of the total sites detected, however. Using another ligand, fNle-Leu-[^{3}H]Phe, these investigators reported the presence of two sites with different affinities. Again, no isotopic vs chemical dilutional dissociation studies were performed to distinguish between negative cooperativity and heterogeneity of binding sites.

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BIOCHEMICAL CHARACTERIZATION OF FORMYLATED PEPTIDE CHEMOATTRACTANT RECEPTORS ON LEUKOCYTES An attempt to characterize chemoattractant receptors biochemically was initially made by Niedel et al (64). These investigators used three different techniques to label the hexapeptide covalently and isolate the N-formylated peptide receptor. All three methods used to label the receptor covalently resulted in the identification of a polypeptide that migrated as a broad band on SDS polyacrylamide gel electrophoresis with an apparent molecular weight between 55,000 and 70,000. Dolmatch & Niedel (65) extended these findings using another radioiodinated photoaffinity label that has a higher labeling efficiency when complexed to the formylated peptide receptor on human neutrophils than did the other compounds. Other workers have succeeded in covalently photolabeling and further characterizing the N-formylated peptide receptor. Schmitt et al (66) synthesized a photoaffinity label and found that it identified a 50,000–60,000-dalton species on polyacrylamide gels run under denaturing conditions. Two major distinct entities of mol wt 50,000 with an isoelectric point of 6.0 and mol wt 60,000 with an isoelectric point of 6.5 were identified. Further studies with native N-formylated peptides in addition to those with covalent peptide labels showed that following binding, the plasma membrane receptors translocate to a Golgi rich fraction (69).

Goetzl et al (68) have approached the characterization of the N-formylated peptide receptor using affinity chromatography (70). Detergent-solubilized human neutrophil plasma membranes were applied to the fMet-Leu-Phe-Sepharose column, washed and eluted with fMet-Leu-Phe. Three predominant

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membrane proteins of approximate molecular weights of 94,000 (MP-1), 68,000 (MP-2), and 40,000 (MP-3) were eluted from the column. MP-2 and MP-3 but not MP-1 bound fMet-Leu-[^{3}H]Phe in equilibrium dialysis chambers. The binding data obtained with these proteins yielded curvilinear Scatchard plots that were interpreted to be representative of binding sites with two different affinities. Kay et al (69) characterized the oligopeptide receptor on the U937 monocyte-like cell line that had been induced to differentiate into macrophage-like cells by treatment with dibutyrl cAMP, dimethyl sulfoxide (DMSO), or lymphocyte-conditioned medium (69). The undifferentiated U937 cells contained no detectable binding of the covalent affinity label, fNle-Leu-Phe-Nle-[^{125}I]-Tyr-Lys. When cells were induced to differentiate, the affinity label was coupled to proteins with molecular weights ranging from 54,000 to 74,000 for dibutyrl cAMP treated cells, 63,000 to 79,000 for conditioned medium-grown cells, and 76,000 to 91,000 for DMSO treated cells (69).

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REGULATION OF BINDING AFFINITY OF THE N-FORMYLATED PEPTIDE RECEPTOR BY GUANINE NUCLEOTIDES Guanine nucleotides play an important role in the regulation of hormone-receptor interactions in certain receptor systems, including the α and β adrenergic (72), glucagon (71), muscarinic-cholinergic (59), and dopaminergic (72) receptors. In some instances, such as the β-adrenergic and prostaglandin receptors, transduction of the hormonal signal that leads to the activation of adenylate cyclase is dependent upon the presence of the guanine nucleotides (70, 73). In adrenergic receptors, guanine nucleotides regulate interconversion between high- and low-affinity receptor states (70). In light of these findings, experiments were performed to determine the effects of guanine nucleotides on the N-formylated peptide chemoattractant receptor. The nonhydrolysable analog of GTP, p[NH]ppG, was incubated with PMN membranes and the binding of fMet-Leu-[^{3}H]Phe was determined. The binding of the ligand was reduced by p[NH]ppG when compared to membranes incubated with buffer alone. Computer-assisted analysis of the data indicated that the decreased binding could be explained by a reduction in the percentage of high-affinity receptors (40). In the presence of buffer alone, membranes expressed an average of 21.3 ± 0.13% high-affinity receptors, but the inclusion of 10^{-4}M p[NH]ppG reduced this to 11.8 ± 0.05% without altering total receptor number. In the presence of p[NH]ppG, the binding data again modeled significantly better to a two-site fit, and K_H and K_L were not different from those of untreated membranes. Therefore, the effect of the guanine nucleotide is to convert receptors originally present in the high-affinity state to a low-affinity state. The guanine nucleotide effects were dose-dependent, in that a half-maximal effect of p[NH]ppG was observed at a concentration of 10^{-6}M, while that of GTP was 5×10^{-6}M and that of GDP was 5×10^{-5}M. Of the nucleotides tested, only GDP, GTP, and the nonhydro-

Annual Reviews

lysable analogs of GTP such as p[NH]ppG and GTPγS decreased the percentage of high-affinity receptors. ATP, ADP, AMP, cAMP, p[NH]ppA, GMP, and cGMP produced no effect on fMet-Leu-[^{3}H]Phe binding (Table 2) (40). Guanine nucleotides produced similar results on the fMet-Leu-[^{3}H]Phe receptor in guinea-pig macrophage membranes (74). Guinea-pig macrophage membrane preparations contain two classes of fMet-Leu-[^{3}H]Phe binding sites with K_Ds of 1.5 ± 0.4nM and 25.5 ± 11.0nM. In the presence of p[NH]ppG, the number of high-affinity receptors was reduced from 29.4% to 8.7% with a concomittant increase in low-affinity receptor sites and no significant change in the total number of binding sites.

Biochemical transduction of the β-adrenergic receptor signal has been shown to be mediated by a nucleotide regulatory protein (N protein) that activates adenylate cyclase (70). A ternary-complex model involving a guanine nucleotide regulatory unit can be applied to the fMet-Leu-[^{3}H]Phe binding data presented herein (Figure 2). We hypothesize that receptor occupancy by fMet-Leu-[^{3}H]Phe facilitates the substitution of GDP by GTP on a nucleotide regulatory subunit (N-unit), thereby producing a N-GTP that subsequently activates an effector molecule(s) or reaction(s). The receptor expresses a low affinity state when coupled to the N-unit carrying GDP or GTP. The high-affinity state of the receptor (R^1) is manifest when the receptor is either free of N or bound to N in the absence of any guanine nucleotide. The effector(s) for the chemoattractant receptor activated by the N-GTP complex is as yet unknown, but candidates would include a phospholipase, protein kinase, methyltransferase or, less likely, adenylate cyclase. Adenylate cyclase acti-

Table 2 Effects of nucleotides on high-affinity FML[^{3}H]P binding to human PMN membranes

Nucleotide[a]	% Inhibition[a]
GTP	68
P[NH]ppG	81
GTPγS	71
GDP	69
GMP	8
cGMP	0
ATP	0
p[NH]ppA	0
ADP	0
AMP	0
cAMP	0

[a]PMN membranes were incubated with 10^{-4} M nucleotide at 25°C for 15 min and then assayed for FML[^{3}H]P binding in the presence of the nucleotides. Results are expressed as % inhibition of the expression of high-affinity FML[^{3}H]P binding sites on membranes preincubated with buffer alone [from (40)].

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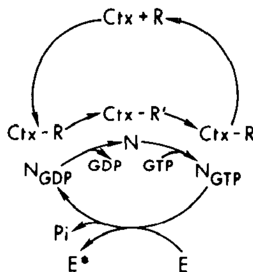

Figure 2 Postulated model for the interaction of guanine nucleotides with a nucleotide regulatory protein and the chemoattractant (CTX)-receptor complex. It is proposed that the affinity of the receptor is affected by its coupling to a regulatory unit (N) that binds guanine nucleotides. The transient high-affinity state (R^1) may occur when the receptor is bound to free N. (E) and (E*) represent the unmodified and modified forms of the putative effector molecule [from (74)].

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vation or inhibition by certain hormones in guinea-pig macrophage membranes does require the presence of guanine nucleotides (73). The chemoattractants fMet-Leu-Phe and C5a, however, neither activated not inhibited the stimulation of adenylate cyclase in these membranes (73) nor in PMN membranes (M. Verghese, R. Snyderman unpublished observations). These findings indicate that although guanine nucleotide regulation of the N-protein is involved in activation of adenylate cyclase in many receptor systems, the chemoattractant receptor-guanine nucleotide-N-unit model may be independent of direct adenylate cyclase regulation. There is precedence for this concept in that it has been shown that light-activation of a photoreceptor 3',5'-cyclic GMP phosphodiesterase in retinal rods requires the binding of GTP to a specific protein (75). Indeed, we now have evidence that fMet-Leu-Phe increases cAMP levels in human PMNs via transient inhibition of phosphodiesterase rather than by activating adenylate cyclase (76). GTP-activated protein-receptor complexes may therefore be generally important for biochemical activation of several transduction systems independent of adenylate cyclase.

REGULATION OF CHEMOATTRACTANT RECEPTOR AFFINITY BY AGONISTS Studies performed in our laboratory and others have indicated that chemoattractants themselves regulate the affinity of the receptors to which they bind. We have recently shown that treatment of human PMN membranes with fMet-Leu-Phe results in an increase in the percentage of high-affinity receptors expressed (41). Preincubation of membranes with 10^{-7}M fMet-Leu-Phe increased the percentage of high-affinity sites from 15% to 48%. A further observation made in these studies was that the new population of high-affinity receptors was not subject to modulation by guanine nucleotides as were the high-affinity N-formylated peptide receptors on untreated membranes. Incubation of intact cells with amounts of fMet-Leu-Phe sufficient to cause desen-

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sitization of chemotaxis also led to the appearance of a new class of high-affinity binding sites. The agonist-induced high-affinity site may thus be an intermediate form of the receptor that triggers internalization or perhaps transduction of a signal for secretion. Support for this concept was reported by Jesaitis et al (77), who have shown that within seconds of ligand binding, the N-formylated peptide receptor exhibits an apparent increase in affinity as judged by a greater than 25–50-fold decrease in the rate of ligand dissociation from the isolated membranes. These slowly dissociating higher-affinity ligand-receptor complexes co-isolated with the detergent-insoluble cellular residues (presumably cytoskeleton) which did not contain cytosolic or Golgi markers. The complexes were in transient association with the "cytoskeleton" with a half-life ranging from 30 sec to 4 min. These authors have speculated that association of ligand-receptor complexes with the cytoskeleton converts the receptor to the high-affinity state and occurs antecedent to endocytosis of the ligand. This association thus may provide the specific mechanochemical force necessary to activate intracellular receptor translocation.

Other studies have also shown that chemoattractants regulate receptor expression on the cell surface. Gallin et al found that treatment of human neutrophils with concentrations of fMet-Leu-Phe that produce submaximal secretion of lysosomal enzymes leads to an increase in the binding of fMet-Leu-[^{3}H]Phe to intact PMNs (78-79). The increased binding was a result of increased numbers of surface binding sites as well as an increase in a nondisplacable pool of specifically bound fMet-Leu-[^{3}H]Phe (80). The enhanced binding correlated with the degree of specific (secondary) granule release. More recent experiments showed that specific granules contain the highest specific fMet-Leu[^{3}H]Phe binding activity (81). These investigators have hypothesized that secretagogs may activate PMNs to enhance their subsequent responses in fMet-Leu-Phe-mediated processes. Specific granules may provide a source of preformed membrane and receptor material which is translocated to the cell surface.

REGULATION OF CHEMOATTRACTANT RECEPTOR AFFINITY AND FUNCTIONAL ACTIVITY BY PHARMACOLOGIC AGENTS: EVIDENCE THAT MOTILITY AND SECRETORY FUNCTIONS ARE REGULATED INDEPENDENTLY The motility and secretory functions of the chemoattractant receptor are stimulated by widely divergent doses of chemotactic factors. For example, with fMet-Leu-Phe, the EC_{50} for chemotaxis is ~ 1 nM while the EC_{50} for secretion of lysozyme is ~ 20 nM. Using pharmacological means, we have presented evidence that these functions of the receptor are regulated differently and that the biological activity of the oligopeptide receptor is affected by its affinity. In several cellular systems, changes in membrane fluidity have been shown to alter receptor-hormone interactions and resultant metabolic processes (82). Membrane

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microviscosity and/or lipid composition in leukocyte membranes can be altered with aliphatic alcohols (38) and polyene antibiotics such as amphotericin B or nystatin (83-84). N-formylated chemoattractant receptor function was therefore evaluated in the presence of these agents. Low doses of aliphatic alcohols, such as n-pentanol and n-butanol increase membrane fluidity in intact human PMNs at concentrations that maintain cellular viability. N-pentanol (0.1%) and n-butanol (0.25%) produced equivalent decreases in the membrane microviscosity parameter in intact cells as measured by diphenylhexatriene fluorescence depolarization (38). The same concentrations of these alcohols produced a shift in the fMet-Leu[^{3}H]Phe receptor average affinity (K_D ± SEM) from 25.7 ± 7.6 nM for the untreated PMNs to 5.2 ± 0.9 and 6.0 ± 0.9 nM for the n-butanol and n-pentanol-treated cells, respectively. No significant changes in the total number of receptors per cell was produced by incubation with alcohols. Thus, under conditions where membrane fluidity was increased, the average affinity of the N-formylated chemoattractant receptor was augmented. The effects of n-butanol and n-pentanol on PMN chemotaxis were studied to determine whether the change in affinity of the receptor was reflected by an alteration in biological activity. In PMNs treated with n-butanol and n-pentanol, migration of the cells to submaximal stimulating doses of fMet-Leu-[^{3}]Phe increased (38). The effects of the alcohols on chemoattractant-stimulated superoxide anion production and lysosomal enzyme secretion were also determined (38). In contrast to the effects on migration, these two functions mediated by the chemoattractant receptor were markedly depressed in the presence of the alcohols. PMA-stimulated O_2^- production was, however, not altered by n-butanol, indicating that the enzymes necessary for the respiratory burst were not affected; rather, the alcohols changed the chemoattractant receptor's transduction mechanism for O_2^- production. Lysosomal enzyme secretion was also greatly decreased by treatment of the cells with n-butanol. Thus, decreased PMN membrane microviscosity produced by aliphatic alcohols is accompanied by altered biological responses of the cells to the chemoattractants, including increased sensitivity of the chemotactic response to lower concentrations of fMet-Leu-Phe but depressed superoxide generation and lysosomal enzyme secretion. Thus, the transductional mechanisms of the chemoattractant receptor can be divided into at least two categories: (*a*) motility-related functions that require low doses of chemoattractants and are augmented by conditions that increase the affinity of the receptor, and (*b*) secretory functions that are decreased when the higher affinity of the receptor predominates.

Further support of the contention that various chemoattractant-mediated functions are regulated differently and that transduction mechanisms are affected by the affinity state of the receptor is provided by studies of the effects of polyene antibiotics on the N-formylated peptide receptor (36). Amphotericin B inhibits chemotaxis (85), presumably by its ability to bind sterols in mem-

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branes and disrupt normal phospholipid:cholesterol interactions (83-84). Since cholesterol synthesis is required for chemotaxis (86) and chemoattractants alter phospholipid metabolism in leukocytes (42, 88), it was hypothesized that amphotericin B produces its effects in leukocytes by altering the lipid environment surrounding the chemotactic factor receptor (36). Both amphotericin B (2–4 μM) and nystatin (10–40 μM) inhibited chemotaxis in a dose-dependent fashion with no loss in cellular viability. The nonpolyene, antifungal agent griseofulvin had no effect on chemotaxis, even at concentrations 25-fold higher than chemotaxis-inhibiting concentrations of amphotericin B. The decreases in the chemotactic response in the presence of amphotericin B may be explained by altered receptor function, in that amphotericin B (1–10 μM) caused a dose-dependent decrease in fMet-Leu[^{3}H]Phe binding in both intact PMN and membrane preparations. Nystatin (5 and 10 μM) also inhibited fMet-Leu[^{3}H]Phe binding to intact cells, but griseofulvin had no effect. No change in the specificity of the N-formylated peptide receptor was observed in the presence of amphotericin B. The abnormal binding of fMet-Leu-[^{3}H]Phe produced by amphotericin B was associated with a decrease in the affinity of the fMet-Leu[^{3}H]Phe receptor. Incubation with 2 μM amphotericin B changed the K_D for the interaction of fMet-Leu[^{3}H]Phe with the receptor from 13 nM to 44 nM, as determined by computer modeling techniques. A slight increase in receptor number per cell was produced by the drug (36). In contrast to the effects on chemotaxis, amphotericin B increased specific granule release as measured by lysozyme secretion initiated by the oligopeptide chemoattractant. There was no significant effect on the release of β-glucuronidase, an azurophilic granule enzyme. The cytoplasmic enzyme marker, lactic dehydrogenase, was not released by amphotericin B treatment. Amphotericin B produced no effect on fMet-Leu-Phe-induced superoxide radical formation. These data show that chemotaxis is decreased, specific granule secretion is increased, and superoxide anion generation is unchanged under conditions where the affinity of the chemoattractant receptor is decreased. Taken together with the effects of the alcohols on chemoattractant oligopeptide receptor affinity and function, the data obtained with amphotericin B and nystatin provide further support for the contention that the affinity state of the receptor reflects its ability to mediate the transduction of different chemoattractant-mediated signals.

The oligopeptide chemotactic factor receptor in human PMN and guinea-pig macrophage membranes thus exists in two affinity states that are in part interconvertible and regulated by guanine nucleotides and by agonist exposure. The high-affinity state of the receptor is not detected in untreated intact cells, presumably because of the high intracellular concentration of guanine nucleotides (74) as well as rapid internalization of the agonist-induced high-affinity state. The average affinity of the receptor in intact cells can, however, be modified by agents that alter the physical state of the PMN membrane. The

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Table 3 Characteristics of the chemotactic and secretory functions of the human PMN oligopeptide receptor

	Chemotactic functions	Secretory functions
EC50 for fMet-Leu-Phe	~ 1 nM	~ 10 nM
Effect of aliphatic alcohols	enhances	inhibits
Effect of polyene antibiotics	inhibits	enhances

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average affinity of the receptor is increased by conditions that decrease membrane microviscosity, and this favors transduction of the chemotactic signal but suppresses lysosomal enzyme release and superoxide production (Table 3). The lower average affinity of the receptor produced by polyene antibiotics favors transduction of signals resulting in secretion and superoxide production. From these studies it is clear that one can differentially modify the biological functions of human PMNs by exposing them to agents that affect physical parameters of membranes and thus receptor function. Differential pharmacologic manipulation of the various functional responses of leukocytes will have important therapeutic implications for inflammatory, neoplastic, and immunodeficiency diseases.

C5a Chemoattractant Receptors

Treatment of serum with the C activator, endotoxin, was shown to produce a 15,000-dalton substance with chemotactic activity (4) that was soon identified as the cleavage product of the fifth component of C, C5a (3, 6, 9). Unquestionable evidence now exists that C5a accounts for most of the chemotactic activity produced by C activation. Human C5a is a glycoprotein, that contains a polypeptide portion of 74 amino acids accounting for a mol wt of 8200 and a carbohydrate portion that accounts for 3000 daltons (14). C5a is chemotactically active in doses as low as 1.0 nM. The most prevalent form of human C5a found in vivo is $C5a_{desarg}$, a molecule from which the COOH-terminal arginine was removed by endogenous carboxypeptidase enzymes (15). $C5a_{desarg}$ is approximately 10-fold less potent than the native C5a molecule. Highly purified, ^{125}I-labeled C5a has been shown to bind to specific receptors on the surface of human neutrophils (89). The binding of [^{125}I]C5a to intact PMNs was saturable but not reversible at 24°C. Half-maximal saturation of C5a binding occurred at 3–7 nM, and the number of sites per cell was estimated to be 100,000–300,000. Structural specificity for the interaction of C5a with its receptor was demonstrated by experiments showing that analogs of this compound, including $C5a_{desarg}$ and C5a(1–69), the latter a carboxypeptidase derivative lacking five residues of the C-terminal portion of the parent molecule, competed for [^{125}I]C5a binding. Native C5a was the most potent inhibitor of binding, followed by $C5a_{desarg}$, then C5a(1–69). C5a(1–69) lacks

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chemotactic activity (90). The synthetic pentapeptide L-methionyl-L-glutaminyl-L-leucyl-glycyl-L-arginine, which mimics the C-terminal linear sequence of C5a, is devoid of both biological activity and ability to interact with the C5a receptor. Based upon these data, Chenoweth & Hugli (91) have proposed that the human C5a polypeptide contains both a receptor "recognition" site in the internal portion of the molecule and an "initiation" or "activation" binding site represented by the C-terminal region. The C5a receptor is distinct from the N-formylated peptide receptor, since these latter compounds do not compete for binding with C5a to the neutrophil membrane (51,91).

Lipid Chemotactic Factors and LTB_4 Leukocyte Receptors

Turner et al (23) first observed that oxidized components of polyenoic fatty acids are chemotactic for neutrophils. Subsequent studies by Goetzl & Pickett (22) further defined the biochemistry and activity of the products resulting from lipoxygenation of arachadonic acid. The leukotriene having the most chemotactic activity for human neutrophils is 5(S),12(R)-dihydroxy-6,8-*trans*-10,14-cis-eicosatetraenoic acid (5,12-diHETE), now known as leukotriene B_4 (24). This compound is chemotactic for human neutrophils at concentrations as low as 1 nM and evokes a maximal response at 20–100 nM. Other lipoxygenation products including 5-HETE, 11-HETE, and 12-HETE were found to be much less potent for producing chemotactic activity. Slow-reacting substance of anaphylaxis (leukotriene C) and several platelet-derived trihydroxytetraenoic acids produced only marginal chemotactic activity (25). Two laboratories have independently described a neutrophil membrane binding site for LTB_4, the lipoxyogenase pathway product of arachidonic acid. Kreisle & Parker (92) isolated endogenously labeled $[^3H]LTB_4$ from human neutrophils and found that binding at 4°C was nonreversible while binding at 37°C resulted in metabolism of the ligand. The specific binding of $[^3H]LTB_4$ ranged from 65 to 80% at 4°C. Since binding of the ligand is not reversible, an apparent dissociation constant (K_D) was calculated based on the 50% inhibitory concentration of $[^{14}C]LTB_4$ and was found to be 200 nM. The number of specific binding sites per cell was estimated at 390,000 (92). Goldman & Goetzl studied LTB_4 binding using similar methodology (93). $[^3H]LTB_4$ (specific activity = 39 ± 19 Ci/mmol) was isolated from neutrophils that had been incubated with $[^3H]$arachidonate and the calcium ionophore A23187. The nonspecific binding of $[^3H]LTB_4$ at a concentration of 0.9 nM measured after 20 min of binding at 0°C was 24%; however, this percentage increased substantially at higher concentrations of $[^3H]LTB_4$. The K_D for the interaction of $[^3H]LTB_4$ with the receptors was calculated by Scatchard analysis and was found to range between 11 nM and 14 nM, depending upon the techniques used to separate bound from free ligand. The number of sites per cell ranged between 26,000 and 40,000. The structural analogs of LTB_4, 5-HETE, and

Annual Reviews

5(5), 12(5)dihydroxyeicosa-6,8,10,trans-14-cis-tetraeonic acid, competitively inhibited the binding of [^{3}H]LTB_4 at doses that evoked a chemotactic response. LTC_4, which is chemotactically inactive, did not inhibit the binding of [^{3}H]LTB_4 at concentrations up to 5×10^{-7}M nor did maximally chemotactic concentrations of fMLP and C5a, indicating that this compound induces chemotaxis via a distinct receptor on leukocytes. Based upon the aforementioned studies, it appears that LTB_4 does interact with binding sites on the surface of leukocytes. However, since the nonspecific binding of this interaction is so high, and binding is largely nonreversible, caution must be exercised in analyzing quantitative binding parameters in this system.

Other Cellular-Derived Chemotactic Factors and Their Cell-Surface Receptors

Human lymphocytes, when incubated with specific antigens, mitogens, or HLA nonidentical lymphocytes produce a 12,500-dalton substance that is chemotactic for monocytes (17, 94). This material has been termed lymphocyte-derived chemotactic factor (LDCF). Production of this factor, which has been shown to be isoelectrically and antigenically distinct from C5a (95), does not require cell division but does depend upon new protein synthesis (94). That this substance is of importance for cell-mediated immune reactions is suggested by in vivo studies in guinea pigs, which have demonstrated that LDCF appears at sites of delayed hypersensitivity reactions just prior to the local influx of macrophages (18). LDCF thus appears to be produced at sites of delayed hypersensitivity in vivo. The difficulty in purifying large quantities of this material from lymphocyte culture supernatants has unfortunately prevented detailed studies of its interaction with a putative cell-surface receptor.

Another cell-derived chemotactic factor termed crystal-induced chemotactic factor (CCF) is produced during phagocytosis of sodium urate or calcium pyrophosphate crystals by human PMNs (20). Since it has been known for some time that intraarticular injection of monosodium urate or calcium pyrophosphate dihydrate crystals precipitates acute attacks of gout and pseudogout, respectively, Spilberg et al hypothesized that the CCF is a primary mediator of local inflammation in these diseases (96). Using [^{125}I]CCF, Spilberg & Mehta (19) demonstrated a specific binding site for this compound on the surface of human PMNs. The binding was rapid and dissociable at 37°C by the addition of excess unlabeled CCF. The K_D for the interaction of [^{125}I] CCF with its binding site was 450 nM and there were 600,000 sites per cell. Synthetic chemoattractants such as fMet-Leu-Phe and Gly-His-Gly as well as activated human plasma containing C5a did not inhibit [^{125}I]CCF binding, suggesting the specificity of the binding site for CCF.

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BIOCHEMICAL TRANSDUCTION OF THE CHEMOATTRACTANT-RECEPTOR SIGNAL

Role of Lipid Metabolism and Methylation Reactions in Chemoattractant-Receptor Signal Transduction

Several chemical pathways have been implicated as being important for transduction of chemotactic, oxidative, and secretory responses induced by chemoattractant receptor occupancy (35). Chemotactic factors produce rapid changes in transmembrane potential (30), ion fluxes (28-29), and cAMP levels (70, 97-98). These alterations have been studied using real-time kinetic analysis, which has determined that cyclic AMP elevation and Ca^{2+} influx occurred in < 5 sec following exposure to chemoattractants, while membrane depolarization and elastase release occurred in 5 sec. Pseudopod extension, superoxide generation, and elastase release occur at 5–10 sec after chemoattractants are introduced (99–100). Alterations in perpendicular light scattering begin immediately and peak within 10 ± 1 sec after introduction of chemoattractants to human PMNs. The light-scattering changes appear to represent PMN membrane responses to chemotactic factors (38). Changes in membrane lipids also appear to be required for chemoattractant-mediated functions. Rapid release of arachidonic acid from membrane phospholipids in leukocytes occurs following chemoattractant receptor occupancy (42, 44). This phenomenon is also observed in other systems such as the thrombin receptor on platelets (101) and the IgE receptor on mast cells (87). The free arachidonic acid is then available for synthesis of prostaglandins, thromboxanes, and leukotrienes, all of which are potent mediators of inflammatory and vasomotor responses. Free arachidonate itself may be an important second messenger of chemoattractant-mediated responses (46 and see below). In human monocytes and PMNs, our laboratory showed that treatment of cells with chemotactic factors produced a preferential loss of phosphatidyl inositol (PI) into which [^{3}H]arachidonic acid had been incorporated (44). No significant change in [^{3}H]arachidonic acid–labeled phosphatidylcholine and phosphotidylethanolamine was noted. These findings suggest that chemoattractants mobilize arachidonate via a phospholipase C activation.

Over the past several years, studies have shown that transmethylation reactions mediated by S-adenosyl-methionine play an important role in the chemotactic response of leukocytes (43, 102). In order to demonstrate this, we performed studies in which methylation reactions were inhibited in cells by incubation with the competitve antagonist of adenosine deaminase, erythro-9-[2-hydroxy-3-nonyl]adenine (EHNA) plus exogenous adenosine and L-homocysteine thiolactone (42). The combination of these agents produced a large increase in S-adenosylhomocysteine, a potent competitive inhibitor of all

Annual Reviews

transmethylation reactions mediated by S-adenosylmethionine (103). Conditions that inhibited 40% or greater of carboxy-O-methylation depressed human monocyte chemotaxis. Similar findings have been observed in human PMNs (45) and guinea-pig macrophages (104). Other compounds such as 3-deazaadenosine also produced increased S-adenosylhomocysteine levels and inhibited chemotaxis. Methylation reactions are also required for other chemoattractant-mediated functions in mononuclear cells, such as superoxide production and arachidonic acid release from phospholipids (44, 104). A possible explanation for the methylation requirement of monocyte chemotaxis is that a reaction of this type is required for the fMet-Leu-Phe-induced activation of the phospholipase that metabolizes phosphatidylinositol (44). Interestingly, the unstimulated, basal activity of this phospholipase may also depend upon a methylation reaction. Other workers have confirmed the methylation requirement of chemoattractant-mediated arachidonic acid release in rabbit PMNs (105).

While attempting to define the precise methylation reaction required for chemotaxis, our laboratory has shown that chemoattractants inhibit the synthesis of methylated phospholipids in guinea-pig macrophages (88). This inhibition results in an increase in the ratio of PE:PC in isolated plasma membranes from chemoattractant-treated guinea-pig macrophages; moreover, this ratio has been found to be increased in lamellapodia of cells responding to a chemotactic gradient (106). Since alterations in phospholipid ratios could affect the activity of chemoattractant receptors, we studied the effects of methylation inhibition on the binding of fMet-Leu-[^{3}H]Phe to intact guinea-pig macrophages (104). The cells were incubated with EHNA, adenosine, and L-homocysteine, and chemoattractant binding data were subjected to computer analysis. Conditions that inhibited methylation reactions by 90% produced a 2.6–4.0-fold reduction in the affinity of the chemoattractant receptor. The K_D for the interaction of fMet-Let-[^{3}H]Phe with the receptor on intact cells was 2.9 $\pm$ 0.2 nM, and the number of receptors per cell was 11,000 $\pm$ 530 in the absence of methylation inhibitors. In the presence of EHNA, adenosine, and L-homocysteine, the K_D was increased to 7.5 $\pm$ 0.6 nM and there was a slight increase in receptor number (12,709 $\pm$ 431). Similar results were noted when 3^{1}deazaadenosine plus homocysteine was used to inhibit methylation. An ongoing methylation reaction thus appears to be required to maintain the oligopeptide chemoattractant receptor in a funtionally active state. Inhibition of methylation lowers the receptor's affinity, and the best explanation for this effect is that inhibition of methylation produces an allosteric alteration in the receptor. The consequence of this alteration is an uncoupling of the receptor from its transductional unit. The nature of this unit is unknown at this time but may be a nucleotide regulatory unit or a phospholipase.

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Potential Role for Protein Kinase C in the Transduction of Chemoattractant Signals

A phospholipid- and calcium-dependent protein kinase (protein kinase C) has recently been identified (107) and found to be widely distributed in tissues. The activity of this enzyme is enhanced by diolein, a substance formed through the action of phospholipase C on membrane phospholipids. We have shown that chemoattractants do not activate adenylate cyclase in leukocyte membranes (73) and have reasoned that protein kinase C may mediate certain chemoattractant-receptor functions. We have found that human PMNs contain substantial protein kinase C activity, which is present in cytosolic fractions of unstimulated cells that have been disrupted by sonication or nitrogen cavitation. Treatment of the cells with doses of PMA sufficient to activate the respiratory burst leads to a translocation of the protein kinase C from the cytosol to a particulate membrane fraction not extractable by 0.1% Triton X 100 (108). The chemoattractant fMet-Leu-Phe, under conditions that initiate secretion and the respiratory burst, also produces translocation of the enzyme to the particulate fraction. In contrast to PMA-induced changes, however, the activity is extractable by 0.1% Triton X 100. Our data are compatible with the notion that following treatment of cells with chemoattractants, protein kinase C becomes loosely embedded in the plasma membrane. PMA treatment on the other hand causes the enzyme to be far more tightly associated with the membrane or with cytoskeletal components associated with the membrane (108). The reason for the difference in extractability of the protein kinase C following treatment of leukocytes with PMA or chemoattractants is not yet known. These findings raise, however, the interesting possibility that the two means of cellular activation, PMA vs chemoattractants, lead to translocation of the enzyme to different sites within the cell. Since PMA-induced activation of the respiratory burst continues indefinitely, whereas chemoattractant-induced activation is quickly desensitized, these studies suggest that the location of activated protein kinase C may affect its regulation and the subsequent biological activities it stimulates. An unanticipated and perhaps widely significant finding is our recent observation that arachidonate itself, as well as other unsaturated long-chain fatty acids such as linoleic acid, are capable of activating leukocyte protein kinase C directly in leukocyte cytosolic fractions in the absence of any other added phospholipids (46, 109). These findings imply that the activation of phospholipases by chemoattractants releases unsaturated fatty acids such as arachidonate, which can serve as second messengers to stimulate protein kinase C, which transduces certain chemotactic signals (Figure 3). Since a number of other receptors such as the thrombin receptor, insulin receptor, and muscarinic-cholinergic receptors also liberate arachidonate from membrane

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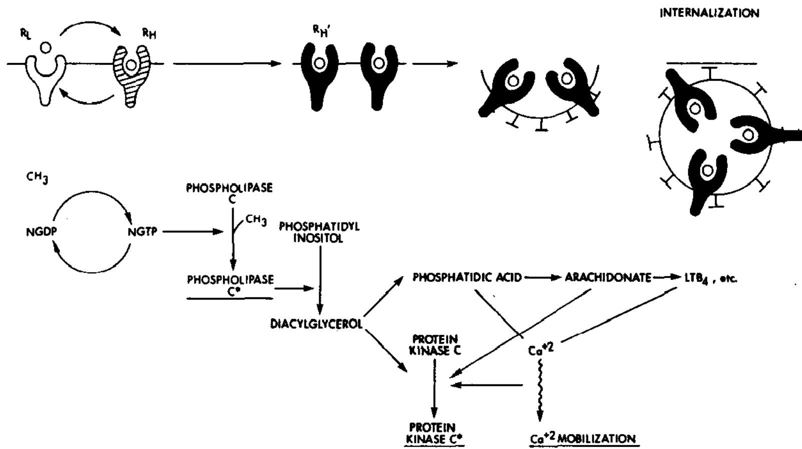

Figure 3 Model of chemoattractantt receptor transduction. It is hypothesized that binding of the chemoattractant to its receptor leads to the formation of a reversible high-affinity state (hatched line receptor) regulated by guanine nucleotides. At high concentrations of the chemoattractant, a nonnucleotide-sensitive high-affinity receptor is formed (blackened receptor). This receptor may be rapidly internalized, perhaps via coated pits, which are present on PMNs(111). Associated with receptor occupancy, phospholipases are activated and intracellular cAMP increases (not shown). Portrayed here is one of the pathways that appears to be activated by chemoattractants in leukocytes. A role for methylation (CH_3) and a nucleotide regulatory unit in activation of phospholipase C is suggested by data discussed in the text of this review [from (110)].

phospholipids, we hypothesize that this phenomenon may be of general relevance to stimulus-response coupling of a large group of cellular receptors.

SUMMARY

Chemoattractant receptors on leukocytes can trigger a number of cellular responses, including cytoskeletal reorganization, changes in cell shape, directed motility, lysosomal enzyme secretion, and activation of the respiratory burst. The dose of chemoattractants required to induce motility-related functions is generally at least ten-fold smaller than the dose required to initiate secretory and respiratory burst activities. This finding and other pharmacological evidence clearly indicate that the two types of functions (i.e. motility and secretion) are regulated differently and can be divergently modified by drugs. The affinity of the oligopeptide chemoattractant receptor on polymorphonuclear leukocytes and macrophages is heterogeneous and dynamically regulated by guanine nucleotides and prior agonist exposure. High- and low-affinity forms of the oligopeptide receptor have been identified by direct binding studies. Our data suggest that low doses of agonists can initiate interconversion of low- and high-affinity states of that portion of chemoattractant receptors regulated by guanine nucleotides. On the other hand, high doses of agonists

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sufficient to induce chemotactic desensitization, lysosomal enzyme secretion, and the respiratory burst lead to the formation of a new population of high-affinity receptors. These binding sites are insensitive to the effects of guanine nucleotides and appear to be rapidly internalized. Transmethylation reactions mediated by S-adenosyl methionine are required for the activation of a phospholipase and release of arachidonate from leukocytes by chemotactic factors. We suggest that release of arachidonate from membrane phospholipids activates and translocates a cytosolic but loosely membrane-associated protein kinase C into the membrane and that this kinase participates in stimulus-response coupling of chemoattractant receptors.

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Literature Cited

1. Metchnikoff, E. 1891. Lecture on phagocytosis and immunity. *Brit. Med. J.* 1:213–217
2. Boyden, S. 1962. The chemotactic effects of mixtures of antibody and antigen on polymorphonuclear leukocytes. *J. Exp. Med.* 115:453
3. Shin, H. S., Snyderman, R., Friedman, E., Mellors, A., Mayer, M. D. 1968. Chemotactic and anaphylatoxic fragment, cleaved from the fifth component of guinea pig complement. *Science* 162:361
4. Snyderman, R., Gewurz, H., Mergenhagen, S. E. 1968. Interactions of the complement system with endotoxic lipopolysaccharide. Generation of a factor chemotactic for polymorphonuclear leukocytes. *J. Exp. Med.* 128:259
5. Snyderman, R., Shin, H. S., Phillips, J. K., Gewurz, H., Mergenhagen, S. E. 1969. A neutrophil chemotactic factor derived from C5 upon interaction of guinea pig serum with endotoxin. *J. Immunol.* 103:413
6. Shin, H. S., Gewurz, H., Snyderman, R. 1969. Reaction of cobra venom factor with guinea pig complement and generation of an activity chemotactic for polymorphonuclear leukocytes. *Proc. Soc. Exp. Bio. Med.* 131:203
7. Ward, P. A., Newman, L. J. 1969. A neutrophil chemotactic factor from human C'5. *J. Immunol.* 102:93
8. Jensen, J. A., Snyderman, R., Mergenhagen S. E. 1969. Chemotactic activity, a property of guinea pig C'5-anaphylatoxin. *Proc. Third Int. Symp. Cellular and Humoral Mechanism in Anaphylaxis and Allergy.* Basel/NY: Karger. p. 265
9. Snyderman, R., Phillips, J. K., Mergenhagen, S. E. 1970. Polymorphonuclear leukocyte chemotactic activity in rabbit and guinea pig serum treated with immune complexes: Evidence for C5a as the major chemotactic factor. *Infect. Immun.* 1:521
10. Snyderman, R., Shin, H. S., Dannenburg, A. M. 1972. Macrophage proteinase and inflammation: The production of chemotactic activity from the fifth component of complement by macrophage proteinase. *J. Immunol.* 109:896
11. Ward, P. A., Hill, J. H. 1970. C5 chemotactic fragments produced by an enzyme in lysosomal granules of neutrophils. *J. Immunol.* 104:535
12. Snyderman, R., Phillips, J. K., Mergenhagen, S. E. 1971. Biological activity of complement in vivo: Role of C5 in the accumulation of polymorphonuclear leukocytes in inflammatory exudates. *J. Exp. Med.* 134:1131
13. Ward, P. A., Zvaifler, N. J. 1971. Complement derived leukotactic factors in inflammatory synovial fluids of humans. *J. Clin. Invest* 50:606
14. Fernandez, H. N., Hugli, T. E. 1976. Partial characterization of human C5a

Annual Reviews

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anaphylatoxin. I. Chemical description of the carbohydrate and polypeptide portions of human C5a. *J. Immunol.* 117:1688

15. Fernandez, H. N., Henson, P.M., Otani, A., Hugli, T. E. 1978 Chemotactic response to human C3a and C5a anaphylatoxins: I. Evaluation of C3a and C5a leukotaxis in vitro and under stimulated in vivo conditions. *J. Immunol.* 120:109
16. Hugli, T. E., Muller-Eberhard, H. J. 1978. Anaphylatoxins. *Adv. Immunol.* 26:1
17. Snyderman, R., Altman, L. C., Hausman, M. S., Mergenhagen, S. E. 1972. Human mononuclear leukocyte chemotaxis: A quantitative assay for mediators of humoral and cellular chemotactic factors. *J. Immunol.* 108:857
18. Postlethwaite, A., Snyderman, R. 1975. Characterization of chemotactic activity produced in vivo by a cell mediated immune reaction in the guinea pig. *J. Immunol.* 114:274
19. Spilberg, I., Mehta, J. 1979. Demonstration of a specific neutrophil receptor for a cell derived chemotactic factor. *J. Clin. Invest.* 63:85
20. Spilberg, I., Gallacher, A., Mehta, J., Mandell, B. 1976. Urate crystal induced chemotactic factor, isolation and partial characterization. *J. Clin. Invest.* 58;815
21. Goetzl, E. J., Austen, K. F. 1975. Purification and synthesis of eosinophilotactic tetrapeptides of human lung tissue: Identification as eosinophil chemotactic factor of anaphylaxis. *Proc. Natl. Acad. Sci. USA* 72:4123
22. Goetzl, E. J., Pickett, W. C. 1980. The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETES). *J. Immunol.* 125:1789
23. Turner, S. R., Campbell, J. A., Lynn, W. S. 1975. Polymorphonuclear leukocyte chemotaxis towards oxidized lipid components of cell membranes. *J. Exp. Med.* 141:1437
24. Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., Smith, M. J. H. 1980. Leukotriene B_4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 286:264
25. Ford-Hutchinson, A. W., Bray, M. A., Cunningham, F. M., Davidson, E. M., Smith, M. J. H. 1981. Isomers of leukotriene B_4 possess different biological potencies. *Prostaglandins* 21:143
26. Deuel, T. F., Senior, R. M., Chang, D., Griffin, G. L., Heinrikson, R. L., Kaiser, E. T. 1981. Platelet factor 4 is chemotactic for neutrophils and monocytes. *Proc. Natl. Acad. Sci. USA* 78:4584
27. Williams, L. T., Antoniades, H. N., Goetzl, E. J. 1983. Platelet-derived growth factor stimulates mouse 3T3 cell mitogenesis and leukocyte chemotaxis through different structural determinants. *J. Clin. Invest.* In press
28. Gallin, J. I., Rosenthal, A. S. 1974. The regulatory role of divalent cations in human granulocyte chemotaxis: Evidence for an association between calcium exchanges and microtubule assembly. *J. Cell. Biol.* 62:594
29. Naccache, P. H., Showell, H. J., Becker, E. L., Shaa'fi, R. I. 1977. Transport of sodium, potassium, and calcium across rabbit polymorphonuclear leukocyte membranes: Effect of chemotactic factor. *J. Cell. Biol.* 73:428
30. Gallin, E. K., Gallin, J. I. 1977. Interaction of chemotactic factors with human macrophages: Induction of transmembrane potential changes. *J. Cell. Biol.* 75:277
31. Smith, C. W., Hollers, J. C., Patrick, R. A., Hassett, C. 1979. Motility and adhesiveness in human neutrophils. Effects of chemotactic factors. *J. Clin. Invest.* 63:221
32. Cianciolo, G. J., Snyderman, R. 1981. Monocyte responsiveness to chemotactic stimuli is a property of a subpopulation of cells which can respond to multiple chemoattractants. *J. Clin. Invest.* 67:60
33. Goldstein, I., Hoffstein, S., Gallin, J., Weissmann, G. 1973. Mechanisms of lysosomal enzyme release from human leukocytes: Microtubule assembly and membrane fusion induced by a component of complement. *Proc. Natl. Acad. Sci. USA* 70:2916
34. Klebanoff, S. J., Clark, R. A. 1978. *The Neutrophil: Function and Clinical Disorders*. NY: North-Holland
35. Snyderman, R., Goetzl, E. J. 1981. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science* 213:830
36. Lohr, K. M., Snyderman, R. 1982. Amphotericin B alters the affinity and functional activity of the oligopeptide chemotactic factor receptor on human polymorphonuclear leukocytes. *J. Immunol.* 129:1594
37. Snyderman, R. 1983. Characterization of the oligopeptide chemoattractant receptor on leukocytes: Binding affinity reflects signal transduction. *Fed. Proc.* 42:2855
38. Yuli, I., Tomonaga, A., Snyderman, R. 1982. Chemoattractant receptor functions in human polymorphonuclear leukocytes are divergently altered by membrane fluidizers. *Proc. Natl. Acad. Sci. USA* 79:5906
39. Koo, C., Lefkowitz, R. J., Snyderman,

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R. 1982. The oligopeptide chemotactic factor receptor on human polymorphonuclear leukocyte membranes exists in two affinity states. *Biochem. Biophys. Res. Commun.* 106:442

40. Koo, C., Lefkowitz, R. J., Snyderman, R. 1983. Guanine nucleotides modulate the binding affinity of the oligopeptide chemoattractant receptor on human polymorphonuclear leukocytes. *J. Clin. Invest.* 72:748
41. Koo, C., Snyderman, R. 1983. The oligopeptide chemoattractant receptor on human neutrophils converts to an irreversible high affinity state subsequent to agonist exposure. *Clin. Res.* 31:4914
42. Hirata, F., Corcoran, B. A., Venkatasubramanian, K., Schiffmann, E., Axelrod, J. 1979. Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. *Proc. Natl. Acad. Sci. USA* 76:2640
43. Pike, M. C., Kredich, N. M., Snyderman, R. 1978. Requirement of S-adenosyl-L-methionine-mediated methylation for human monocyte chemotaxis. *Proc. Natl. Acad. Sci. USA* 75:3928
44. Pike, M. C., Snyderman, R. 1981. Transmethylation reactions are required for initial morphologic and biochemical responses of human monocytes to chemoattractants. *J. Immunol.* 127:1444
45. Snyderman, R., Pike, M. C., Kredich, N. M. 1980. Role of transmethylation reactions in cellular motility and phagocytosis. *Mol. Immunol.* 17:209
46. McPhail, L. C., Snyderman, R. 1984. Mechanisms of regulating the respiratory burst of leukocytes. In *Contemporary Topics in Immunobiology*, ed. R. Snyderman. NY: Plenum. In press
47. Freer, R. J., Day, A. R., Radding, J. A., Schiffmann, E., Aswanikumar, S., Showell, H. J., Becker, E. L. 1980. Further studies on the structural requirements for synthetic peptide chemoattractants. *Biochemistry* 19:2404
48. Showell, H. J., Freer, R. J., Zigmond, S. H., Schiffmann, E., Aswanikumar, S., Corcoran, B. A., Becker, E. L. 1976. The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J. Exp. Med.* 143:1154
49. Marasco, W. A., Phan, S. H., Krutzsch, H., Showell, H. J., Feltner, D. E., Nairn, R., Becker, E. L., Ward, P. A. 1983. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. 5th Int. Cong. Immunol. Sci., Kyoto, Japan. (Abstr.)
50. Schiffmann, E., Corcoran, B. A., Wahl, S. M. 1975. N-formylmethionyl peptides as chemoattractants for leukocytes. *Proc. Natl. Acad. Sci. USA* 72:1059
51. Williams, L. T., Snyderman, R., Pike, M. C., Lefkowitz, R. J. 1977. Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* 74:1204
52. Aswanikumar, S., Corcoran, B., Schiffman, E., Day, A. R., Freer, R. J., Showell, H. J., Pert, C. B. 1977. Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. *Biochem. Biophys. Res. Commun.* 74:810
53. Niedel, J. E., Kahane, I., Cuatrecasas, P. 1979. Receptor-mediated uptake and degradation of ^{125}I chemotactic peptide by human neutrophils. *J. Biol. Chem.* 254:10700
54. Niedel, J. E., Kahane, I., Cuatrecasas, P. 1979. Receptor-mediated internalization of fluorescent chemotactic peptide by human neutrophils. *Science* 205:1412
55. Snyderman, R., Fudman, E. J. 1980. Demonstration of a chemotactic factor receptor on macrophages. *J. Immunol.* 124:2754
56. Weinberg, J. B., Muscato, J. J., Niedel, J. 1981. Monocyte chemotactic peptide receptor. *J. Clin. Invest.* 68:621
57. Benyunes, M. C., Snyderman, R. 1984. Characterization of an oligopeptide chemoattractant receptor on human blood monocytes using a new radioligand. *Blood*. In press
58. Williams, L. T., Lefkowitz, R. J. 1978. *Receptor Binding Studies in Adrenergic Pharmacology*. NY: Raven
59. Burgisser, G., DeLean, A., Lefkowitz, R. J. 1982. Reciprocal modulation of agonist and antagonist binding to muscarinic cholinergic receptor by guanine nucleotides. *Proc. Natl. Acad. Sci. USA* 79:1732
60. DeLean, A., Hancock, A. A., Lefkowitz, R. J. 1982. Validation and statistical analysis of computer modeling method for quantitative analysis of radioligand binding data for mixtures of pharmacological receptor subtypes. *Mol. Pharmacol.* 21:5
61. Hancock, A. A., DeLean, A., Lefkowitz, R. J. 1979. Quantitative resolution of beta-adrenergic receptor subtypes by selective ligand binding. Application of a computerized model fitting technique. *Mol. Pharmacol.* 16:1
62. Seligmann, B. E., Fletcher, M. P., Gallin, J. I. 1982. Adaptation of human neutrophil responsiveness to the chemoat-

Annual Reviews

tractant N-formylmethionylphenylalanine. *J. Biol. Chem.* 257:6280
63. Mackin, W. M., Chi-Kuang, H., Becker, E. L. 1982. The formyl peptide chemotactic receptor on rabbit peritoneal neutrophils. *J. Immunol.* 129:1608
64. Niedel, J., Davis, J., Cuatrecasas, P. 1980. Covalent affinity labeling of the formyl peptide chemotactic receptor. *J. Biol. Chem.* 255:7063
65. Dolmatch, B., Niedel, J. 1983. Formyl peptide chemotactic receptor: Evidence for an active proteolytic fragment. *J. Biol. Chem.* 258:7570
66. Schmitt, M., Painter, R. G., Jesaitis, A. J., Preissner, K., Sklar, L. A., Cochrane, C. G. 1983. Photoaffinity labeling of the N-formyl peptide receptor binding site of intact human polymorphonuclear leukocytes. *J. Biol. Chem.* 258:649
67. Painter, R. G., Schmitt, M., Jesaitis, A. J., Sklar, L. A., Aissnar, K., Cochrane, C. G. 1982. Photoaffinity labeling of the N-formyl peptide receptor on human polymorphonuclear leukocytes. *J. Cell. Biochem.* 20:203
68. Goetzl, E. J., Foster, D. W., Goldman, D. W. 1981. Isolation and partial characterization of membrane protein constituents of human neutrophil receptors for chemotactic formylmethionyl peptides. *Biochemistry* 20:5717
69. Kay, G. E., Lane, B. C., Snyderman, R. 1983. Induction of selective biological responses to chemoattractants in a human monocyte-like cell line. *Infect. Immun.* 41:1166
70. Stadel, J. M., DeLean, A., Lefkowitz, R. J. 1982. Molecular mechanisms of coupling in hormone receptor-adenylate cyclase systems. *Adv. Enzymol.* 53:1
71. Lad, P. M., Welton, A. F., Rodbell, M. 1977. Evidence for distinct guanine nucleotide sites in the regulation of the glucagon receptor and of adenylate cyclase activity. *J. Biol. Chem.* 252:5942
72. DeLean, A., Kilpatrick, B. F., Caron, M. G. 1982. Dopamine receptor of the porcine anterior pituitary gland. *Mol. Pharmacol.* 22:290
73. Verghese, N. W., Snyderman, R. 1983. Hormonal regulation of adenylate cyclase in macrophage membranes is regulated by guanine nucleotides. *J. Immunol.* 180:869
74. Snyderman, R., Pike, M. C., Edge, S., Lane, B. 1983. A chemoattractant receptor on macrophages exists in two affinity states regulated by guanine nucleotides. *J. Cell. Biol.* In press
75. Wheeler, G. L., Bitensky, M. W. 1977. A light-activated GTPase in vertebrate photoreceptors: Regulation of light-activated cyclic GMP phosphodiesterase. *Proc. Natl. Acad. Sci. USA* 74:4238
76. Verghese, M., McPhail, L. C., Fox, K., Snyderman, R. 1984. Chemoattractants trigger rapid, transient inactivation of phosphodiesterase in leukocytes: A novel mechanism for receptor-mediated enhancement of cAMP. *Clin. Res.* In press
77. Jesaitis, A. J., Naemura, J. R., Sklar, L. A., Cochrane, C. G., Painter, R. G. 1983. Rapid modulation of N-formyl chemotactic peptide receptors on the surface of human granulocytes: Formation of slowly dissociating ligand-receptor complexes in transient association with cell cytoskeleton. *J. Cell. Biol.* In press
78. Gallin, J. I., Wright, D. G., Schiffmann, E. 1978. Role of secretory events in modulating human neutrophil chemotaxis. *J. Clin. Invest.* 62:1364
79. Fletcher, M. P., Gallin, J. I. 1980. Degranulating stimuli increase the availability of receptors on human neutrophils for the chemoattractant fMet-Leu-Phe. *J. Immunol.* 124:1585
80. Fletcher, M. P., Seligmann, B. E., Gallin, J. I. 1982. Correlation of human neutrophil secretion, chemoattractant receptor mobilization and enhanced functional capacity. *J. Immunol.* 128:941
81. Fletcher, M. P., Gallin, J. I. 1983. Human neutrophils contain an intracellular pool of putative receptors for the chemoattractant N-formylmethionylleucylphenylalanine. *Blood* 62:792
82. Heron, D. S., Shinitsky, M., Hershkowitz, M., Samuel, D. 1980. *Proc. Natl. Acad. Sci. USA* 77:7463
83. Andreoli, T. E. 1973. On the anatomy of amphotericin B-cholesterol pores in lipid bilayer membranes. *Kidney Int.* 4:337
84. Lampen, J. O. 1969. Amphotericin B and other polyenic antifungal antibiotics. *Am. J. Clin. Path.* 52:138
85. Bjorksten, B., Ray, C., Quie, P. G. 1976. Inhibition of human neutrophil chemotaxis and chemiluminescence by amphotericin B. *Infect. Immunol.* 14:315
86. Pike, M. C., Snyderman, R. 1980. Lipid requirements for leukocyte chemotaxis and phagocytosis: Effects of inhibitors of phospholipid and cholesterol synthesis. *J. Immunol.* 124:1963
87. Ishizaka, T., Hirata, F., Ishizaka, K., Axelrod, J. 1980. Stimulation of phospholipid methylation, Ca^{+2} influx, and histamine release by bridging of IgE receptors on rat mast cells. *Proc. Natl. Acad. Sci. USA* 77:1903
88. Pike, M. C., Kredich, N. M., Snyderman, R. 1979. Phospholipid methylation

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in macrophages is inhibited by chemotactic factors. *Proc. Natl. Acad. Sci. USA* 76:2922

89. Chenoweth, D. E., Hugli, T. E. 1978. Demonstration of a specific C5a receptor on intact polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* 75:3943
90. Chenoweth, D. E., Erikson, B. W., Hugli, T. E. 1979. Human C5a-related synthetic peptides as neutrophil chemotactic factors. *Biochem. Biophys. Res. Commun.* 86:227
91. Chenoweth, D. E., Hugli, T. E. 1980. Human C5a and C5a analogs as probes of the neutrophil C5a receptor. *Mol. Immunol.* 17:151
92. Kreisle, R. A., Parker, C. W. 1983. Specific binding of leukotriene B_4 to a receptor on human polymorphonuclear leukocytes. *J. Exp. Med.* 157:628
93. Goldman, D. W., Goetzl, E. J. 1982. Specific binding of leukotriene B_4 to receptors on human polymorphonuclear leukocytes. *J. Immunol.* 129:1600
94. Snyderman, R., Meadows, L., Amos, D. B. 1977. Characterization of human chemotactic lymphokine production induced by mitogens and mixed leukocyte reactions using a new microassay. *Cell. Immunol.* 30:225
95. Altman, L. C., Chassey, B., Mackler, B. F. 1975. Physicochemical characterization of chemotactic lymphokines produced by human T and B lymphocytes. *J. Immunol.* 110:18
96. Spilberg, I., Rosenberg, D., Mandell, B. 1977. Induction of arthritis by purified cell-derived chemotactic factor: Role of chemotaxis and vascular permeability. *J. Clin. Invest.* 59:582
97. Simchowitz, L., Fishbein, L. C., Spilberg, I., Atkinson, J. P. 1980. Induction of a transient elevation in intracellular levels of adenosine-3',5'-cyclic monophosphate by chemotactic factors: An early event in human neutrophil activation. *J. Immunol.* 124:1482
98. Smolen, J. E., Korchak, H. M., Weissmann, G. 1980. Increased levels of cyclic adenosine-3',5' monophosphate in human polymorphonuclear leukocytes after surface stimulation. *J. Clin. Invest.* 65:1077
99. Painter, R. G., Sklar, L. A., Jesaitis, A. J., Schmitt, M., Cochrane, C., G. 1984. Activation of neutrophils by N-formyl chemotactic peptides. *Fed. Proc.* In press
100. Sklar, L. A., Jesaitis, A. J., Painter, R. G., Cochrane, C. G. 1982. Ligand/receptor internalization: A spectroscopic analysis and a comparison of ligand binding, cellular response, and internalization by human neutrophils. *J. Cell. Biochem.* 20:192
101. Lapetina, E. G., Billah, N. M., Cuattrecasas, P. 1981. The initial action of thrombin on platelets: Conversion of phosphatidylinositol to phosphatidic acid preceding the production of arachidonic acid. *J. Biol. Chem.* 256:5037
102. O'Dea, R. F., Viveros, O. H., Aswanikumar, S., Schiffmann, E., Chiang, P. K., Cantoni, G. L., Axelrod, J. 1978. A protein carboxymethylation stimulated by chemotactic peptides in leukocytes. *Fed. Proc.* 37:1656
103. Kredich, N. M., Martin, D. W., Jr. 1977. Role of S-adenosyl-homocysteine in adenosine mediated toxicity in cultured mouse T lymphoma cells. *Cell* 12:931
104. Pike, M. C., Snyderman, R. 1982. Transmethylation reactions regulate affinity and functional activity of chemotactic factor receptors on macrophages. *Cell* 28:107
105. Bareis, D. L., Hirata, F., Schiffmann, E., Axelrod, J. 1982. Phospholipid metabolism, calcium flux, and the receptor-mediated induction of chemotaxis in rabbit neutrophils. *J. Cell. Biol.* 93:690
106. Pike, M. C., Snyderman, R. 1981. Alterations of new methylated phospholipid synthesis in the plasma membranes of macrophages exposed to chemoattractants. *J. Cell. Biol.* 91:221
107. Castagna, M., Yoshima, T., Kaibachi, S., Kikkawa, U., Nishizuka, Y. 1982. Direct activation of calcium activated phospholipid dependent protein kinase by tumor-promoting phorbol esthers. *J. Biol. Chem.* 257:7847
108. McPhail, L. C., Wolfson, M., Snyderman, R. 1984. Protein kinase C (PKC) and neutrophil activation: PKC becomes tightly membrane associated when cells are stimulated with phorbol myristate acetate (PMA). *Fed. Proc.* (Abstr.) submitted
109. McPhail, L. C., Clayton, C. C., Snyderman, R. 1984. A potential second messenger role for unsaturated fatty acids: Activation and modulation of Ca^{+2} dependent protein kinase. *Science*. In press
110. Pike, M. C., Snyderman, R. 1984. Leukocyte chemoattractant receptors. In *The Receptors*, ed. P. M. Conn. NY: Academic. In press
111. Davis, B. J., Walter, R. J., Pearson, C. B., Becker, E. L., Oliver, J. M. 1982. Membrane activity and topography of fMet-Leu-Phe-treated polymorphonuclear leukocytes: acute and sustained responses to chemotactic peptides. *Am. J. Path.* 108:206.

Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359

ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395

IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429

COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461

C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:257-281. Downloaded from arjournals.annualreviews.org
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THE CELL BIOLOGY OF MACROPHAGE ACTIVATION[1]

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I. INTRODUCTION

The fundamental concept of Mϕ activation dates to the observations of Elie Metchnikoff, who noted over 75 years ago that mononuclear phagocytes from animals resistant to certain bacterial infections had increased competence for ingesting and killing these microbes (1). During the 1960s, George Mackaness and his colleagues elegantly elucidated the basis of cellular immunity to facultative and obligate intracellular parasites and termed "activated" the large, angry Mϕ that finally effect such immunity [for reviews, see (2,3)]. In the early 1970s, Evans & Alexander and Hibbs & Remington found that these angry Mϕ were also extremely efficient at destroying neoplastic, eukaryotic cells as well [for references, see (3)]. The activated Mϕ has thus come to be defined as one capable of mediating antitumor or antimicrobial effects (3).

The basic cellular physiology of the Mϕ has been extensively studied. Indeed, mononuclear phagocytes have proven to be a successful and widely used tool in cell biology. Since the elegant and seminal studies of Cohn and colleagues in the 1960s on the development and maturation of Mϕ (4), the endocytic and regulatory receptors, the plasma membrane proteins, the secretory products, and the intracellular constituents of these cells have been exam-

[1]Abbreviations: ADCC—antibody dependent cellular cytotoxicity; APR—antigen presentation; CAD—classic ADCC; CP—cytolytic protease; CTX—chemotaxis; IA—I-region associated antigen; IL—interleukin; IFN—interferon; Mab—monoclonal antibody; MAF—macrophage activating factor; MPS—mononuclear phagocyte system; Mϕ—macrophage(s); MTC—macrophage-mediated tumor cytotoxicity; NAD—novel ADCC; PHG—phagocytosis; PKc—protein kinase C; PMA—phorbol myristate acetate; ROI—reactive oxygen intermediate(s).

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Annual Reviews

ined in considerable detail. As it turns out, a great many if not most of these physiologic and biochemical properties or capacities can be markedly up- or down-regulated, particularly during Mϕ activation. Thus, an important strategy for ascertaining the mechanisms by which activated Mϕ recognize and kill tumor cells and microbes has emerged: Define the physiologic and metabolic alterations that uniquely characterize activated Mϕ. From such studies, a new concept of activation has emerged: acquisition of competence to mediate or complete a complex function (i.e. modulation to an enhanced ability for performing a multistep task) [see (3) for review]. Inherent in this definition is an important distinction between complex functions such as microbial kill and physiologic capacities such as number of FcRI (see section II, below).

Mononuclear phagocytes, after extensive development in the marrow, migrate to the tissues via the blood (5). Once established in the tissues, Mϕ can be subjected to a barrage of stimulatory and suppressive signals, particularly in instances of altered homeostasis such as inflammation, that profoundly alter their morphology, metabolism, and physiology and hence activate them for numerous functions. Activation thus represents a useful model for studying the regulation and expression of Mϕ modulation in the tissues. As we begin to define completely the physiologic capacities required for each function and the signals specifically inducing and suppressing each capacity, we shall thus also acquire a clearer understanding of the cellular and molecular biology of Mϕ development.

Our purpose in this chapter is to assess current progress in attaining these goals. After briefly reviewing the distinctions and relationships between capacities and functions and the general development of the mononuclear phagocyte system, we consider some of the numerous capacities of Mϕ and their regulation. Then, we describe the way various combinations of these capacities can be combined to permit activation for several functions. The majority of data presented pertain to murine Mϕ. [NB: The activation of human Mϕ is distinct from that of murine Mϕ in numerous regards. For references, see (6).]

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II. CAPACITIES VERSUS FUNCTIONS: DISTINCTION BY CIRCUIT MODELS

Analysis of Mϕ development can be greatly facilitated by distinguishing functions from capacities [reviewed in (3)]. A *capacity* can be defined as a single attribute (i.e. ability, characteristic, or property), usually quantified biochemically or immunologically as a specific feature of the cell's composition or metabolism (see Table 1). A *function* can be defined as completion of a complex action (i.e. task or role), usually quantified by determining in a physiologic assay the rate or extent of completion. Capacities generally represent the

Annual Reviews

Table 1 Representative capacities and functions of Mϕ

Capacities
1. Number of FcRI
2. Affinity of receptors for N-formylated peptides
3. Expression of Ia antigen
4. Degree of selective binding of tumor cells
5. Amount of plasminogen activator secreted
6. Amount of H_2O_2 secreted
7. Content of acid phosphatase
Functions
1. Direct cytolysis of tumor cells
2. Classic ADCC of tumor cells
3. Novel ADCC of tumor cells
4. Phagocytosis
5. Processing and presentation of antigen
6. Chemotaxis

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full expression of separate and independently regulated gene products; functions represent the result of operative interactions of these products. Mϕ activated for a specific function will be designated by a subscript (i.e. activation_{MTC} connotes competence for macrophage-mediated tumor cytotoxicity or MTC).

This distinction may also be analyzed by modeling the completion of a function as a simple control circuit (Figure 1) [for details, see (3)]. The input of such a circuit is the initiating signal. The components of the circuit are the requisite capacities, while the output is completion of the function. Capacities are thus components of a circuit, while functions are outputs. As corollaries to this model, (a) capacities subserve and hence are less complex than functions, and (b) activation for a given function is equivalent to induction of all requisite capacities for that function (3). It should be emphasized that circuits of varying complexities can be constructed and then placed in hierarchies. For example, phagocytosis (PHG), itself a function, would be a component of a more complex circuit, the output of which was the kill of an intracellular parasite.

This definition of capacities is useful in a reductionist approach to analyzing the complex problem in cellular control posed by Mϕ development in the tissues. It is obvious, however, that the present physiologic bases for defining

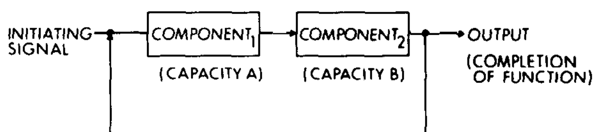

Figure 1 A circuit model of macrophage function. See text for details.

Annual Reviews

the various capacities vary considerably from capacity to capacity (see, for example, Table 1). Thus, capacities are presently best defined operationally by their predictive relationship to certain functions (see section V, below).

III. GENERAL DEVELOPMENT OF THE MONONUCLEAR PHAGOCYTE SYSTEM (MPS)

Elements of the MPS originate in the bone marrow, where their development has been extensively reviewed [see (3,5)]. These developmental changes, which result in acquisition of endocytic competence and surface expression of both complement and Fc receptors, are expressed by all elements of the MPS and are relatively stable for the remainder of their history. These developmental changes are often quantitative rather than qualitative. Mononuclear phagocytes, after leaving the marrow, pass through the blood as monocytes and hence into the various tissues of the body, where they are termed the resident tissue Mϕ. Functional heterogeneity exists in most populations of Mϕ [for review, see (3)], but these differences can be generally accounted for by differences in degree of maturation. To date, no convincing evidence indicates that the MPS comprises distinct subpopulations of distinctly differentiated Mϕ (7,8).

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A cardinal feature of the MPS is that Mϕ can change further once in the tissues (Figure 2). Most Mϕ natively resident in the tissues represent a quiescent population of cells that superficially resemble monocytes in many ways, such as morphology, degree of spreading, phagocytic competence, and diminished capacity to respond to lymphokines (in regard to activation$_{MTC}$; see section V, below) [for review, see (3)]. Certainly, resident tissue Mϕ are very distinct from Mϕ taken from sites of inflammation (so-called inflammatory Mϕ, see below). By appropriate stimulation, profound alterations can be induced in the physiology of the resident cells (e.g. 30-fold increases in content of acid hydrolases) (3). For these reasons, models of Mϕ development have often assumed a progression from monocyte to resident tissue Mϕ to inflammatory Mϕ, etc (Figure 2) (3). Studies in recent years on murine monocytes have called this model into question. In terms of content of the ectoenzyme

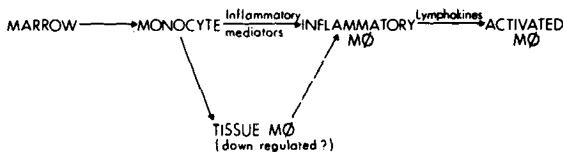

Figure 2 A general model of macrophage development. Monocytes arise in the marrow and migrate into the tissues, where they are down-regulated. In inflammation, monocytes and young macrophages populate the inflammatory site. The influx of T cells and generation of lymphokines there drive the macrophages further.

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5′nucleotidase, responsiveness to lymphokines for induction of Ia antigens, expression of the transferrin receptor, and competence for secretion of prostaglandins (9,11; J. E. Weiel, T. A. Hamilton, D. O. Adams, unpublished), murine monocytes closely resemble young Mφ from sites of inflammation and are distinct from resident peritoneal Mφ. In addition, content of B-galactosidase, believed to be an enzymatic marker of Mφ age rather than of activation, is high in resident peritoneal Mφ and low in mononuclear phagocytes of the marrow, monocytes, and inflammatory Mφ (12). These data have led to the hypothesis that mononuclear phagocytes are at least partly developed upon emigration from the marrow and that many of their properties are down-regulated in the tissues by local suppressor substances (E. R. Unanue, personal communication). Prostaglandins derived from the Mφ are an intriguing candidate for this role [see (14)].

Mφ taken from sites of nonimmunologically mediated inflammation (termed *inflammatory Mφ*) are, in any case, very distinct from resident tissue histiocytes (15); alveolar Mφ, continually exposed to multiple inhaled elements of the environment, are a conspicuous exception (8). The alterations in cellular physiology that distinguish resident from inflammatory Mφ can be striking (i.e. differences of 3–800-fold in various capacities) and encompass increases in size, content of acid hydrolases, secretion of neutral proteases, and alterations in content of several ectoenzymes (4,15). Inflammatory Mφ have also gained competence or increased competence (i.e. become activated) for chemotaxis (CTX) and for PHG via Fc and complement receptors (see section V, below).

The signals that induce the changes characterizing inflammatory Mφ remain to be defined precisely (3). Such Mφ have been exposed in vivo to complex, exogenous inducers of inflammation such as thioglycollate broth and to numerous endogenous inflammatory mediators such as proteases, complement components, and antibodies (3). The elegant studies of Cohn two decades ago, which initially defined the cell biology of Mφ maturation, showed that substances active at the Mφ surface induce many of the alterations typical of the inflammatory Mφ (4). Induction of these various characteristics is not tightly linked, in that stimulation of one capacity does not necessarily lead to stimulation of a second [for review, see (3)].

A third distinctive group comprises Mφ taken from sites of immunologically mediated inflammation. In addition to possessing the characteristics borne by inflammatory Mφ, the mononuclear phagocytes exposed to lymphokines (LK) and other signals in vivo are competent (i.e. activated) to present antigen to T lymphocytes, to kill tumor cells, and to destroy facultative/obligate intracellular parasites (see section V). Capacities unique to these Mφ are detailed subsequently (section V). In fact, activation of murine Mφ for lytic function is more complex than this. Development of Mφ activated$_{MTC}$, for example,

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proceeds by a series of operationally defined stages (16,17,18). In brief, young inflammatory Mϕ *(responsive Mϕ)*, though not resident peritoneal Mϕ, respond readily to LK and thus gain responsiveness to a second signal, such as traces of endotoxin. The LK-treated Mϕ, termed *primed Mϕ*, are not cytolytic. Exposure of primed Mϕ to a second signal, such as traces of endotoxin, renders them fully $activated_{MTC}$ as determined by acquisition of cytolytic competence. It should be noted that in some instances the $activation_{MTC}$ of Mϕ in vitro with LK and endotoxin does not fully reproduce the changes induced in Mϕ isolated from sites of immunologically mediated inflammation in vivo (19).

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IV. MACROPHAGE CAPACITIES AND THEIR REGULATION

Overview

Circuit models provide a powerful tool for dissecting, level by level, the cellular and biochemical bases of Mϕ activation [for details, see (3)]. The number of capacities possessed by Mϕ is almost staggering. In terms of secretion, they are now known to release over 75 defined molecules, while their plasma membranes bear over 30 distinct receptors (20). Furthermore, most of these capacities are altered in development. For example, an extensive, selective, and closely coordinated remodeling of the protein composition of the plasma membrane constitutes a major feature of activation [for review, see (21)]. For reasons of brevity, the discussion to follow focuses on capacities chosen because they are (*a*) known to be altered in the course of activation and/or (*b*) already established as important determinants of Mϕ function. In most cases, details of the biochemistry and molecular biology governing expression of the capacities are limited. In this section we also emphasize the regulatory signals that enhance or diminish these capacities.

Mϕ capacities important to functional maturation can be separated into three broad categories. The first contains those intracellular constituents important to the fundamental metabolism of the cell and to the degradation of ingested materials. The second, the ability to recognize and interact with the extremely varied molecules in the external environment of the Mϕ, is mediated largely by specific receptors/glycoproteins on the cell surface. The third is the ability to synthesize, package, and secrete a wide variety of molecular products, which act both intra- and extracellularly. These three types of capacities often operate in a highly coordinated fashion. For example, secretory output of a particular product may depend upon both the intracellular competence for synthesis and/or secretion and the number, affinity, or coupling efficacy of the surface receptors to the specific triggering stimulus. Some critical Mϕ capacities and the factors controlling them are summarized below (see Table 2).

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Intracellular Capacities

At least three categories of intracellular structures are of obvious central importance in the execution of complex functions by Mϕ. The first and most thoroughly studied of these is the vacuolar system, encompassing phagosomes, pinosomes, endosomes, primary/secondary lysosomes, and phagolysosomes (22). Even in resident tissue Mϕ, the endocytic function of Mϕ results in internalization of the entire Mϕ plasma membrane nearly two times per hour (estimated time of complete turnover is ~33 min) (22). Despite this extensive turnover of membranes, proteins of the surface plasma membrane are remarkably similar to those of the interior, save for the selective interiorization of a receptor following its engagement and endocytosis with a particle coated with the specific ligand (22). In inflammatory Mϕ or Mϕ undergoing substantial phagocytic challenge, this membrane flow is remarkably enhanced (15). Associated with the formation of primary and secondary lysosomes is the accumulation within these structures of over 40 hydrolytic enzymes necessary to degradation of phagocytosed particles and molecules. The acquisition of increased numbers of lysosomes and their associated enzymes is induced by a variety of signals including phagocytic stimuli and lymphokines (4,23). Such stimuli can also induce discharge of lysosomal contents into the extracellular compartment (23).

The second category of intracellular structures is the Mϕ cytoskeletal apparatus (24). This system of filaments and tubules obviously participates in phagocytic and secretory functions and is also necessary for selective binding of neoplastic cells (24,25). The proteins comprising this complex system, including actin, myosin, acumentin, and gelsolin, represent nearly 15% of total cellular protein (24). While the quantity of these constituents may not vary during activation, their functions can be subtly and closely controlled in response to specific signals. For example, phagocytosis mediated by complement receptors is controlled by the relative lateral mobility of these sites in the plasma membrane (see the section on activation for other functions, below). Likewise, microtubules are not necessary for phagocytosis per se, but subsequent intracellular events do not proceed in an organized fashion in their absence (26).

The third category is the mitochondrial system, the associated respiratory chain, and the enzymes of the Emden-Meyerhoff path and hexose monophosphate shunt (27). Most Mϕ depend principally upon aerobic glycolysis, though alveolar Mϕ depend much more upon mitochondrial respiration. Inflammatory Mϕ do contain increased numbers of mitochondria (4). Phagocytosis generally triggers a respiratory burst and activates a membrane-associated oxidase that results in generation of superoxide anion near the plasma membrane (28). Because phagocytosis is not necessarily accompanied by a respiratory burst

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Table 2 Macrophage capacities and their regulation

Capacity	Expression at stage of activation$_{MTC}$	Regulation and consequences	References
Intracellular enzymes			
Lysosomal hydrolases	Responsive, primed, and activated Mϕ	(*a*) ↑ dramatically in responsive cells (i.e. 30-fold) in response to sterile inflammatory agents in vivo and in vitro (*b*) Digestible particulates increase production (*c*) ↑ induced in vitro in response to LK (MAF)	14, 23
Surface receptors and proteins			
Fc receptors (FcRI, FcRII)	All stages	(*a*) ↑ number of receptors in inflammatory vs resident Mϕ (4–5×) (*b*) ↑ phagocytic function in inflammatory Mϕ, regulatory signals unknown (*c*) ↑ function for ADCC (both classic and novel forms) in appropriately activated Mϕ (*d*) ↑ numbers induced by α, β and γ IFN	30, 34, 116
Complement receptor (CR1, CR3)	All stages, but ↑ number of CR3 on resident Mϕ	(*a*) ↑ phagocytic function in responsive, primed, and activated Mϕ, due to altered mobility in plasma membrane (*b*) Induced by T cell–derived lymphokine distinct from IFN_γ	36, 37
Man-Fuc-GlcNac receptor	Resident and responsive Mϕ	(*a*) ↓ numbers in primed and activated Mϕ (*b*) Induced by T cell–derived lymphokine (*c*) ↓ ingestion of zymosan	41, 42, 43, 44
Ia (Class II histocompatibility antigens)	Small percentage of Mϕ at all stages, but present on most primed and activated Mϕ	(*a*) ↑ expression and function in primed and activated Mϕ (*b*) Induced by T cell–derived lymphokine (MAF) (*c*) Develops independently of tumoricidal capacity (*d*) ↓ by exposure to PGE_2 (*e*) Permits effective interaction with T cells → effective APR	45, 46
fMet-Leu-Phe receptor	All stages (human Mϕ)	(*a*) Affinity of receptor regulates response (*b*) High-affinity → CTX (*c*) Low-affinity → secretion of ROI, PGs, lysosomal hydrolases	55, 56, 57, 58

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C_5A receptor	All stages	(*a*) ↑ in resident Mϕ, ↓ in inflammatory Mϕ (*b*) Mediates CTX, secretion of IL1	39, 40
Transferrin receptor	Monocytes and responsive Mϕ	(*a*) ↑ monocytes and responsive Mϕ (*b*) ↑ in culture (*c*) ↓ on resident Mϕ [(*a*)–(*c*): regulatory signals unknown] (*d*) ↓ primed and activated Mϕ (*e* ↓ induced by MAF, IFN_γ	47
Mϕ tumor binding	Primed and activated Mϕ	(*a*) ↑ as responsive Mϕ are primed in vivo or in vitro (*b*) Induced by MAF/IFN_γ (*c*) Retained even as tumoricidal capacity decays	3, 25
5′nucleotidase	Resident Mϕ	(*a*) ↓ in monocytes, responsive, primed, and activated Mϕ (*b*) Decrease coincides with acquisition of responsiveness to lymphokines	49
CSF-1	All stages	(*a*) ↓ in LPS treated Mϕ (*b*) Regulates morphology, differentiation, and proliferation but responses differ among different populations of Mϕ	59, 60, 61, 63
Acetylated LDL receptor	All stages	(*a*) Not regulated quantitatively between stages (*b*) Induces secretion of plasminogen activator, other neutral proteases, and cytolytic proteinase (*c*) Triggers conversion of primed to activated Mϕ	42, 53, 54
α-2 macroglobulin-protease complex	All stages	(*a*) Not regulated quantitatively between stages (*b*) Inhibits protease secretion including plasminogen activator and cytolytic proteinase (*c*) Blocks tumor cytolysis	42, 53
Lactoferrin receptor	All stages	(*a*) Not regulated quantitatively between stages (*b*) Inhibits Mϕ colony formation by blocking secretion of CSF from Mϕ	42, 56
F4/80 antigen	Resident and responsive Mϕ	(*a*) ↓ expression in primed Mϕ in response to T lymphocyte–derived LK MAF	44, 50, 51
ACM.1 antigen	Primed and activated Mϕ	(*a*) Qualitative marker of primed and activated Mϕ-regulatory signals unknown (*b*) Antibody does not block cytolytic function	52

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Table 2 Macrophage capacities and their regulation (continued)

Capacity	Expression at stage of activation$_{MTC}$	Regulation and consequences	References
M99 antigen	Resident and responsive Mϕ	(*a*) Qualitative marker of resident, responsive Mϕ-regulatory signals not known (*b*) Relationship to other, similar markers unknown	52
Secretory capacities			
Lysozyme	All stages	(*a*) Constitutive secretion (*b*) Not retained in cells	67
Neutral proteases Elastase Collagenase Plasminogen activator	Responsive, primed, and activated Mϕ	(*a*) ↑ quantitatively coincident with conversion from resident to responsive Mϕ, signal unknown (*b*) Ligands for acetylated LDL receptor trigger ↑ secretion	14, 23, 54
Cytolytic proteinase	Primed and activated Mϕ only	(*a*) Actual secretion is qualitative and quantitative marker of activated Mϕ (*b*) T cell–derived LK primes for secretion (MAF, γIFN) (*c*) Secretion triggered by LPS or by ligands for acetylated LDL receptor (*d*) Mediates selective cytolysis of tumor cells	3, 54
Arachidonic acid metabolites	All stages	(*a*) Substantial qualitative and quantitative variation in metabolites between Mϕ in different stages (*b*) Resident and responsive Mϕ have high total capacity and largest variety of metabolites. Mϕ primed or activated in vivo have ↓ total secretion and make only PGE_2 (*c*) Regulatory signals unknown	11, 19

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Reactive oxygen secretion	Some responsive, primed, and fully activated Mϕ	(*a*) Mild increase in secretory capacity in responsive Mϕ. Some eliciting agents induce substantial capacity (e.g. casein) (*b*) 2–3 day treatment with IFN_{γ} induces increased capacity (*c*) NADPH oxidase altered in Mϕ activated for O_2^- generation (*d*) Secretion regulated both by total capacity and triggering efficiency (i.e. triggering agents vary in efficacy)	77, 79, 80, 81, 82
Complement components	All stages	(*a*) Qualitative and quantitative variation in capacity among different stages (*b*) ↑ secretion in Mϕ *activated in vivo by chronic infection* (*c*) ↑ secretion by sterile inflammatory agents in vivo (*d*) Some ↑ in vitro with *LPS and adrenergic agonists*	83, 84
Monokines			
Lymphocyte activating factor (endogenous pyrogen) Interferon_C	All stages	(*a*) ↑ secretion seen in response to a large variety of exogenous signals (i.e. viruses, bacteria, fungi, lymphokines, polynucleotides, other pharmacologic agents) (*b*) Loosely regulated—common Mϕ function	85, 86, 87

Annual Reviews

(29), the additional energy required for this function apparently derives from glycolysis. ATP generated glycolytically is stored in a large pool of creatine phosphate, which provides the immediate energy source for mobility-dependent functions (29).

Cell-Surface Capacities

The plasma membrane of Mϕ contains a large number and variety of proteins, glycoproteins, and glycolipids cardinal to their regulation and effector functions. Over 30 specific receptors, for example, are now documented (20). Of particular importance, expression of many of these components is extensively revised during Mϕ development [for review, see (21)].

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RECEPTORS INVOLVED IN PHAGOCYTOSIS The most thoroughly studied Mϕ cell-surface capacities are those associated with phagocytosis. At least three categories of externally oriented receptors in the plasma membrane have been clearly implicated in the capacity to ingest particles bearing appropriate recognition structures: (*a*) receptors for the Fc regions of various immunoglobulins; (*b*) receptors for the third component of complement; and (*c*) the receptor for mannose/fucose/N-acetylglucosamine-terminated glycoproteins. In addition to participation in phagocytic function, these receptors can also trigger a variety of responses, including secretion of prostaglandins, lysosomal hydrolases, and reactive oxygen intermediates (ROI).

Receptors for Fc Because study of the structure and function of Fc receptors is an exceptionally large field (30), our discussion focuses on changes in Fc receptors during Mϕ development. Peritoneal Mϕ express at least two major classes of Fc receptor: (*a*) FcRI, which recognize the γ2a isotype of IgG and (*b*) FcRII, which recognize the γ1 and γ2b isotypes (30). In addition, substantial evidence indicates the existence of specific binding sites for the γ3 isotype of IgG and for IgE (31,32). FcRI and FcRII may be coupled to different signal-transduction mechanisms: FcRI occupancy is reported to enhance phospholipase A_2 activity and the related arachidonic acid cascade, while FcRII occupancy may mediate elevation of intracellular cyclic AMP (33). These observations imply that the function of specific classes of Fc receptors may be an important focus for regulatory control. Furthermore, a variety of studies have shown that the number of FcRI and FcRII copies per Mϕ can vary with the stage and type of maturation achieved in vivo and that this variation can be reproduced in vitro by exposure to preparations of β or γ interferon (34,35). Specifically, the number of FcRI and FcRII increases in inflammatory as opposed to resident peritoneal Mϕ, while the number of FcRI increases and the number of FcRII decreases in BCG-elicited Mϕ (21). Too, the number of FcRI appears

Annual Reviews

to be more readily altered than the number of FcRII (based on observations in vitro). Thus, the Mϕ functional capacities mediated by Fc receptors can be regulated both by the nature of the immunoglobulin class involved and by exposure of the Mϕ to extracellular signals.

Receptors for complement At least two cell-surface receptors on Mϕ recognize the third component of complement and fragments thereof: CR1 and CR3 (36). These receptors appear to recognize different portions of the C3 molecule, but substantial cross-reactivity has been observed in their interaction with C3b and C3bi (36). This confusing area has been clarified by the use of monoclonal antibodies that specifically block function of CR3 (37). Mϕ in all stages of development have the capacity to bind C3-coated particles, though it has been reported that expression of CR3 is lost by Mϕ elicited with inflammatory stimuli (38). More importantly, the function of CR1 (and possibly that of CR3) is substantially altered during development. Resident Mϕ bind but do not ingest C3b- and C3bi-coated particles, while inflammatory Mϕ in further stages of development bind and phagocytize via these receptors (for details, see the section on activation for other functions, below).

Mononuclear phagocytes also bear receptors for the C5a-derived anaphylatoxin, a 74-residue glycoprotein cleaved from C5 during complement activation (39). The number of receptors for C5a can vary widely among different populations of Mϕ, such that resident peritoneal Mϕ express 4–5 times the number of receptors seen on inflammatory Mϕ. Engagement of this receptor induces secretion of interleukin-1 and initiates chemotaxis (39,40).

Receptor for mannose-fucose Several laboratories have now described a binding site on peritoneal Mϕ that recognizes glycoproteins having terminal mannose, N-acetylglucosamine, or fucose residues (41,42). While the function of this receptor remains uncertain, it can provide an additional means of binding and phagocytosing certain microbial elements (43). Ligands for the receptor competitively inhibit the binding and ingestion of zymosan particles by inflammatory Mϕ (43). Surprisingly, however, the number of receptor sites is substantially diminished in Mϕ in the later stages of activation (42,44), and this downshift in the mannose receptor can be achieved by exposure in vitro to MAF (44).

RECEPTORS/PROTEINS MEDIATING NONPHAGOCYTIC FUNCTIONS

Ia antigen The expression of class II histocompatibility antigens by mononuclear phagocytes has been clearly associated with the presentation of antigen by Mϕ to thymus-derived lymphocytes (45). Beller, Unanue and colleagues, in a series of elegant studies, have established that expression of Ia antigens

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Annual Reviews

is tightly regulated in response to several exogenous signals (46). LK induce expression of Ia, while prostaglandin E_2, perhaps secreted by Mϕ, reduces expression (46).

Receptor for transferrin In recent studies from this laboratory, we have identified and characterized a Mϕ cell-surface receptor for the iron-carrying glycoprotein transferrin (47). Expression of the transferrin receptor is markedly elevated in monocytes and Mϕ taken from sites of sterile inflammation; resident Mϕ and Mϕ from sites of immunologically mediated inflammation have 3–10-fold fewer binding sites (47; T. A. Hamilton, D. O. Adams, unpublished). Down-regulation of this receptor can be readily induced in vitro in response to physiologic concentrations of MAF or recombinant IFNγ. The downshift in expression in response to discrete signals thus provides a valuable tool for elucidating the mechanism of action of IFNγ on responsive Mϕ.

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Binding site for tumor cells Activated Mϕ selectively bind tumor cells to their surface, and this capacity is an essential part of cytolytic function (see section V, below, for details). Although the biochemical determinants of enhanced tumor binding have not yet been identified, several lines of evidence indicate participation of a trypsin-sensitive cell-surface binding site, partaking many characteristics of a receptor (25). The requirement for metabolic energy on the part of the Mϕ in binding tumor cells does, however, distinguish this capacity from the simple interaction between receptor and a soluble ligand.

5′Nucleotidase The ectoenzyme 5′nucleotidase has been extensively characterized as a marker of Mϕ development (49). While resident peritoneal Mϕ exhibit high levels of the enzyme, content is dramatically reduced in responsive, primed, and fully activated Mϕ. The decrease has been attributed to increased membrane recycling and to decreased synthesis of the enzyme. There is an inverse correlation between the expression of 5′nucleotidase and the inducibility of Ia antigen by exposure to MAF (see 46). Thus, activity of this enzyme may be strictly regulated, not in response to MAF but perhaps by other signals that lead simultaneously to increased MAF responsiveness. The role of 5′nucleotidase in the cells' economy and the functional significance of its down-regulation in mature Mϕ remain to be established.

Other surface antigens Numerous attempts have been made to generate monoclonal antibodies that distinguish specific cell-surface molecules on Mϕ in different states of development [for review, see (37)]. To date, only three such monoclonal antibodies (Mab) have been produced. First, expression of the F4/80 antigen, a glycoprotein of Mr ~ 160,000, is diminished on Mϕ in the later stages of activation (44). Mϕ, primed or activated, have less than 20% of the levels expressed by resident or responsive Mϕ (44). This change could be

Annual Reviews

induced in vitro by treatment with crude LK preparations. In guinea pigs, a major surface glycoprotein of Mr ~ 160,000 (gp160) is similarly down-regulated in activated Mϕ (50). The downshift of gp160 may result from proteolytic cleavage of gp160 into two fragments of Mr 78,000 and 82,000, both of which remain in the plasma membrane (51). While the functional consequence of this change is obscure, it does document a specific biochemical change associated with activation that is not apparently mediated by altered gene expression. Second, AcM.1 distinguished Mϕ in an activated stage (elicited in vivo with pyran copolymer or *P. acnes*) from resident Mϕ or Mϕ elicited with sterile inflammatory agents like thioglycollate broth or proteose-peptone (52). Third, MM9 binds to resident and responsive Mϕ but not to activated$_{MTC}$ Mϕ (52). While AcM.1 could eliminate cells responsible for mediating MTC following treatment with complement, the antibodies were unable to block cytolytic function (52). Expression of any of these antigens has not been associated to date with specific functions.

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SURFACE RECEPTORS INVOLVED IN REGULATION

Receptor for acetylated proteins and lipoproteins Mϕ have a receptor that binds acetylated/maleylated proteins and lipoproteins (53). While this receptor is not altered as Mϕ proceed down the activation$_{MTC}$ cascade (42), its engagement stimulates secretion of neutral proteases, including plasminogen activator and cytolytic proteinase (CP) from primed Mϕ (54). The potential role of this receptor in Mϕ participation in atherogenesis has recently been reviewed (53).

Receptor for α2 macroglobulin The ability of Mϕ to bind and ingest α2 macroglobulin-protease complexes can serve a scavenging function by eliminating excess protease activity from inflammatory sites. Expression of the receptor remains constant throughout development of activation (42). In contrast to the acetylated lipoprotein receptor, ligand binding to the α-2 macroglobulin receptor shuts off secretion of neutral proteases such as plasminogen activator, CP, and other mixed proteases (54). This receptor could thus serve an autoregulatory role by feedback inhibition of protease secretion, when substantial amounts of these enzymes are in the Mϕ microenvironment.

Receptor for formylated peptides Mononuclear phagocytes of many species, though not of mice, bear receptors for N-formylated peptides (55). Binding and induction of biological activity by these compounds are coordinately and tightly linked to the structure of their three terminal amino acids; N-fMet-Leu-Phe is the most potent tripeptide yet synthesized (56). The number of such receptors is similar on many types of Mϕ obtained in vivo (55,57), though expression de novo of the receptor can be induced in the U937 human Mϕ-like cell line by exposure to LK (58). Engagement of these receptors can stimulate the respiratory burst and discharge of lysosomal enzymes or can

Annual Reviews

initiate chemotaxis (55). Induction of secretory function versus chemotaxis may be regulated by the affinity of the receptor population (see Snyderman & Pike in this volume for detailed review) (55).

Receptor for Mϕ growth factor Colony-stimulating factor, specific for Mϕ development (CSF-1), binds to a specific, high-affinity receptor on the Mϕ cell surface (59). This receptor not only initiates proliferation but also induces morphologic changes such as spreading in mature Mϕ and differentiation along the monocyte-Mϕ lineage in immature myeloid stem cells (60). Essentially all Mϕ populations contain some cells capable of proliferative response to CSF-1, though the relative proportion varies substantially (61). Inflammatory or responsive Mϕ exhibit the greatest proliferative response to CSF-1, while resident and fully activated Mϕ populations are much less responsive. Nevertheless, all Mϕ populations exhibit substantial morphologic changes in response to CSF-1, indicating the presence of functioning receptors (T. A. Hamilton, unpublished observations). Exposure of inflammatory Mϕ to ng levels of LPS can down-regulate the CSF-1 receptor (63). Thus, expression of this receptor may be constitutive, but its degree of expression appears to be under regulatory control.

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Receptor(s) for other lymphokines Analysis of putative receptors for lymphokines such as MAF and MIF has proven difficult, because these compounds have not yet been purified to homogeneity in large quantity. A membrane glycolipid with properties of the putative MIF receptor has recently been isolated (64). Too, a receptor for IFNγ, which appears to be one potent MAF (see section on activation for tumor cytolysis, below), has now been identified on fibroblasts (65). Thus, information on the display of these putative receptors by various populations of Mϕ may be shortly forthcoming and will be of particular interest.

Receptor for lactoferrin The receptor for lactoferrin on mononuclear phagocytes does not vary among Mϕ in different stages of tumoricidal activation (42). However, lactoferrin, a secretory product of neutrophils, does inhibit colony formation in Mϕ cultures, apparently by inhibiting secretion of colony-stimulating factor (66).

Secretory Capacities

ENZYMES

Lysosomal hydrolases The selective release of lysosomal acid hydrolases by mononuclear phagocytes occurs in response to numerous exogenous stimuli and has been extensively reviewed (23). Phagocytic particles can stimulate enzyme release, as can LK preparations containing MAF.

Annual Reviews

Lysozyme Lysozyme, which mediates digestion of bacterial cell walls, is a well-documented secretory product of Mϕ. Unlike lysosomal hydrolases, which are largely retained within the Mϕ and only released upon appropriate stimulation, lysozyme is secreted continuously (67). Furthermore, essentially all populations of Mϕ produce and secrete large amounts of lysozyme (67).

Neutral proteases A third category of digestive enzymes secreted by Mϕ are the neutral proteases, including collagenase, elastase, plasminogen activator, and a CP. Unlike the enzymes discussed previously, secretion of the neutral proteases is closely and differentially regulated during activation (68). While resident macrophages are poor secretors of most proteases, inflammatory Mϕ secrete substantial amounts. In the case of both collagenase and plasminogen activator, low secretory cells have very low levels of intracellular enzyme, suggesting appropriate signals induce both synthesis and secretion of these products.

Release of neutral proteases, like many other secretory products of Mϕ (see Table 3), is regulated in two steps: (*a*) An initial signal prepares or primes the Mϕ for secretion; and (*b*) a subsequent signal triggers actual release of the product in question (68). Priming for neutral protease secretion can be induced by LK such as MAF. Among the other molecules that prime are proteases themselves and endotoxin in vivo. Secretion by primed Mϕ can be variously triggered, depending on the protease, by ligands that bind to the receptor for acetylated proteins, endotoxin, or phagocytic challenge (68,54). Of interest, secretion of this same set of neutral proteases can be shut off by binding of α2 macroglobulin-trypsin complexes to their specific receptor (54). This effect was most readily observed in fully activated Mϕ where protease secretion was highest, yet could also be observed in inflammatory and primed Mϕ as well.

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Table 3 Effect of various signals on regulation of various macrophage secretory capacities[a]

Secretory product	Priming signal(s)	Effect of priming signals	Triggering signals
Arachidonate metabolites	BCG or *C. parvum* in vivo	↓ capacity	Zymosan, immune complexes
Reactive oxygen species (O_2^-, H_2O_2)	LK, IFNγ in vitro; BCG or *C. parvum* in vivo	↑ capacity	PMA, zymosan, immune complexes
Plasminogen activator	LPS in vivo	↑ capacity	Phagocytic challenge; LPS
Cytolytic proteinase	LK or IFNγ in vitro; pyran copolymer in vivo	↑ capacity	LPS, maleyl BSA, tumor cell binding

[a]Data from references 14, 19, 28, 54, 68, 75, 77, and 92.

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ARACHIDONIC ACID METABOLITES Mφ are a major source of these products and, by consideration of their importance in homeostasis, it is clear that their release constitutes an important aspect of function [for review, see (14)]. Of particular note, prostaglandins of the E series can play an autoregulatory role by limiting expression of certain capacities in Mφ necessary for several types of tumor cytolysis (14). The biochemical and enzymatic pathways involved in synthesis of the various arachidonic acid products have been extensively studied [for review, see (69,70)]. Metabolites of arachidonic acid produced by murine Mφ include prostacyclin (PGI_2), thromboxane B_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, leukotriene C_4 (slow reacting substance of anaphylaxis), and leukotriene B_4, a potent mediator of chemotaxis (19). Human monocytes show a somewhat more complex pattern of release (11). Release is triggered by engagement of a variety of receptors via stimulants such as zymosan and antigen-antibody complexes.

The enzymes responsible for metabolism of arachidonic acid are obvious targets for external regulation of this capacity (69,70). First, availability of arachidonic acid for metabolism depends on phospholipase A_2, which cleaves arachidonic acid from its stored form in the cell's pool of neutral phospholipid. Release and metabolism of arachidonic acid depend upon availability of Ca^{2+}, which is presumably required for the Ca^{2+}-dependent phospholipase A_2. Second, levels of cyclooxygenase and lipooxygenase control the pattern of metabolism for the released arachidonic acid. Third, tumor-promoting phorbol diesters, including phorbol 12,13 myristate acetate (PMA), stimulate secretion (71). In light of recent results from several laboratories indicating PMA acts by stimulating a phospholipid, Ca^{2+}-dependent protein kinase (PK_c) (72,73), this enzyme may also be involved.

Resident or inflammatory Mφ (elicited with proteose-peptone) have the highest total secretory capacity (using zymosan as a stimulant) and make the largest number of individual products (19). Mφ from animals treated with BCG or *P. acnes* release ~ 20% of the level produced by resident/inflammatory Mφ and have a greatly diminished capacity to produce all products but prostaglandin E_2. Similar changes, however, could not be reproduced in vitro with LK (19). Secretion of PGE_2 is enhanced in guinea-pig Mφ immunologically activated in vivo (74). Thus, Mφ from immunologically stimulated animals have altered arachidonate metabolism, but factors in addition to LK are apparently involved in regulating these changes.

REACTIVE OXYGEN INTERMEDIATES Secretion of reactive oxygen species (e.g. O_2^-, H_2O_2, OH·), like arachidonic acid metabolism, is a highly complex capacity, which is still only poorly understood at the molecular level (28). Based strictly on a priori considerations, it would appear to depend upon at least three subcapacities: (*a*) number or affinity of receptors for the triggering ligand; (*b*) levels of the oxidase complex needed to produce O_2^- and other

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reactive oxygen intermediates (ROI), and (*c*) efficiency of coupling between the receptors and the oxidases. Engagement of Fc receptors, complement receptors, and receptors for mannose terminal glycoproteins can stimulate an oxidative burst (28). PMA also potently stimulates the oxidative burst (75), presumably by bypassing the need for receptor-ligand coupling and directly stimulating PK_c. The principal enzyme that generates reactive oxygen species is a NADPH oxidase located, at least in its active form, principally at or near the plasma membrane. Associated with the complex is a novel cytochrome (b_{-245}), which is missing or nonfunctional in some forms of chronic granulomatous disease (76).

Capacity for the oxidative burst is clearly enhanced in Mϕ developed beyond the resident stage (75). Specifically, Mϕ activated_{MTC} in vivo by BCG or *P. acnes* produce more ROI in response to PMA than do Mϕ in earlier stages of activation. LK, specifically IFNγ, can potently induce capacity to generate increased amounts of ROI in response to PMA (77). However, induction of this capacity does not correlate precisely with induction of tumoricidal function [for review, see (3)]. For example, certain inflammatory Mϕ that do not exhibit activation_{MTC} have substantial ROI secretory capacity (101). The time course of LK-mediated induction of cytolytic function also differs significantly from induction for pharmacologically mediated release of H_2O_2 [see (3)]. Finally, Mϕ from different inbred strains of mice acquire and maintain H_2O_2 secretory capacity independent of tumoricidal function (79).

Generation of O_2^- and other ROI by external stimuli requires the activation of a NADPH oxidase, as unstimulated macrophages have no detectable activity of this enzyme (80,81). The rapidity of enzyme activation (within seconds in some instances) essentially rules out its synthesis de novo (81). Activatability of the enzyme is also induced rapidly (5–20 min) after application of appropriate activating signals, suggesting that de novo enzyme synthesis is not required for increased capacity to release ROI (82). When resident Mϕ and inflammatory Mϕ which had a 10-fold greater secretory capacity for O_2^- were analyzed, a 2–3-fold increase in oxidase activity was observed in the inflammatory Mϕ (80). This increase was accounted for by altered kinetic parameters of the oxidase (i.e. decreased K_m and increased V_{max}) plus elevated cellular NADPH levels. These and other observations indicate that Mϕ activation may lead to modification of existing oxidase molecules rather than to generation of new ones.

OTHER SECRETORY PRODUCTS

Complement components Mϕ secrete multiple components of the complement system, including members of both classical and alternative pathways (83). These include C1q, C2, C4, C3, C5, factor B, factor D, properdin, C3b INA (I), and β1H (H). Elevated complement secretion can be induced in various populations of Mϕ by host infection with *Listeria monocytogenes* or

Annual Reviews

BCG, while sterile inflammatory agents can also increase production of complement in vitro. Cholinergic and adrenergic receptor agonists stimulate synthesis and secretion following in vitro exposure (84).

Interleukin 1 (IL-1, lymphocyte activating factor or endogenous pyrogen) Mononuclear phagocytes are a major source of IL-1 and release it in response to a wide variety of stimuli including viruses, bacterial cells, fungi and fungal products, mycobacteria, spirochetes, mycoplasmas, lymphokines, polynucleotides, and other pharmacologic agents (85,86). IL-1 appears to be a central mediator of the Mϕ's role in lymphocyte stimulation, in induction of the febrile response, and potentially in the muscle wasting associated with fever (85,86).

Interferon Mononuclear phagocytes are significant producers of interferon, class I (β) (87). IFN secretion by Mϕ can be stimulated by the same wide range of substances that induce secretion of IL-1, including viruses, microbial agents, and LK.

Regulation of Capacities

Several general concepts govern regulation of Mϕ capacities. First is the inducing signal, and this must be considered from several vantage points. (*a*) It is often difficult to identify precisely which signal governs a particular capacity. Mϕ obtained from sites of inflammation in vivo have been exposed to a wide variety of signals, including proteases, complement components, antibodies and immune complexes, phagocytosed particles, LK, and the complex inflammatory stimulant itself (e.g. thioglycollate broth, mycobacteria) (3). Furthermore, even studies conducted in vitro have often employed only crude supernatants as sources of MAF and other LK. Thus, conclusions regarding the exact nature of regulatory signals must be viewed with caution. (*b*) The quantity of a signal applied also represents an important determinant, since the same signal may have different effects when used at different concentrations. (*c*) The capacity of a particular population of Mϕ to respond to a particular signal will depend upon the history of the Mϕ themselves. (*d*) The same signal can affect different capacities in different ways (see Table 3). (*e*) Kinetics play a major role in determining the response to a given signal. Acquisition of cytolytic competence manifested by acquisition of capacity to secrete CP and capacity to bind tumor cells requires from 6–8 hr following a minimal 2-hr pulse of MAF (88). After this treatment, the capacity to secrete CP decays within 24 hr, even in continued presence of the initiating signals, while capacity to bind tumor cells is retained substantially longer (89). As a consequence of the same brief LK pulse, enhanced expression of Ia antigen is also induced but requires 72 hr or longer for full expression (45). During this same time interval, the capacity to generate H_2O_2 is also being induced but requires continual exposure to MAF (90). Thus, exposure to MAF initiates

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a complex and tightly controlled sequence of events, varying significantly in kinetics of induction, exposure, and maintenance.

Although beyond the scope of this chapter, it should be emphasized that important factors in regulating Mϕ development and function are secretory products released from the Mϕ themselves [for review, see (14)]. Complement components, ROI, interferons, coagulation products, fibronectin, prostaglandins, leukotrienes, antiproteases, and proteases, all secreted by Mϕ, are significant regulators of Mϕ function—either directly or indirectly via interactions with other cells or molecules. These potent regulatory molecules can impinge upon Mϕ to initiate many complex positive and negative feedback loops. As just one example, the increased release of complement constituents by immunologically activated Mϕ can lead to generation of C3b, which in turn can bind to its receptor and initiate release of prostaglandins that can dampen lytic functions of the activated Mϕ (14).

An important concept that hinders easy understanding of regulation is the heterogeneity of Mϕ within any given population [for reviews, see (3,8)]. Individual capacities are not necessarily expressed uniformly by all cells in a given population of Mϕ. Capacities known to exhibit heterogeneity include Fc receptor–mediated functions (PHG and ADCC), marker enzymes, Ia antigen expression, complement receptor expression (CR3), and antitumor function (both direct cytotoxicity and cytostasis). While there is little evidence to indicate that such heterogeneity derives from populations of Mϕ exhibiting distinct differentiation patterns (7), the expression of heterogeneity may nevertheless contribute to variable function in a particular experiment.

To date we lack quantitative biochemical information on the molecular bases of the particular Mϕ capacities, the discrete signals that induce and suppress the expression of these capacities, and the biological mechanisms that transduce these signals into appropriate cellular alterations in metabolism. One important regulatory focus, however, has been identified as the receptor. Obviously, the number of a given receptor profoundly affects the outcome between a population of Mϕ and a given concentration of that ligand. An important new concept is that a shift in a receptor's affinity can alter the function induced by ligand occupancy [for review, see (55)].

V. MACROPHAGE FUNCTIONS AND THEIR REGULATION

Activation for Tumor Cytolysis

Mϕ, when appropriately activated, selectively and efficiently lyse neoplastic cells in a contact-dependent, nonphagocytic process requiring several days (3). This has been termed Mϕ-mediated tumor cytotoxicity (MTC). Completion of MTC is a multistep process encompassing: (*a*) binding of tumor cells by the activated Mϕ; and (*b*) secretion by the Mϕ of lytic mediators, including

Annual Reviews

a novel cytolytic protease (CP). Multiple lines of evidence indicate that each step is necessary but not sufficient for completion of cytolysis, so that target lysis by activated Mϕ in MTC ultimately requires expression of selective target binding and secretion of CP [for review, see (3)].

The binding of cells by Mϕ is of two sorts: (*a*) a rapid, weak, and nonselective binding observed between all cell pairs such as inflammatory macrophages and tumor cells or activated Mϕ and lymphocytes; and (*b*) a slower, strong, and selective binding between activated Mϕ and tumor cells only [for review, see (92)]. The selective, as opposed to the nonselective, binding requires trypsin-sensitive structures on the Mϕ and is inhibited by colchicine, vinblastine, and cold. Multiple lines of evidence suggest this binding is mediated by receptor(s) on the activated Mϕ; the recognition structure(s) on the tumor cells is contained within the plasma membrane and appears to be shared by at least three murine tumors of disparate origin. Initiation of selective binding, which triggers more extensive and rapid release of CP, has been suggested to mediate target recognition in MTC as well as to provide a space between Mϕ and targets that concentrates and protects lytic mediators. The extent of both selective and nonselective binding can be precisely quantified by use of radioactively labeled tumor cells.

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The target injury step is inhibited in a variety of ways that suggest it involves secretory release by the activated Mϕ (3). Mononuclear phagocytes secrete a wide variety of lytic products [for review, see (93)]. To date, the role of each of these toxic products in MTC against adverse types of tumor cells remains to be established precisely. Multiple lines of evidence, however, indicate the novel enzyme CP plays a major role (92). CP, a neutral serine protease of Mr ~40,000, is secreted only by activated Mϕ; its lytic potency is high (the lytic concentration 50% is 10^{-9}M) and directed principally against neoplastic cells. The amount of CP secreted by a given number of Mϕ can be precisely quantified in lytic units by probit analysis of cytolytic dose curves. Of interest, pretreatment of tumor cells with extremely small concentrations of H_2O_2 ($\sim 10^{-6}$M) synergistically potentiates the lytic effects of CP, indicating that interaction between disparate mediators of cytolysis may occur in MTC [see (93)].

Murine Mϕ acquire activation MTC in a series of stages that are defined operationally by their requirements for and responsiveness to the signals needed to induce full cytolytic competence (16,17,18) (Table 4). Young mononuclear phagocytes, though not resident peritoneal Mϕ, respond well to treatment with LK by gaining sensitivity to a second signal such as endotoxin. The LK-treated Mϕ, termed primed Mϕ, are not cytolytic but become so when exposed to very small amounts of a second signal (i.e. ng of endotoxin per ml).

Considerable progress has been made recently on molecular identification of the signals regulating activation$_{MTC}$. The functional effect in crude LK

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Table 4 Stages in activation$_{MTC}$ of murine Mϕ[a]

	Resident Mϕ	Responsive Mϕ	Primed Mϕ	Activated Mϕ
Operational definition	Do not respond to lymphokine or endotoxin by becoming cytolytic	Respond to lymphokine followed by endotoxin by becoming cytolytic	Respond to endotoxin by becoming cytolytic	Fully cytolytic
Capacity for selective binding of tumor cells			++++	++++
Prepared to secrete cytolytic protease			++++	++++
Secrete cytolytic protease				++++
Kill tumor cells in MTC				++++

[a]Data from (92,136).

containing supernatants that induce priming has been termed MAF and has generally been associated with protein of Mr ~40,000 (16). Recently, biochemical and immunological techniques have strongly indicated this activity is IFNγ (94). Although receptors for interferons have been demonstrated on other cell types, their existence on mononuclear phagocytes remains to be established formally. The molecular relationship, if any, of a molecule of Mr ~22,000 secreted by a T-cell line also remains to be established (95). Endotoxin is the most widely used triggering signal, and its active part appears to be the lipid A portion (96), consistent with its putative action via membranous intercalation rather than with a surface receptor (97). Maleylated proteins, acting via the receptor for acetylated proteins, also serve as triggers (54). The signals that induce such activation relate tightly to the signals that induce the two capacities necessary for completing MTC (i.e. the capacity for selective binding and the capacity for secretion of CP) (92). Capacity for selective binding of tumor cells, expressed by primed and activated Mϕ, is induced by LK in vitro under the same conditions required to induce priming for cytolysis. Competence for secretion of CP, by contrast, is acquired in two stages: an initial signal (i.e. LK) prepares the Mϕ for secretion and a second signal (i.e. traces of endotoxin) triggers actual release of the enzyme.

Annual Reviews

The existence of inbred strains of mice whose Mϕ cannot be activated for MTC permits a critical test of several of these interpretations. Mϕ elicited by BCG in C3H/HeJ mice, which do not respond to endotoxin, do not normally secrete CP but do bind tumor cells selectively (98). Exposure of such Mϕ to maleylated proteins, which trigger the release of several proteases including CP from primed Mϕ taken from normal strains of mice, induces release of CP and concomitantly cytolysis of bound tumor cells (54). The data confirm the role of CP in MTC and point up the tight relationship between signals that govern activation and those that regulate the involved capacities.

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Activation for ADCC

The ability of mononuclear phagocytes to lyse antibody-coated erythrocytes has long been established, and the competence of Mϕ to lyse antibody-coated tumor cells has been established in the last several years [for reviews, see (99,100)]. The classic form of antibody-dependent cellular cytotoxicity (ADCC) by Mϕ, like that by neutrophils or K cells, is rapid and generally mediated by polyclonal antisera. In general, Mϕ must be activated for lysis of antibody coated cells. ADCC by activated Mϕ can be divided into two steps: (*a*) target recognition and binding; and (*b*) target cytolysis (101).

Binding of antibody-coated targets by Mϕ, which resembles that observed in MTC morphologically and in terms of strength of attachment, results from interaction between antibodies and Fc receptors (101). Various reports from several laboratories have indicated that either FcRI or FcRII are more important in ADCC (102,103). In one study employing a polyclonal, xenogeneic antiserum against the tumor targets, inhibition of either FcRI by trypsinization of the Mϕ or of FcRII by a Fab fragment of the 24G.2 monoclonal antibody inhibited target binding by ~50% (W. Johnson, E. Pure, D. O. Adams, unpublished). Inhibition of FcRI inhibited subsequent cytolysis by ~80%, while inhibition of FcRII reduced lysis by only ~20%. These data imply that both FcRI and FcRII can mediate ADCC but that the former may be more efficient. Establishment of the binding is rapid and proceeds at 4°C but is inhibited by cytochalasin B (101). This binding is necessary but not sufficient for cytolysis and is mediated equally well by inflammatory and activated Mϕ.

The lytic step in ADCC, inhibited by incubating Mϕ and bound targets at 23° or 4°C, is completed only by Mϕ activated for ADCC (101). The studies cited above imply a critical role for FcRI in this step. Since ADCC can be inhibited by monensins (105), a critical role for secretion in the lytic step is implied. Nathan and colleagues have adduced compelling evidence that H_2O_2 plays a major role in mediating lysis (106), though the possibility that other as yet unidentified mediators participate remains open [see (103)]. It is perhaps worth emphasizing that engagement of Fc receptors stimulates profuse release of H_2O_2 from Mϕ (28).

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Mϕ can readily be activated for the classic form of ADCC by activating them for MTC (101). Close inspection of the relationship between the two forms of activation, however, indicates that they are clearly separable by stability and by induction requirements (100). Too, Mϕ elicited by casein, which are responsive Mϕ in terms of activation MTC, are activated for classic ADCC (101). Depending on the target, other inflammatory Mϕ can also be activated for ADCC (W. J. Johnson, D. O. Adams, unpublished).

Evidence from numerous laboratories documents a particular potency of murine antibodies of the IgG2a isotype, generally in cooperation with host Mϕ, in mediating destruction of tumors (108,109,110,111). When the lytic interaction between Mϕ, tumor cells and antibodies (either polyclonal or monoclonal) of the IgG2a isotype is examined in vitro, extensive cytolysis in the presence of the antibodies (i.e. ADCC) can ensue (112). Profound differences, however, distinguish this novel ADCC (NAD) reaction from the classic form described above (113). Lysis of tumor cells by NAD requires 1–3 days, rather than the 4–6 hr or less in the classic form (113). The Mϕ $\text{activated}_{\text{NAD}}$ are either responsive or primed Mϕ but represent only selected subtypes of these Mϕ (i.e. they are elicited only by certain inflammatory or priming stimuli, whose common characteristics are not yet defined). Of particular interest, Mϕ $\text{activated}_{\text{NAD}}$ can be modulated in vitro to Mϕ activated $_{\text{MTC}}$ by application of LK plus endotoxin concomitantly. This treatment completely shuts off competence to mediate NAD. NAD is mediated principally by antibodies of the IgG2a isotype, either polyclonal or monoclonal; but certain conspicuous exceptions exist.

NAD, like classic ADCC, is a two-step reaction (113). Binding can be mediated by antibodies of either the IgG2a or IgG2b isotypes and by a wide variety of Mϕ. The lytic step appears to be critical, since it is mediated only by Mϕ activated $_{\text{NAD}}$ and depends upon FcRI. Since the lytic step can be inhibited by procedures which inhibit release of ROI (i.e. anaerobiosis or glucose deprivation), a role for these products in lysis is implied.

The precise signals regulating and the exact capacities required for classic ADCC and for NAD remain to be established. Indeed, several intriguing paradoxes must be resolved. As just one example, why do Mϕ $\text{activated}_{\text{CAD}}$ and Mϕ $\text{activated}_{\text{NAD}}$ differ so strikingly, when both appear to involve FcRI and release of ROI?

Activation for Microbial Destruction

The central role of activated mononuclear phagocytes in host protection against facultative and obligate intracellular bacterial invaders in vivo is extremely well established (114). Activated Mϕ are also important in vivo in the destruction of certain viruses and extracellular parasites (115,116). Until recently, however, a variety of technical problems have hindered precise quantification

Annual Reviews

of microbicidal function in vivo and hence delineation of its capacities and their regulation. Although these problems have now been overcome for the most part—principally by use of nonbacterial pathogens that do not replicate extracellularly and that can be precisely enumerated by microscopy, large gaps in our understanding of microbial destruction remain to be filled. It should be emphasized that Mϕ activation for microbicidal function actually encompasses multiple subtypes of activation. For example, experimental manipulations of various sorts can cleanly separate competence to kill toxoplasma from competence to kill tumor cells (117).

The mechanisms by which Mϕ recognize various microorganisms are presently the least understood part of microbicidal function, partially because the number of pertinent variables is so large. First, many parasites (e.g. schistosomes) are killed extracellularly after contact mediated by antibodies or by as yet undefined means (118); kill of such extracellular parasites in the presence of antibody has been likened to the ADCC reaction against tumor cells (119). Second, other invaders, opsonized with antibody and/or complement fragments or not, are endocytosed by the Mϕ via Fc, C3, mannose, and other as yet undefined surface receptors. Microbes, like other particles [for review, see (120)], are presumably bound at the Mϕ surface and then phagocytosed, after which they are contained in a membrane-bound compartment that is doubtless the endosome (121). From this point, the microorganisms may have several fates: (*a*) The microorganism may lyse the phagosomal membrane and enter the cytosol; (*b*) the microorganism may prevent the fusion of lysosomes with the phagosome, so that the ingested microbe remains within a simple phagosome; or (*c*) lysosomal fusion may occur to form phago-lysosomes (122,123). In addition to our general lack of understanding of the molecular rules governing the intracellular traffic of endosomes (121), various microbes have evolved means of actively determining the fate of this organelle [for review, see (124)]. The disparate systems of microbial recognition remain to be formally organized with respect to the microbe, its specific portal of entry, and its ultimate home in the Mϕ. This cellular decision is, however, critical because it has a profound impact on the fate of the ingested microbe. Microbes are generally killed after the formation of a phago-lysosome and the generation of a respiratory burst (125), though there are conspicuous exceptions to this principle. On the one hand, formation of a phago-lysosome and generation of ROI is not sufficient to kill all ingested microbes (125). On the other hand, it is possible that microbes residing in the cytosol are actually destroyed there and only subsequently come to reside in phago-lysosomes (126). At present, little available evidence indicates which, if any, of these mechanisms of recognition, endocytosis, and intracellular shuttling of phagocytosed organisms are altered in activated Mϕ.

A major antimicrobial mechanism of activated Mϕ is the production and intracellular release of reactive oxygen species, such as O_2^-, H_2O_2, and

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OH· (125,127,128). The ability of activated Mϕ to kill species of *Leishmania, Toxoplasma, Trypanosomes, Mycobacteria,* and *Candida* correlates very closely with their capacity for PMA-triggered release of H_2O_2 (125,128). Other lines of evidence also strongly argue for a central role of activated oxygen intermediates in destruction of these organisms [for reviews, see (125,128)]. The precise species of oxygen intermediates critical to kill remain to be established, but attention has been drawn to the importance of the peroxidase-H_2O_2-halide antimicrobial system and to generation of hydroxyl radical (OH·) by the Haber-Weiss reaction or by interaction with Fenton's reagent (iron) (127). Mϕ also may destroy microbes by nonoxidative means such as acidification of phagosomes, cationic peptides, interferon, lysozyme, complement components, and two novel cationic proteins, though the importance of each of these in various forms of activation for microbial kill remains to be established (128,129). In sum, one characteristic that is critical for microbial kill and that clearly marks Mϕ activated for destroying many diverse organisms is extent of pharmacologically mediated release of H_2O_2.

The induction of Mϕ activation for microbicidal function has now been elucidated in some detail. Activation for kill of rickettsia or leishmania, like activation for MTC, is a two-step reaction: (*a*) an initial priming by LK; and (*b*) a subsequent triggering by an additional signal such as traces of endotoxin (130,131). The two types of activation further resemble one another in kinetics of induction and maintenance and in the presence of similar defects in certain inbred strains of mice. The two types of activation, however, can be clearly distinguished from one another by disparate requirements for young mononuclear phagocytes from sites of inflammation to respond to the LK signal and by the requirement for the continuous presence of LK and targets to achieve maximum activation$_{MTC}$ (131). Finally, separation of crude LK by molecular sieving indicates that only one of the three fractions capable of inducing activation for intracellular kill of rickettsia and leishmania (LK of Mr of ~130,000, ~50,000, and ~10,000) is actually capable of inducing activation for extracellular kill of schistosomes and tumor cells (in an assay of MTC) (LK of Mr of ~50,000) (131). The molecular identity of these LK remains to be established, but IFNγ has recently been shown to induce the capacity for PMA-triggered release of H_2O_2 (77).

The capacities necessary for activation for microbicidal kill are now beginning to be defined in detail. It already appears, however, that there are multiple types of activation for the kill of different microbes and hence multiple critical capacities.

Activation for Other Functions

Phagocytosis is significantly increased in inflammatory as opposed to resident peritoneal Mϕ [for review, see (15)]. The increase in Fc-mediated PHG doubtless results, in part, from the ~6-fold increase in number of FcRI and the ~4-

fold increase in number of FcRII (30). Of note, inflammatory Mϕ also contain an increased intracellular pool of creatinine phosphate, which serves as a major source of energy for phagocytosis (29). The increased competence of inflammatory Mϕ to take up particles via C3 receptors is a different but particularly interesting story (132). Resident and inflammatory Mϕ alike bind complement-coated erythrocytes, but only the latter ingest them. As it turns out, complement receptors on resident Mϕ are not freely mobile in the plane of the plasma membrane and so cannot cluster about a bound particle to permit "zippering" and thus endocytosis. By contrast, the receptors are mobile in inflammatory Mϕ. Mobility of the complement receptors is closely and complexly regulated (132).

Mϕ can be activated for two other functions, which are reviewed in this volume (55 and 133) and are thus but briefly mentioned here. Mϕ from sites of inflammation are more chemotactically active than resident peritoneal Mϕ (133). A minimum number of receptors for chemotactic peptides (i.e. ~10,000 copies per cell) is required for effective chemotaxis to such peptides (58), but other as yet unidentified capacities are required as well (134). The processing of antigen by Mϕ offers a final example (135). This function requires uptake and partial digestion of the antigen and then its effective presentation to T lymphocytes via cell–cell interaction. The capacity for "processing" is displayed constitutively by numerous types of Mϕ (i.e. resident, inflammatory, etc). The capacity for cell–cell interaction requires expression of Ia on the Mϕ, which is closely regulated by LK and thus expressed only by Mϕ in the later stages of activation. It may be noted, however, that expression of Ia and priming for MTC are not synonymous and may even be mutually exclusive (136).

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VI. CONCLUSIONS AND PERSPECTIVES

Mϕ possess numerous physiologic properties that may be modulated during activation. These include alterations in intracellular constituents, plasma membrane components, and secretion. The expression of these capacities, regulated by numerous classes of signal molecules, is closely related to the numerous functions of Mϕ. Although the capacities now recognized are disparate in terms of their actual molecular definitions, they relate closely to one another in their operational ability to predict functional competence. Regulation of the various capacities is complex, and most can be down- as well as up-modulated. Thus, as Mϕ persist in the tissues and are exposed to various signals, some capacities are turned on and others turned off.

A purist's definition of Mϕ activation restricts use of the term to development of antimicrobial or antineoplastic function—or even to the former solely. We have employed a broader definition, based upon a reductionist analysis,

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in which activation represents increased competence to perform a complex function. The application of circuit modeling, in which performance of the function results from expression of certain requisite cellular and biochemical capacities, leads to a more precise definition: *Mϕ activation* is the result of appropriate modulation of specific capacities (i.e. either induced or diminished expression) such that functional competence is either increased or acquired where it did not previously exist.

Is this model of activation valid? Analysis of the many capacities of Mϕ (see Tables 1 and 2) makes several points in support of the model. Many of the capacities are regulated (both up and down) or are mediators of regulation through a diverse collection of extracellular signals. This same group of signals has clearly been associated with the induction or acquisition of complex functions. In extension of the concept, many of the defined capacities can be closely related to one or more complex functions. Perhaps the best example of this comes from analysis of $\text{activation}_{\text{MTC}}$, where the capacities are fully defined and where there is close agreement between expression of the defined capacities and lytic competence. Evidence from studies on other examples of functional activation are consistent with the model, but the full list of capacities and the signals that regulate them must be established before we can evaluate the concept completely.

The fundamental conception of Mϕ development in the tissues has been a linear progression of increasing expression of capacities and functions [for review, see (3)]. More recent models have suggested a linear progression, having the resident-tissue Mϕ set off to one side (Figure 1). We suggest a more complex situation may pertain (Figure 3). We surmise that the circulating monocyte represents the starting point of most Mϕ development outside the marrow. Coincident with its extravisation into the tissue spaces and in the absence of activating signals, the new tissue Mϕ is suppressed by local regulatory factors into a largely quiescent state, associated with a progressive loss of response to activating signals. In the presence of altered homeostasis such as inflammation, further development of the new tissue Mϕ will proceed variably in response to a variety of inductive signals. Some of the potential paths of activation are multistep and encompass a stepwise acquisition of functions; others have but one step. Too, some paths run parallel with one another, while some are mutually exclusive. We envision that most, if not all, of these states of activation are not only reversible but may actually be shut off by active suppressor signals, perhaps generated by the Mϕ themselves. This raises the intriguing possibility that an inductive signal that turns on activation also sets in motion the ultimate production of the suppressive signals that finally turn it off. We do not yet have the data to speculate on whether the Mϕ revert to their initial state or are blocked from all or certain forms of activation after their first form of activation is shut off. The evidence presently

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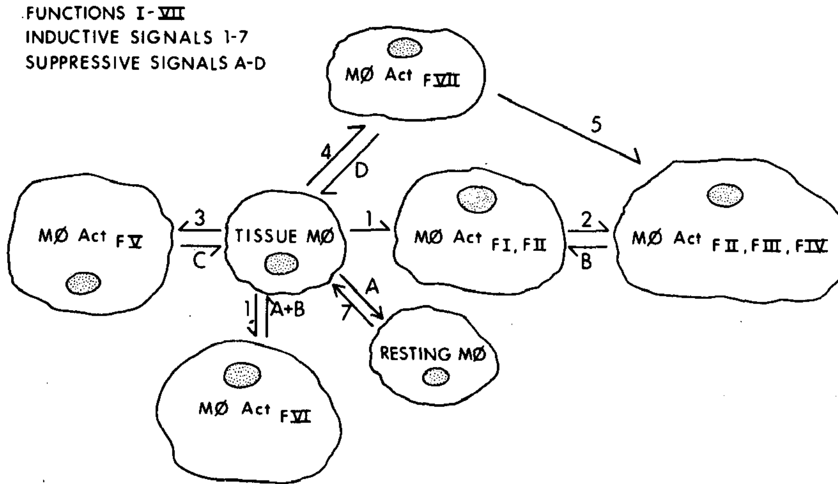

Figure 3 A speculative model of macrophage development. At center is a newly emigrated monocyte. Under suppressive signal A, it is down-regulated to a resident macrophage (this cell may be subsequently up-regulated). Depending on which inductive signal is received, the young tissue macrophage may be modulated in several ways and thus be activated for various functions. Most modulations are ultimately suppressed.

available in support of this overall model is of two sorts. First, the various capacities of Mϕ do not all develop synchronously but rather develop asynchronously and even disparately. Second, functional competence for spontaneous cytolysis and novel ADCC appear to be mutually exclusive (see Table 5, below). Critical testing of this model will thus be a major question over the next several years.

Table 5 Competence of Mϕ to mediate several functions by stage of activation$_{MTC}$[a]

	Resident Mϕ	Responsive Mϕ	Primed Mϕ	Activated$_{MTC}$ Mϕ
MTC	–	–	–	+ + + +
Cytostatis	–	–	–	+ + + +
Classic ADCC	–	+ + or – (depending on the target)	+ + or – (depending on the target)	+ + +
Novel ADCC	–	+ + + + or – (depending on the agent used to elicit the Mϕ)	+ + + + or – (depending on the agent used to elicit the Mϕ)	–
Chemotaxis	±	+ + +	+ + +	+ + +
Phagocytosis	±	+ + + +	+ + + +	+ +
Antigen presentation	–	–	+ + +	+ + +

[a]Data from (15, 45, 92, 99, 104, 107, 133, 135, 136).

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The study of Mϕ activation has now entered an exciting era, where this fascinating problem in cellular development and regulation can be addressed in terms of specific alterations in cellular and molecular biology. Of the broad range of questions that will be asked, we emphasize five. First, what is the molecular basis of each capacity? Second, what signals and signal transduction systems regulate each capacity? Third, what suppressor signals, particularly those derived from Mϕ, shut off activation? Fourth, how does the past history of a Mϕ govern its activation (i.e. what is the potential for activation of one type after the cell has been activated for another function)? Fifth, how much control, if any, of Mϕ development in the tissues is regulated in the genome and what are the mechanisms of control?

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Literature Cited

1. Metchnikoff, E. 1905. *Immunity to Infectious Diseases*. Cambridge/London/NY: Cambridge Univ. Press
2. Mackaness, G. B. 1970. The monocyte and cellular immunity. *Sem. Hematol.* 7:172–84
3. Adams, D. O., Marino, P. 1983. Activation of mononuclear phagocytes for destruction of tumor cells as a model for study of macrophage development. In *Contemporary Topics in Hematology-Oncology*, Vol. III, ed. A. S. Gordon, R. Silber, J. LoBue. NY: Plenum. In press
4. Steinman, R. M., Cohn, Z. A. 1974. The metabolism and physiology of the mononuclear phagocytes. In *The Inflammatory Process*, ed. B. W. Zweifach, L. Grant, R. T. McCluskey, 1:449–510. NY: Academic. 2nd ed.
5. Van Furth, R. 1980. Cells of the mononuclear phagocytes system. Nomenclature in terms of sites and conditions. In *Mononuclear Phagocytes. Functional Aspects*, ed. R. Van Furth, pp. 1–40. The Hague: Martinus-Nijhoff
6. Fischer, D. G., Hubbard, W. J., Koren, H. S. 1981. Tumor cell killing by freshly isolated peripheral blood monocytes. *Cell. Immunol.* 58:426
7. Gordon, S., Hirsch, S. Differentiation antigens and macrophage heterogeneity. In *Macrophages and Natural Killer Cells*, ed. S. J. Norman, E. Sorkin, pp. 391–400. NY: Plenum
8. Walker, W. S. 1976. Functional heterogeneity of macrophages. In *Immunobiology of the Macrophage*, ed. D. S. Nelson, pp. 91–110. NY: Academic
9. Beller, D. I., Ho, K. 1982. Regulation of macrophage populations: V. Evaluation of the control of macrophage IA expression *in vitro*. *J. Immunol.* 129:971
10. Deleted in proof
11. Pawlowski, N. A., Kaplan, G., Hamill, A. L., Cohn, Z. A., Scott, W. A. 1983. Arachidonic acid metabolism by human monocytes. Studies with platelet-depleted cultures. *J. Exp. Med* 158:393–412
12. Bersuker, I., Goldman, R. 1983. On the origin of macrophage heterogeneity: A hypothesis. *J. Reticuloendo. Soc. (JRES)* 33:207–20
13. Deleted in proof
14. Bonney, R. J., Davies, P. 1984. Possible autoregulatory functions of the secretory products of mononuclear phagocytes. *Contemp. Top. Immunobiol.* 14:199–223
15. Cohn, Z. A. 1978. The activation of mononuclear phagocytes: fact, fancy, and future. *J. Immunol.* 121:813–16
16. Meltzer, 1981. Tumoricidal cytotoxicity by lymphokine-activated macrophages: development of macrophage tumoricidal activity requires a sequence of reactions. *Lymphokines* 3:319–43.
17. Hibbs, J. B., Taintor, R. R., Chapman, H. A., Weinberg, J. B. 1977. Macrophage tumor killing: influence of the local environment. *Science* 197:279–82.
18. Russell, S. W., Doe, W. F., McIntosh, A. J. 1977. Functional characterization of a stable, noncytolytic stage of macrophage activation in tumors. *J. Exp. Med.* 146:1511
19. Scott, W. A., Pawlowski, N. A., Murry, H. W., Andreach, M., Zrike, J., Cohn,

Annu. Rev. Immunol. 1984.2:283-318. Downloaded from arjournals.annualreviews.org
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Z. A. 1982. The regulation of arachidonic acid metabolism by macrophage activation. *J. Exp. Med.* 155:1148–61

20. Nathan, C. F., Cohn, Z. A. Cellular components of inflammation: monocytes and macrophages. In *Textbook of Rheumatology*, ed. W. Kelly, E. Harris, S. Ruddy, R. Hedge. NY: W. B. Saunders. 2nd ed. In press
21. Ezekowitz, R. A. B., Gordon, S. 1984. Alterations in surface properties by macrophage activation. Expression of receptors for Fc and mannose terminal glycoproteins and differentiation antigens. *Contemp. Top. Immunobiol.* 14:33–56
22. Steinman, R. M., Mellman, I. S., Muller, W. A., Cohn, Z. A. 1983. Endocytosis and recycling of plasma membrane. *J. Cell. Biol.* 96:1–27
23. Page, R. C., Davies, P., Allison, A. C. 1978. The macrophage as a secretory cell. *Int. Rev. Cytol.* 52:119
24. Stossel, T. P. 1981. Actin filaments and secretion: The macrophage model. *Meth. Cell Biol.* 23:215–30
25. Somers, S. D., Mastin, J. P., Adams, D. O. 1983. The binding of tumor cells by murine mononuclear phagocytes can be divided into two, qualitatively distinct types. *J. Immunol.* 131:2086–93
26. Bhisey, A. N., Freed, J. J. 1971. Altered movement of endosomes in colchicine-treated macrophages. *Exp. Cell. Res.* 64:430–33
27. Axline, S. 1970. Functional biochemistry of the macrophages. *Sem. Hematol.* 7:142
28. Johnston, R. B., Jr. 1981. Enhancement of phagocytosis-associated oxidase metabolism as a manifestation of macrophage activation. *Lymphokines* 3:33–56
29. Loike, J. P., Kozler, V. F., Silverstein, S. C. 1979. Increased ATP and creatine phosphate turnover in phagocytosing mouse peritoneal macrophages. *J. Biol. Chem.* 254:9558
30. Unkeless, J. C., Fleit, H., Mellman, I. S. 1981. Structural aspects and heterogeneity of immunoglobin Fc receptors. *Adv. Immunol.* 31:247
31. Melewicz, F. M., Spiegelberg, H. L. 1980. Fc receptors for IgG on a subpopulation of human peripheral blood monocytes. *J. Immunol.* 125:1026
32. Diamond, B., Yelton, D. E. 1981. A new Fc receptor on mouse macrophages binding IgG_3. *J. Exp. Med.* 153:514–19
33. Nitta, T., Suzuki, T. 1982. Biochemical signals transmitted by Fcγ receptor. Triggering mechanisms of the increased synthesis of adenosine-3′5′-cyclic monophosphate mediated by $Fc_{\gamma 2a}$ and $Fc_{\gamma 2b}$ receptors of a murine macrophage-like cell line (P388 D1). *J. Immunol.* 129:2708–14
34. Vogel, S. N., Finbloom, D. S., English, K. E., Rosenstreich, D. L., Langreth, S. G. 1983. Interferon-induced enhancement of macrophage Fc receptor expression. B-interferon treatment of C3H/HeJ macrophages results in increased numbers and density of Fc receptor. *J. Immunol.* 130:1210
35. Rhodes, J., Jones, D. H., Bleehen, N. M. 1983. Increased expression of human monocyte HLA-DR antigens and Fc_γ receptors in response to human interferon *in vivo*. *Clin. Exp. Immunol.* 53:739–43
36. Rabellino, E. M., Ross, G. D., Polley, M. J. 1978. Membrane receptors of mouse leukocytes. I. Two types of complement receptors for different regions of C3. *J. Immunol.* 120:871–79.
37. Springer, T. A., Unkeless, J. C. 1984. Analysis of macrophage differentiation and function with monoclonal antibodies. *Contemp. Top. Immunobiol.* 14:1–31
38. Walker, W. S., Yen, S-E. 1982. Complement receptor phenotypes of culture-derived murine macrophages. *J. Cell Physiol.* 110:277–84
39. Goodman, M. G., Chenoweth, D. E., Weigle, W. D. 1982. Induction of interleukin 1 secretion and enhancement of humoral immunity by binding of human C5a to macrophage surface C5a receptors. *J. Exp. Med.* 156:912
40. Snyderman, R., Phillips, J. K., Mergenhagen, S. E. 1971. Biological activity of complement *in vivo*? Role of C5 in accumulation of polymorphonuclear leucocytes in inflammatory exudates. *J. Exp. Med.* 134:1131
41. Stahl, P., Schlesinger, P., Rodman, J. S., Doebber, T. 1976. Recognition of lysosomal glycosidases *in vivo* inhibited by modified glycoproteins. *Nature* 264:86–88.
42. Imber, M., Pizzo, S. V., Johnson, W. J., Adams, D. O. 1982. Selective diminution of the binding of mannose by murine macrophages in the later stages of activation. *J. Biol. Chem.* 257:5129–35
43. Sung, S-S. T., Nelson, R. S., Silverstein, S. C. 1983. Yeast mannans inhibit binding and phagocytosis of zymosan by mouse peritoneal macrophages. *J. Cell Biol.* 95:160–66
44. Ezekowitz, R. A. B., Gordon, S. 1982. Down-regulation of mannosyl receptor-mediated endocytosis and antigen F4/80 in bacillus calmette-guerin activated mouse macrophages. Role of T lymphocytes and lymphokines. *J. Exp. Med.* 155:1623
45. Unanue, R. 1981. The regulatory role of

Annual Reviews

Annu. Rev. Immunol. 1984.2:283-318. Downloaded from arjournals.annualreviews.org
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macrophages in antigenic stimulation. Part II: Symbiotic relationship between lymphocytes and macrophages. *Adv. Immunol.* 31:1

46. Beller, D. I., Unanue, E. R. 1982. Reciprocal regulation of macrophage and T cell function by way of soluble mediators. In *Lymphokines*, ed. S. Mizel, 6:25–45. NY: Academic
47. Hamilton, T. A., Weiel, J. E., Adams, D. O. 1984. Expression of the transferrin receptor is modulated in macrophages in different stages of functional activation. *J. Immunol.* In press
48. Deleted in proof
49. Edelson, P. J. 1981. Macrophage plasma membrane enzymes as differentiation markers of macrophage activation. *Lymphokines* 3:57
50. Remold-O'Donnell, E., Lewandrowski, K. 1982. Decrease of the major surface glycoprotein GP160 in activated macrophages. *Cell Immunol.* 70:85
51. Remold-O'Donnell, E., Lewandrowski, K. 1982. Macrophage surface component GP160: sensitivity to plasmin and other proteases. *J. Immunol.* 128:1541–44
52. Taniyama, T., Tokunaga, T. 1983. Monoclonal antibodies directed against mouse macrophages in different stages of activation for tumor cytotoxicity. *J. Immunol.* 131:1032–37
53. Brown, M. S., Goldstein, J. L. 1983. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. *Ann. Rev. Biochem.* 52:233–61
54. Johnson, W. J., Pizzo, S. V., Imber, M. J., Adams, D. O. 1982. Receptors for maleylated proteins regulate the secretion of neutral proteases by murine macrophages. *Science* 218:574–76
55. Snyderman, R., Pike, M. C. 1984. Chemoattractant receptors on phagocytic cells. *Ann. Rev. Immunol.* 2:257–81
56. Snyderman, R., Goetzl, E. 1981. Molecular and cellular mechanisms of leucocyte chemotaxis. *Science* 213:830–37
57. Snyderman, R., Fudman, E. J. 1980. Demonstration of a chemotactic factor receptor on macrophages. *J. Immunol.* 124:2754–57
58. Pike, M. C., Fischer, D. G., Koren, H. S., Snyderman, R. 1980. Development of specific receptors for N-formylated chemotactic peptides in a human monocyte cell line stimulated with lymphokines. *J. Exp. Med.* 152:31–40
59. Guilbert, L. J., Stanley, E. R. 1980. Specific interaction of murine colony stimulating factor with mononuclear phagocytic cells. *J. Cell Biol.* 85:153–59
60. Metcalf, D. 1982. Regulation of macrophage production. *Adv. Exp. Biol. Med.* 155:33–48
61. Lin, H. S., Stewart, C. C. 1974. Peritoneal exudate cells. I. Growth requirement of cells capable of forming colonies in soft agar. *J. Cell. Physiol.* 83:369–78
62. Deleted in proof
63. Chen, B. D-M., Lin, H. S., Shen, H. 1983. Lipopolysaccharide inhibits the binding of colony stimulating factor (CSF-1) to murine peritoneal macrophages. *J. Immunol.* 130:2256–60
64. Liu, D. Y., Petschek, K. D., Remold, H. G., David, J. R. 1982. Isolation of a guinea pig macrophage glycolipid with the properties of the putative migration inhibitory factor receptor. *J. Biol. Chem.* 257:159–62
65. Anderson, P., Yip, Y. K., Vilchek, J. 1983. Human interferon γ is internalized and degraded by cultured fibroblasts. *J. Biol. Chem.* 758:6497–502
66. Broxmeyer, H. E., Smithyman, A., Eger, R. R., Meyers, P. A., DeSousa, M. 1978. Identification of lactoferrin as the granulocyte-derived inhibitor of colony-stimulating activity production. *J. Exp. Med.* 148:1052–67
67. Gordon, S., Todd, J., Cohn, Z. A. 1974. In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. *J. Exp. Med.* 139:1228–48
68. Gordon, S. 1978. Regulation of enzyme secretion by mononuclear phagocytes: Studies with macrophage plasminogen activator and lysozyme. *Fed. Proc.* 37:2754–58
69. Sammuelsson, B., Goldyne, M., Granström, E., Hamberg, M., Hammarström, S., Malmsten, C. 1978. Prostaglandins and thromboxanes. *Ann. Rev. Biochem.* 47:997–1029
70. Hammarström, S. 1983. Leukotrienes. *Ann. Rev. Biochem.* 52:355–77
71. Humes, J. L., Sadowski, S., Galavage, M., Goldenberg, M., Subers, E., Bonney, R. J., Kuehl, F. A., Jr. 1982. Evidence for two sources of arachidonic acid for oxidative metabolism by mouse peritoneal macrophages. *J. Biol. Chem.* 257:1591–94
72. Niedel, J. E., Kuhn, L. J., Vandenbark, G. R. 1983. Phorbol diester receptor copurifies with protein kinase C. *Proc. Natl. Acad. Sci. USA* 80:36–40
73. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., Nishizuka, Y. 1982. Direct activation of calcium activated phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J. Biol. Chem.* 257:7847–51
74. Friedman, S. A., Remold-O'Donnell, E.,

Annual Reviews

Annu. Rev. Immunol. 1984.2:283-318. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Piessens, W. F. 1979. Enhanced PGE_2 production by MAF treated peritoneal exudate macrophages. *Cell. Immunol.* 42:213–18

75. Nathan, C. F., Root, R. K. 1977. Hydrogen peroxide release from mouse peritoneal macrophages. Dependence on sequential activation and triggering. *J. Exp. Med.* 146:1648–62
76. Segal, A. N., Cross, A. R., Garcia, R. C., Borregaard, N., Velerius, N. H., Soothill, J. F., Jones, O. T. G. 1983. Absence of cytochrome b_{-245} in chronic granulomatous disease: A multicenter European evaluation of its incidence and relevance. *New Engl. J. Med.* 308:245
77. Nathan, C. F., Murray, H. W., Wiebe, M. E., Rubin, B. Y. 1983. Identification of $interferon_{\gamma}$ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.* 158:670–89
78. Deleted in proof
79. Cohen, M. S., Taffet, S. M., Adams, D. O. 1982. The relationship between secretion of H_2O_2 and completion of tumor cytotoxicity by BCG-elicited murine macrophages. *J. Immunol.* 128:1781–85
80. Sasada, M., Pabst, M. J., Johnston, R. B., Jr. 1983. Activation of mouse peritoneal macrophages by lipopolysaccharide alters the kinetic parameters of the superoxide producing NADPH oxidase. *J. Biol. Chem.* 258:9631–35
81. McPhail, L. C., Synderman, R. 1983. Activation of the respiratory burst enzyme in human polymorphonuclear leukocytes by chemoattractants and other soluble stimuli: Evidence that the same oxidase is activated by different transductional mechanisms. *J. Clin. Invest.* 72:192–200
82. Johnston, R. B., Jr., Chadwick, D. A., Cohn, Z. A. 1981. Priming of macrophages for enhanced oxidative metabolism by exposure to proteolytic enzymes. *J. Exp. Med.* 153:1678–83
83. Bentley, C., Zimmer, B., Hadding, U. 1981. The macrophage as a source of complement components. *Lymphokines* 4:197–231
84. Whaley, K., Lappin, D., Barkas, T. 1981. C2 synthesis by human monocytes is modulated by a nicotinic cholinergic receptor. *Nature* 293:580–83
85. Simon, P. L., Willoughby, W. F. 1982. Biochemical and biological characterization of rabbit interleukin I (IL-1). *Lymphokines* 6:47–64
86. Buracos, V. I., Rodeman, H. P., Dirarello, C. A., Goldberg, A. L. 1983. Stimulation of muscle protein degradation and prostaglandin E_2 release by leukocytic pyrogen (Interleukin 1). *New Engl. J. Med.* 308:553–58
87. Neumann, C. 1982. Mononuclear phagocytes as producers of interferon. *Lymphokines* 7:165–202
88. Marino, P. A., Adams, D. O. 1982. The capacity of activated murine macrophages for augmented binding of neoplastic cells: Analysis of induction by lymphokine containing MAF and kinetics of the reaction. *J. Immunol.* 128:2816–23
89. Adams, D. O., Marino, P. A. 1981. Evidence for a multistep mechanism of cytolysis by BCG-activated macrophages: The interrelationship between the capacity for cytolysis, target binding, and secretion of the cytolytic factor. *J. Immunol.* 126:981–87
90. Nathan, C. F., Nogueira, N., Juangbhich, C., Ellis, J., Cohn, Z. A. 1979. Activation of macrophages in vivo and in vitro. Correlation between hydrogen peroxide release and killing of *T. Cruzi*. *J. Exp. Med.* 149:1056–68
91. Adams, D. O., Johnson, W. J., Marino, P. A. 1982. Mechanisms of target recognition and destruction in macrophage-mediated tumor cytotoxicity. *Fed. Proc.* 41:2212–21
92. Johnson, W. J., Somers, S. D., Adams, D. O. 1983. Activation of macrophages for tumor cytotoxicity. *Contemp. Top. Immunobiol.* 14:127–46
93. Adams, D. O., Nathan, C. F. 1983. Molecular mechanisms operative in cytolysis of tumor cells by activated macrophages. *Immunol. Today* 4:166–70
94. Schreiber, R. D., Pace, J. L., Russell, S. W., Altman, A., Katz, D. H. 1983. Macrophage activating factor produced by a T cell hybridoma: Physicochemical and biosynthetic resemblance to γ-interferon. *J. Immunol.* 131:826–30
95. Meltzer, M. S., Benjamin, W. R., Ferrar, J. J. 1982. Macrophage activation for tumor cytotoxicity: Induction of macrophage tumoricidal activity by lymphokines from El-4, a continuous T-cell line. *J. Immunol.* 129:2802–7
96. Doe, W. F., Yang, S. T., Morrison, D. C., Betz, S. J., Henson, P. M. 1979. Macrophage stimulation by bacterial lipopolysaccharides. II. Evidence for differentiation signals delivered by Lipid A and by a protein rich fraction of lipopolysaccharides. *J. Exp. Med.* 148:557
97. Morrison, D. C., Rudback, J. A. 1981. Endotoxin-cell membrane interactions leading to transmembrane signalling. *Contemp. Top. Mol. Immunol.* 8:187–218
98. Adams, D. O., Marino, P. A., Meltzer, M. S. 1981. Characterization of genetic defects in macrophage tumoricidal capacity: Identification of murine strains with abnormalities in secretion of cyto-

Annual Reviews

lytic factors and ability to bind neoplastic targets. *J. Immunol.* 126:1843–47

99. Adams, D. O., Lewis, J. G., Johnson, W. J. 1984. Multiple modes of cellular injury by macrophages: requirement for different forms of effector activation. *Prog. Immunol.* 5. In press
100. Adams, D. O., Cohen, M., Koren, H. S. 1984. Activation of mononuclear phagocytes for cytolysis: parallels and contrast between activation for tumor cytoxicity and for ADCC. In *Macrophage-Mediated Antibody Dependent Cellular Cytoxicity*, ed. H. S. Koren, pp. 155–68 NY: Plenum
101. Johnson, W. J., Bolognesi, D., Adams, D. O. 1984. Antibody dependent cytolysis (ADCC) of tumor cells by activated murine macrophages is a two step process: quantification of target binding and subsequent target lysis. *Cell. Immunol.* In press
102. Nathan, C. F., Brukner, L. H., Kaplan, G., Unkeless, J., Cohn, Z. A. 1980. Role of activated macrophages in antibody-dependent lysis of tumor cells. *J. Exp. Med.* 152:183
103. Ralph, P., Nakoinz, I., Diamond, B., Yelten, D. 1980. All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes. *J. Immunol.* 125:1885–88
104. Deleted in proof
105. Pedersen, B., Norrild, B., Krebs, H. J. 1982. The effect of monensin on the antibody-dependent cell-mediated killing. *Cell. Immunol.* 72:208–14
106. Nathan, C., Cohn, Z. A. 1980. Role of oxygen-dependent mechanisms in antibody-induced lysis of tumor cells by activated macrophages. *J. Exp. Med.* 152:198
107. Deleted in proof
108. Key, M. E., Haskill, J. S. 1984. Antibody dependent cellular cytotoxicity (ADCC): A potential antitumor defense mechanism in situ in a murine mammary adenocarcinoma. See Ref. 100, pp. 243–62
109. Matthews, T. J., Collins, J. J., Roloson, G. J., Thiel, H.-S., Bolognesi, D. P. 1981. Immunologic control of the ascites form of murine adenocarcinoma 755. IV. Characterization of the protective antibody in hyperimmune serum. *J. Immunol.* 126:2332–36
110. Herlyn, D., Koprowski, H. 1982. Ig2a monoclonal antibody inhibits human tumor cell growth through interaction with effector cells. *Proc. Natl. Acad. Sci. USA* 79:4761–65
111. Badger, C. C., Bernstein, I. D. Therapy of murine leukemia with monoclonal antibody against a normal differentiation antigen. *J. Exp. Med.* 157:828–42
112. Langlois, A. S., Mathews, T., Roloson, G. J., Thiel, H. J., Collins, J. S., Bolognesi, D. P. 1981. Immunological coating of the ascites for the murine adenocarcinoma 755V. Antibody-directed macrophages mediate tumor cell destruction. *J. Immunol.* 126:2337–41
113. Johnson, W., Steplewski, X., Koprowski, H., Adams, D. O. 1984. A distinctive cytolytic interaction between tumor cells and murine macrophages directed by antibodies of the IgG2a isotype. (Manuscript submitted)
114. North, R. J. 1974. Cell mediated immunity and the response to infections. In *Mechanisms of Cell-Mediated Immunity*, ed. R. T. McClusky, S. Cohen, pp. 185–219. NY: John Wiley
115. Lefford, M. J. 1983. Immunity to facultative intracellular parasites. In *The Reticuloendothelial System: A Comprehensive Treatise. Volume IV. Immunopathology*, ed. N. R. Rose, B. V. Siegal, pp. 103–143. NY: Plenum
116. Nelson, D. S. 1978. Macrophages as effectors of cell-mediated immunity. In *Phagocytes and Cellular Immunity*, ed. H. Gadebusch, pp. 57–100. Cleveland, OH: CRC
117. Axline, S. G. 1970. Functional biochemistry of the macrophages. *Sem. Hematol.* 7:142
118. James, S. L., Lazdins, J. K., Hiery, S., Natovitz, P. 1983. Macrophages as effector cells of protective immunity in murine schistosomiasis. VI. T-cell dependent, lymphokine-mediated activation of macrophages. *J. Immunol.* 131:1481–86
119. Capron, A., Dessaint, J.-P., Hague, A., Capron, M. 1982. Antibody-dependent cell-mediated cytotoxicity against parasites. *Prog. Allergy* 34:234–67
120. Silverstein, S., Cohn, Z. A. 1977. Endocytosis. *Ann. Rev. Biochem.* 46:669–722
121. Hopkins, C. R. 1983. The importance of the endosome in intracellular traffic. *Nature* 304:684–85
122. Jones, T. C. 1980. Interactions between murine macrophages and obligate intracellular protozoa. *Am. J. Pathol.* 101:127–32
123. Mosser, D. M., Edelson, P. J. 1984. Mechanisms of microbial entry and endocytosis by mononuclear phagocytes. *Contemp. Top. Immunobiol.* 14:71–96
124. Edelson, P. J. 1982. Intracellular parasites and phagocytic cells: Cell biology and pathophysiology. *Rev. Infect. Dis.* 4:124
125. Murray, H. W. 1984. Activation of macrophages to display enhanced oxidative and antiprotozoal activity. *Contemp. Top. Immunobiol.* 14:97–115
126. Nogueira, N., Cohn, Z. A. 1984. Acti-

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Annual Reviews

vation of mononuclear phagocytes for the destruction of intracellular parasites: Studies with *T. Cruzii*. *Contemp. Top. Immunobiol.* 14:117–26

127. Klebanoff, S. S. 1982. Oxygen-dependent cytotoxic mechanisms of phagocytes. *Adv. Host. Defense Mechan.* 1:111–62
128. Nathan, C. F. 1984. Mechanisms of macrophage antimicrobial activity. *Trans. R. Soc. Trop. Med. Hyg.* In press
129. Lehrer, R. I., Szklarek, D., Selsted, M. E., Fleischmann, J. 1981. Increased content of microbicidal cationic peptides in rabbit alveolar macrophages elicited by complete Freund adjuvant. *Infect. Immun.* 33:775
130. Buchmuller, Y., Mauel, J. 1979. Studies on the mechanisms of macrophage activation. II. Parasite destruction in macrophages activated by supernates from concanavalin A-stimulated lymphocytes. *J. Exp. Med.* 150:359–70
131. Nacy, C. A., James, S. L., Osler, C. N., Meltzer, M. S. 1984. Activation of macrophages to kill *Ricketsiae* and *Leishmania:* Disassociation of intracellular microbicidal activities and extracellular destruction of neoplastic and Helmieth tests. *Contemp. Top. Immunobiol.* 14:147–70
132. Griffin, F. M. 1984. Activation of macrophage complement receptors for phagocytosis. *Contemp. Top. Immunobiol.* 14:57–70
133. Meltzer, M. S., Jones, E. E., Boetcher, D. A. 1975. Increased chemotactic responses of macrophages from BCG-infected mice. *Cell. Immunol.* 17:268
134. Verghese, M., Synderman, R. 1983. Hormonal activation of adenylate cyclase in macrophage membranes is regulated by guanine nucleotides. *J. Immunol.* 130:869.
135. Unanue, E. R. 1984. Antigen-presenting function of the macrophage. *Ann. Rev. Immunol.* 2:395–428
136. Blumental, E. J., Roberts, W. K., Vasil, A., Talmage, E. 1983. Macrophage activation: Dissociation of cytotoxic activity from Ia-A antigen expression. *Proc. Natl. Acad. Sci. USA* 80:2031–35
137. Johnson, W. J., Marino, P. A., Schreiber, R. D., Adams, D. O. 1983. Sequential activation of murine mononuclear phagocytes for tumor cytolysis: Differential expression of markers by macrophages in the several stages of development. *J. Immunol.* 131:1038–43

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:283-318. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:283-318. Downloaded from arjournals.annualreviews.org
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INTERLEUKIN 2

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INTRODUCTION

Compared to the last twenty years of lymphokine research, the past two years of effort directed towards understanding the T-lymphocytotrophic hormone, interleukin 2 (IL-2) (1–3), have resulted in an exponential increase in our grasp of the importance of intercellular communicating molecules. As in many other fields of biology, breakthroughs have resulted from the application of monoclonal antibodies and molecular biology to this area of research. In addition, an endocrinologic approach has provided fresh insight into the mechanism whereby IL-2 promotes T-cell proliferation. Consequently, the tools are at last available for new experimental approaches to fundamental questions in immunology and cell biology.

The idea that lymphocytes are stimulated to undergo DNA duplication and mitosis by antigen triggering of specific receptors has slowly evolved to the understanding that while this concept is correct, the process is carried forward by discernible molecular mechanisms. Through painstaking cellular and biochemical experiments a consensus has finally been reached that IL-2 is a critical biologic activity, released from antigen-triggered lymphocytes within hours of activation, that functions to mediate a switch in T cells from G_1 into the proliferative phases (i.e. S, G_2, and M) of the cell cycle. However, until recently, we could only speculate about whether IL-2 *activity* resulted from the action of a single molecule or a series of molecules. Moreover, the mechanism of IL-2 action and the immunologic relevance of IL-2 activity remained obscure.

To proceed beyond a phenomenologic level of experimentation it was necessary to formulate molecular approaches to IL-2 and its mechanism of inter-

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Annual Reviews

action with T cells. The cDNA nucleotide sequence encoding for IL-2, the genomic structure, and the amino acid sequence of the IL-2 molecule responsible for IL-2 activity are now known (4–7). A single-copy cellular gene codes for a 15,420-dalton polypeptide that provides the signal for T-cell DNA duplication and subsequent mitosis. The knowledge that IL-2 activity can be ascribed to a single protein rather than a family of molecules aided considerably the design of experiments to explore the mechanism of IL-2 interaction with T cells.

Even before the isolation of cDNA clones that encode mRNA capable of translating IL-2 activity, conventional biochemical approaches suggested that IL-2 activity might result from a single peptide (8). These experiments provided the foundation for biosynthetic radiolabeling experiments followed by isolation of the radiolabeled peptide so that IL-2–T-cell binding experiments could be performed (9,10). The initial experiments promptly revealed that IL-2 binds to a site on a membrane receptor molecule. Consequently, for the first time we understood that T-cell mitosis results from the interaction of a single polypeptide ligand and a single class of high-affinity, thermodynamically independent membrane receptors.

The awareness that antigen-triggered T-cell clonal expansion is mediated by a polypeptide hormone-receptor mechanism has fueled the imaginations of investigators schooled in pharmacology, enzymology, and endocrinology. The molecular and cellular reagents are now available with which to approach such questions as genetic or acquired defects in the secretion of IL-2 or the antigen-triggered appearance of the IL-2 receptor. Moreover, it may now be possible to uncover agonists and antagonists of the IL-2-receptor interaction that will have physiologic and pathophysiologic relevance to the T-cell immune response. It is fortunate that we now stand before these thresholds of investigation, since we are facing two new diseases that involve T-cell defects, the acquired immunodeficiency syndrome (AIDS) and adult T-cell leukemia (ATL). It is premature to imagine a primary role for defective IL-2 secretion or responsiveness in AIDS and ATL. However, as clinical studies are initiated, our basic understanding of the T-cell immune response and the physiologic role of IL-2 will surely expand rapidly.

IL-2 GENETICS

It is refreshing to observe, in a rapidly expanding and highly competitive area of research, the series of experiments reported by Tanaguchi and co-workers (4), who successfully isolated the first cDNA clone that encodes for IL-2 biologic activity; especially as these investigators approached the problem without (*a*) knowledge of the fact that a single peptide was responsible for IL-2 activity, (*b*) information about the primary structure of the polypeptide, and

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Annual Reviews

(*c*) the availability of antibodies reactive with IL-2. Although new advances in molecular cloning techniques should facilitate future experiments designed to isolate cDNA coding for biologically active lymphokines, it is instructive to recount the cloning strategy of Tanaguchi et al (4). Because the only means available for detecting IL-2 activity was the IL-2 bioassay, which depends upon the IL-2 concentration–dependent promotion of proliferation of cloned cytolytic T-lymphocyte lines, the investigators detected IL-2 mRNA by using *X. laevis* oocytes. The use of the oocyte translation assay was a wise choice. Although the IL-2 bioassay is remarkably sensitive (the lower limit of detection is 1–10 pM) (10), in vitro systems (e.g. the reticulocyte and wheat germ systems) translate polypeptides in concentrations several orders of magnitude below the limits of detection in the bioassay.

Probably the most important decision of Tanaguchi et al (4), which determined their ultimate success, was to create a selected cDNA library. Polyadenylated RNA extracted from a lectin-induced, high IL-2 producer human leukemic T-cell line was size fractionated on a sucrose density gradient. Each fraction was tested for IL-2 mRNA by oocyte translation, and only positive fractions were used to develop the cDNA library. On the basis of the quantity of RNA excluded, a 10-fold enrichment of IL-2 mRNA was achieved. Subsequent experiments revealed the wisdom of this choice. Upon isolation of IL-2 cDNA and rescreening of the cDNA library, the frequency of IL-2-positive clones was found to be 1 in 2,000. Consequently, the task of screening an unselected cDNA library would have been exceedingly laborious and time-consuming, assuming that an IL-2 cDNA would only be present with a frequency of 1:20,000.

The nucleotide sequence of the IL-2 cDNA predicts a polypeptide of 153 amino acids containing an amino-terminal signal sequence of 20 amino acids. Thus, the mature secreted protein, predicted from the cDNA nucleotide sequence, contains 133 amino acids constituting a calculated molecular weight of 15,420.5 daltons. This value is in good agreement with the estimated molecular size of the secreted peptide that we (8) and others (11) had obtained by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Mr = 15,500, reducing conditions). Additional information obtained from the predicted IL-2 amino acid sequence is also of interest. Previous experiments employing neuraminidase and various glycosidases indicated that IL-2 is sialylated and glycosylated to a variable degree, thus accounting for an approximate 1000-dalton increase in size and three separately charged species detectable by isoelectric focusing (8). The absence of potential N-glycosylation sites (AsN-X-Ser or AsN-X-Thr) from the predicted amino acid sequence indicated that any carbohydrate associated with the molecule had to be O-linked.

Subsequent to the publication of the complete nucleotide sequence for IL-2, many other groups, who were screening cDNA libraries by various means,

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rapidly identified IL-2 cDNA clones using oligonucleotide probes synthesized on the basis of the published sequence. Consequently, over the past several months, many biotechnology companies have engineered high IL-2 producer cDNA bacterial isolates and are beginning to make recombinant IL-2 available to the research community. IL-2 in milligram and gram quantities will thus be available for in vitro, in vivo, and clinical trials. As this reagent is employed in future experiments, investigators should insist on demonstrated criteria for estimates of purity to avoid ambiguous results from biological experiments designed to detect new IL-2 activities (i.e. other than the promotion of T-cell mitosis).

Of considerable importance to the interpretation of the biologic and physiologic implications of IL-2 is the question of IL-2 genetic and polypeptide diversity. When investigators had employed unfractionated conditioned media or partially purified IL-2 preparations for immunologic experiments it remained unclear whether different T-cell subsets respond to the same or different molecules. It should be appreciated that the bioassay used to define IL-2 activity employs cloned murine cytolytic IL-2-dependent T cells (12). Thus, it became controversial whether helper T-cells or suppressor T cells also responded to the moiety isolated and defined as the activity that promotes cytolytic T cell growth. It was difficult to approach this question at the protein level because, as already stated, biochemical analysis produced evidence for molecular heterogeneity even when the activity was restricted to cytolytic T cells. An alternative approach to the question of IL-2 molecular heterogeneity was available once a cDNA encoding for IL-2 biological activity was available. Using the Il-2 cDNA it was possible to identify, isolate, and sequence the IL-2 gene and to determine its organization in normal and neoplastic T cells (5).

The IL-2 gene is divided into 4 exons separated by intervening sequences and is comprised of 4930 base pairs (bp). Exon 1 contains a 5′ nontranslated region and codes for the first 49 amino acids, 20 of which constitute the signal polypeptide. A short intervening sequence (91 bp) separates this exon from exon 2, 60 (bp) long, which codes for the next 20 amino acids. The second and third exons are separated by a long intervening sequence of 2292 bp. Exon 3 (144 bp), which codes for the next 48 amino acids, is again followed by a long intervening sequence of 1364 bp. The fourth and final exon codes for the remaining 36 amino acid residues followed by a termination codon, TGA. Sequence determination of the entire genome and comparison with the cDNA revealed that the sequence includes 292 nucleotides upstream from the translation-initiation site and extends 297 bp downstream from the polyadenylation signal. A promotor sequence, 'TATAAA,' occurs 77 bp upstream from the translation-initiation site, and transcription starts 53 nucleotides from the initiation site.

Restriction endonuclease digestion followed by Southern blot analysis of cellular DNAs prepared from normal and neoplastic lymphocytes indicates

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that there is only a single copy of the human IL-2 gene (5). Although this result simplifies the interpretation of controversial experiments that suggested there might be more than one IL-2 polypeptide, it remains an open question whether posttranslational modification results in IL-2 molecules that have preferential affinities for different functional T-cell subsets. Further analysis of this problem will, therefore, involve experiments using well-defined IL-2 molecules and separated or cloned T-cell subsets. In addition, experiments using cloned genomic and cDNA together with transfection approaches should allow discrimination of the critical regulatory mechanisms that result in IL-2 gene expression after antigen activation of the T-cell antigen receptor. Moreover, all available evidence indicates that IL-2 gene expression is transient after antigen activation. Consequently, future experiments will identify the factors and mechanisms of down-regulation of IL-2 gene expression that may be operative and that may be important for the regulation of T-cell clonal expansion.

IL-2 MONOCLONAL ANTIBODIES

The second major recent development in the IL-2 field has been the successful generation of IL-2-reactive monoclonal antibodies (6). In addition to such obvious uses for monoclonal antibodies as in IL-2 immunoassays, monoclonal immunoadsorptive purification of IL-2 is a powerful and cost-effective method for obtaining homogeneous polypeptide. It is noteworthy that premature reports appeared in the literature purporting development of IL-2-reactive monoclonal antibodies (13,14). However, in each instance, the data failed to demonstrate that the hybridoma products were in fact antibodies reactive with IL-2. Although the products demonstrated neutralizing activity, no experiments on a competitive relationship between IL-2 and the antibodies were offered. Moreover, experiments to exclude any suppressive effects of the hybridoma products on general cellular metabolism were absent from the reports. Finally, the hybridoma products were ineffective when used for the immunoaffinity purification of IL-2.

The successful generation of IL-2 monoclonal antibodies relied ultimately on the accumulated knowledge of the molecular characteristics of IL-2 and its mechanism of interaction with T cells. The primary advances, which should be generally applicable to lymphokines, included the development of a rapid, quantitative, unambiguous bioassay to monitor biochemical purification, the identification of cellular sources of IL-2 so that large quantities could be obtained in serum-free medium, and the development of IL-2 immunoassays to facilitate the identification of anti-IL-2-secreting hybridomas.

Several monoclonal antibodies reactive with IL-2 have been developed, three of which have been sufficiently characterized. Two antibodies (DMS-1, DMS-2), derived from separate hybridoma cultures, appeared similar in initial experiments: both were of the IgG_1 isotype and both neutralized IL-2 activity

Annual Reviews

in equimolar concentrations. Subsequent experiments have indicated that these two antibodies recognize the same epitope on IL-2 (R. C. Budd, K. A. Smith, unpublished). In enzyme-linked immunoassays, the antibodies do not complement one another, and they compete for IL-2 binding in equimolar concentrations. Of interest, DMS-1 and DMS-2 cross-react with murine IL-2 and IL-2 derived from normal human mononuclear cells (JURKAT-derived IL-2 served as the immunogen) but do not react with IL-2 derived from rat splenocytes.

In equilibrium and kinetic binding experiments, DMS-1 and DMS-2 bind radiolabeled IL-2 with a similar affinity ($K_d = 2 \times 10^{-7}$ M) and demonstrate relatively slow rate constants. At near saturating concentrations DMS-1 and DMS-2 reach equilibrium within 10 min at 37°C but require 2 hr at 4°C. The third monoclonal antibody, of the IgG_{2a} isotype (designated DMS-3), binds to IL-2 with a higher affinity ($K_d = 2 \times 10^{-8}$ M) and with much faster rate constants than DMS-1 and DMS-2 (equilibrium is attained within 20 sec at 4°C). Knowledge of the equilibrium dissociation constants and the rate constants for association and dissociation at different temperatures aids considerably the use of the monoclonal antibodies. For example, DMS-3 functions effectively as an immmunoadsorbent at 4°C, whereas DMS-1 and DMS-2 adsorbent columns must be used at 37°C to achieve effective binding. These differences can be attributed to the rate constants for association, which differ markedly for these antibodies. The salient feature remains the extreme effectiveness of immunoaffinity purification, provided the antibodies are utilized properly. IL-2 can be purified to homogeneity (as determined by SDS-PAGE, reversed-phase liquid chromatography, and amino acid sequence analysis) in a single step and in large quantities using antibodies coupled to a solid gel support in concentrations of 15–30 mg/ml gel.

IL-2 STRUCTURE

Studies of the structure-activity relationship of IL-2 are embryonic at this time, since well-characterized purified IL-2 has only recently become available in sufficient quantities for such studies. However, knowledge that IL-2 is the product of a single gene and is variable glycosylated has focused attention on the question of the physiologic role, if any, of the carbohydrate components of the polypeptide. Studies performed with different classes of IL-2 derived from lectin-stimulated human tonsil cells indicate no discernible differences imparted to the growth-promoting activity of variably sialylated IL-2 molecules, as assayed using cloned murine CTLL or lectin-activated human peripheral-blood lymphocytes (8). The idea that the carbohydrate component plays no part in the interaction with T cells has also been recently confirmed by testing recombinant IL-2. Using cloned murine cytolytic T cell lines, we find that recombinant IL-2 appears to effect T-cell proliferation at identical con-

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centrations when compared to glycosylated, JURKAT-derived IL-2 (K. A. Smith, unpublished). Thus, the active site of the molecule appears to reside in the polypeptide rather than the carbohydrate component.

It has been postulated that the physiologic role of the carbohydrate resides in the mechanism of clearance of IL-2 (8). The rapid removal of circulating desialylated glycoproteins is well documented and is mediated by a carbohydrate recognition system present in hepatocytes (15). The initial step involves specific binding to the asialoglycoprotein receptor of hepatocytes, which recognizes glycoproteins having terminal galactose or glucose residues on their oligosaccharide chains. Thus one might anticipate that desialylated IL-2, like erythropoietin, could be susceptible to rapid, physiologic hepatic clearance (16). However, from the potential immunotherapeutic standpoint, recombinant IL-2, which totally lacks a carbohydrate component, might be expected to have a prolonged in vivo half-life, since it should not be susceptible to hepatic removal.

The active site of the IL-2 polypeptide will receive a great deal of attention in the future as attempts are made to identify an IL-2 fragment that mediates IL-2 activity. If successful, these studies may allow the construction of antagonists of IL-2 action that may be of use as immunosuppressive agents. Moreover, synthetic peptides may be useful and cost-effective immunopotentiating agents. Finally, definition of the tertiary structure of IL-2 should now be approachable.

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THE MECHANISM OF THE IL-2–T-CELL INTERACTION

The third significant advance in IL-2 research has been the understanding of the mechanism of interaction of the IL-2 polypeptide with activated T cells. Soon after the discovery of IL-2 activity in lymphocyte-conditioned medium and the development of the quantitative IL-2 bioassay, absorption experiments revealed that IL-2 activity could be removed from conditioned media in a time-, temperature-, and cell concentration–dependent fashion only by activated T cells (17–19). The cellular specificity of IL-2 absorption suggested that IL-2 might bind to activated T cells by means of specific membrane receptors in a fashion analogous to that of polypeptide hormones. However, to proceed beyond the relatively crude absorption experiments and to provide molecular information relevant to the mechanism of the IL-2–T cell interaction, it was necessary to develop a radiolabeled IL-2 binding assay.

In a series of experiments utilizing biosynthetically radiolabeled IL-2, highly purified by conventional biochemical procedures (9) and by monoclonal antibody affinity adsorption (10), IL-2 bound specifically to activated T cells by means of a site on a membrane molecule that satisfies all the criteria ascribed to authentic hormone receptors (i.e. high affinity, saturability, ligand and tissue

Annual Reviews

specificity). Of particular significance to the biological implications of IL-2 activity, the affinity of the receptor for IL-2 is quite high (K_d = 5–20 pM). Thus, IL-2 receptors are fully saturated at a concentration of 1×10^{-10} M (1.5 ng/ml). Moreover, subsequent experiments revealed that the concentrations that bind to IL-2 receptors coincide exactly with those that promote T-cell proliferation. Consequently, as would be predicted from the thermodynamic principles of a single ligand and a single class of high-affinity receptors, at equilibrium the rate of T-cell proliferation is dependent on the concentrations of free IL-2 available to the cells. These observations reduce the variables that regulate the rate and magnitude of T-cell clonal expansion to only three: the free IL-2 concentration, the cellular IL-2 receptor concentration, and the affinity of the receptor for IL-2. These findings cleared the way for further experiments exploring the biologic and immunologic implications of the IL-2-receptor interaction. Moreover, for the first time it was apparent how an immunologically nonspecific, but T-cell specific growth hormone functioned in the immune response. Antigenic specificity of T-cell clonal expansion is insured because unstimulated T cells do not express IL-2 receptors.

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CHARACTERIZATION OF THE IL-2 RECEPTOR

The ability to determine quantitatively the number and affinity of IL-2 binding sites in different cell populations was crucial in demonstrating the ligand and tissue specificity of the IL-2-receptor interaction. However, a qualitative assay of the density of IL-2 receptors at the level of individual cells was a prerequisite before the consequences of IL-2-receptor binding to all of the cells within the population could be evaluated. In addition, methods were required to explore the molecular nature of the IL-2 receptor. Fortunately, a monoclonal antibody (anti-Tac) with specific reactivity for antigen- or lectin-activated T cells had been developed by Takashi Uchiyama and co-workers (20,21). Thus, in collaborative experiments anti-Tac was found to block radiolabeled IL-2-receptor binding in a concentration-dependent manner (22). Moreover, anti-Tac suppresses IL-2-dependent T-cell proliferation at concentrations remarkably coincident with those that block IL-2-receptor binding.

These observations suggest strongly that anti-Tac reacts with the IL-2 receptor, although it remains unclear whether the epitope recognized is part of the IL-2 binding site: IL-2 does not compete for anti-Tac binding even when used in a 100-fold molar excess over that required to saturate IL-2 binding sites fully (D. A. Cantrell, K. A. Smith, unpublished). However, biochemical evidence favors the interpretation that the Tac antigen and the IL-2 receptor are parts of the same molecule. Radiolabeled IL-2 covalently cross-linked to the IL-2 receptor$^+$ cell membranes identifies a 60,000-dalton moiety when analyzed by fluorography after SDS-PAGE (23), and immunoprecipitation of

Annual Reviews

radiolabeled cell membranes using anti-Tac also identifies a 60,000-dalton species (22). Moreover, two additional monoclonal antibodies have recently been described that appear to be reactive to the murine and rat IL-2 receptors (24; T. Malek, personal communication). Both of these antibodies recognize a membrane molecule in the range of 55,000–60,000 daltons (T. Malek, T. Diamanstein, personal communication). Consequently, it appears that the IL-2 binding site resides on a 60,000-dalton membrane glycoprotein that does not contain intrachain disulfide bonds and does not appear to be noncovalently linked to other molecules (22,23).

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THE IMMUNOLOGIC RELEVANCE OF THE IL-2-RECEPTOR HORMONE SYSTEM

Since the discovery of the IL-2-receptor system, immunologists have been puzzled by the concept that an antigen-nonspecific factor might be the only mechanism that promotes T-cell clonal expansion, since T-cell clonal expansion is antigen-specific. However, the IL-2 hormone-receptor system is unique: IL-2 receptors are not expressed until the antigen receptor is triggered. Thus, the immune specificity of T-cell clonal expansion is generated by the restriction of IL-2 receptor expression only to antigen-activated T-cell clones. Moreover, the transient expression of IL-2 receptors after antigen activation insures the conservation of the clonality of T-cell immune responses.

Because IL-2 binding and biological response experiments indicated that the IL-2 receptor density might be one of the critical variables determining the extent of the T-cell proliferative response, IL-2 receptor expression was examined sequentially after immune stimulation (25). Human peripheral-blood mononuclear cells activated polyclonally by a mitogenic lectin were found to express IL-2 receptors asynchronously. Although a minority (30%) of cells express IL-2 receptors within 24 hr of activation, the IL-2 receptor density per cell remains low. In the absence of cell-cycle progression, the proportion of IL-2 receptor-positive cells gradually increases; the IL-2 receptor density per cell also increases. Since IL-2 receptor expression precedes the onset of DNA synthesis, these observations suggest that a critical threshold of IL-2-receptor density is required prior to the commitment to cell-cycle progression. Upon removal of the activating lectin, IL-2 receptor levels progressively decline; the rate of proliferation diminishes in parallel. Repetitive exposure to lectin results in a more rapid reexpression of maximal IL-2 receptor levels, which is then followed by an accelerated resumption of proliferation. Identical results are obtained after alloantigen stimulation and upon stimulation of cloned human T cells with clonotypic monoclonal antibodies (S. Meuer, D. A. Cantrell, E. Reinherz, K. A. Smith, unpublished).

The immunologic relevance of these findings resides in the understanding

Annual Reviews

that IL-2 receptor expression, and thus IL-2 responsiveness, are strictly antigen dependent. Moreover, the findings suggest that after cell division in response to IL-2, the resulting daughter cells must be triggered by antigen once again to express IL-2 receptors. Consequently, if these concepts can be extrapolated to the in vivo behavior of T cells, one might imagine that as antigen is cleared, previously activated T-cell clones would lose IL-2 receptors and revert to G_0 resting T cells. In this context a "memory" T cell can be thought of as a T cell that had once expressed IL-2 receptors but has subsequently lost them.

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THE BIOLOGIC RELEVANCE OF IL-2-RECEPTOR INTERACTION FOR THE REGULATION OF CELL-CYCLE PROGRESSION

An intriguing observation that has been made repeatedly when both prokaryotic and eukaryotic cells have been studied concerns the extreme variability of cell-cycle times of genetically homogeneous cell populations (26). Of particular interest, the division rates of individual cells follow a normal distribution, and as division rate is the reciprocal of the time elapsed between cell divisions, the cell population is distributed logarithmically with respect to cell-cycle progression. Since there is no relationship between the division rate from one generation to the next, the cell population retains the same mean growth rate over many cell divisions. Although several models have been proposed to explain these phenomena, the critical determinants of cell-cycle progression have remained obscure.

Subsequent to the demonstration that the IL-2-receptor interaction is the crucial event that mediates T-cell proliferation, a striking observation was that IL-2 receptor density is heterogeneous and distributed log-normally within T-cell populations (25). This distribution of cellular IL-2 receptor density could not be explained by changes within the cell cycle, since synchronized G_0/G_1 cells demonstrated the same heterogeneity. In addition, clonal variation could not account for the distribution, as cloned cells were found to be identical to polyclonal T-cell populations. Since IL-2-promoted T cell–cycle progression is IL-2-concentration dependent and yields symmetrically sigmoid logarithmic dose-response curves, it appeared likely that the logarithmic distribution of IL-2 receptor density was responsible for this phenomenon. Moreover, should this supposition be correct, the absolute number of IL-2-occupied receptors would determine cell-cycle progression and account for the rate-normal distribution of T cell–cycle times.

In a series of experiments using synchronized IL-2 receptor$^+$ T-cell populations, we found cell-cycle progression to be dependent upon only three variables: IL-2 concentration, IL-2 receptor density, and the duration of the IL-2 receptor interaction (D. A. Cantrell, K. A. Smith, unpublished). Thus,

Annual Reviews

high-density IL-2 receptor cells progress from G_0/G_1 into the proliferative phases of the cell cycle prior to cells with a low density of IL-2 receptors. Perhaps the most definitive demonstration of the critical role of IL-2 receptor density was obtained through cell-sorting experiments. Synchronized IL-2 receptor$^+$ populations, separated into high- and low-density IL-2 receptor subsets, differ markedly in the time required to enter the proliferative phases of the cell cycle. Moreover, at limiting IL-2 concentrations, only the high-density IL-2 receptor–positive cells are triggered to undergo DNA replication. Among other things, these findings explain the symmetrically sigmoid nature of the IL-2 log-dose response curve. Under equilibrium conditions, as IL-2 concentration is varied, the logarithmic distribution of IL-2 receptors within the population dictates a logarithmic IL-2 concentration–dependent response.

Especially noteworthy is the observation that a critical threshold of IL-2-receptor interactions is necessary to promote T cell–cycle progression. Even though IL-2-receptor binding comes to equilibrium within 15 min, several hours of IL-2-receptor interaction are required to initiate cell-cycle progression. These experimental findings point to the accumulation of a critical concentration of intracellular signals that ultimately determine the quantal response of DNA replication. As well, since others have shown that continuous protein synthesis is required during G_1 for cell-cycle progression, these observations provide an explanation for the seemingly long interval (10–12 hr) between IL-2 binding and the S-phase transition. Since all cells examined (26), including bacteria, yeasts, protozoa, and mammalian and avian cells, display growth characteristics identical to those of proliferating T-cell populations, these data indicate that the intracellular molecular reactions that lead to DNA replication are common to all life forms. Thus, future investigation of these reactions using cloned IL-2-dependent T cells may provide new information relevant to both normal and neoplastic cell growth.

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THE IL-2-RECEPTOR SYSTEM AND T-CELL MALIGNANCIES

Soon after the discovery that antigen- or lectin-activated normal T cells proliferate in response to IL-2, the IL-2-receptor hormone system was explored as a possible mechanism responsible for neoplastic T-cell growth. The initial experiments were disappointing. Leukemic cells isolated from patients with acute lymphoblastic leukemia (ALL) and cell lines derived from these patients could not be found to produce IL-2 or to express IL-2 receptors (27). In addition, more recent experiments using cloned IL-2 cDNA have shown no transcriptional activity of the IL-2 gene (5). In retrospect, these findings are consonant with the data derived from examination of normal thymocytes, which the T-ALL cells phenotypically resemble. Immature thymocytes do not

Annual Reviews

produce IL-2 spontaneously, nor are they capable of producing IL-2 in response to lectin or antigen (28). Moreover, immature T cells do not express IL-2 receptors when examined using the radiolabeled IL-2 binding assay (9).

In contrast to the lack of involvement of IL-2 or IL-2 receptors in the growth of T-ALL cells, in some instances an autocrine mechanism involving the secretion and response to IL-2 is operative to promote autonomous neoplastic T-cell growth. In a detailed series of experiments, using a neoplastic T-cell line derived from a gibbon ape with a retrovirus-induced T-cell lymphoma, the rate of cell proliferation was found to be dependent on the IL-2-receptor mechanism: Glucocorticoids suppress IL-2 secretion and cell proliferation, and this suppressive effect can be overcome if purified IL-2 is supplied exogenously (27). These findings establish conclusively that continuous in vitro neoplastic T-cell growth can be operative through the IL-2-receptor mechanism. Yet to be explored are the in vivo consequences of this autocrine response. However, the implications of the involvement of the IL-2-receptor system in leukemogenesis are obvious: Therapeutic agents that block IL-2 production or IL-2 responsiveness may be useful in the treatment of some T-cell leukemias.

In the past several years a new disease has become recognized that may involve the IL-2 receptor mechanism. First reported from Japan by Yodoi and colleagues (29), a rapidly progressive T-cell leukemia (adult T-cell leukemia, ATL) is a malignancy manifested by neoplastic transformation of T cells that express phenotypic markers characteristic of mature helper T cells (i.e. $T3^+$, $T4^+$, $T8^-$). It is perhaps harmonious that the first isolate of a unique human retrovirus was made from cells derived from a patient with ATL by the same group that first demonstrated the long-term growth of T cells. Highly purified IL-2 supported the growth of neoplastic T cells from patients suffering from ATL (30, 31). Of interest, a cell line (designated HUT-102) that was initially IL-2-dependent became IL-2-independent (31). Poiesz and co-workers detected reverse transcriptase in the medium of HUT-102 cells, and subsequent experiments revealed that the cells produced a unique retrovirus (designated human T-cell leukemia virus, HTLV) (32). Of particular interest to the IL-2-receptor mechanism known to be operative in normal T-cell proliferation, the cells from patients with ATL respond to IL-2 in the absence of an immune IL-2-receptor-inducing signal such as lectin or antigen (31). These findings suggest that the neoplastic T cells express IL-2 receptors in the absence of antigen-receptor triggering.

Within the past two years the cells from many patients with ATL have been examined for IL-2-receptor expression. There is a strict correlation between the presence of IL-2 receptors and HTLV. These findings have prompted an examination for evidence of an IL-2-receptor autocrine mechanism to explain ATL neoplastic T-cell growth. Although an initial report by Gootenberg et al (33) suggested that the HUT-102 cell line produced and responded to IL-2, a

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more extensive survey of several HTLV$^+$ IL-2 receptor$^+$ cell lines indicates that the IL-2 gene is generally not expressed. Northern blot analysis using cloned IL-2 cDNA has failed to detect IL-2 mRNA in most of the cell lines examined (R. Gallo, personal communication). Moreover, in situ cDNA-RNA hybridization has not detected a subpopulation of cells that produce IL-2. Thus, an autocrine mechanism does not appear to be operative in the autonomous growth of most of these neoplastic T-cell lines.

Examination of IL-2 receptor expression by ATL cell lines has revealed some intriguing differences when compared to lectin-activated normal T cells. HTLV$^+$ cell lines express a 50–100-fold higher density of IL-2 receptors than do normal T cells (H. M. Wang, K. A. Smith, unpublished). Identical results have been obtained employing quantitative equilibrium binding analysis using radiolabeled IL-2 and radiolabeled anti-Tac. In addition, IL-2 receptors of the leukemic cell lines bind IL-2 with a lower affinity than do normal cells. At this time, the reasons for these differences are not readily apparent. However, it could be postulated that leukemic cell IL-2 receptors are abnormal, and behave as though they are ligand-triggered in the absence of IL-2. Consequently, further studies comparing the structure and function of IL-2 receptors expressed by normal and leukemic T cells appear warranted.

The role of the retrovirus in IL-2 receptor expression by the ATL cells remains obscure. The entire HTLV genome has been cloned and sequenced by Yoshida and co-workers (34) and does not appear to contain a v-onc insert. Open reading frames that may code for several peptides are present at the 3′ end of the viral genome, but the significance of these nucleotide sequences is not yet evident. Proviral insertion and promotion of the IL-2 receptor gene expression is one obvious possiblity that has yet to be excluded. Whatever the relationship between HTLV and IL-2 receptor expression, it is anticipated that further investigation of the growth regulatory events of IL-2 receptor$^+$ neoplastic T cells may be fruitful. This is especially noteworthy in light of the critical role that the IL-2 receptor plays in mediating cell-cycle progression by normal T cells.

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CONCLUSION

In the past few years the investigation of the mechanism of T-cell proliferation has matured to the molecular level. Consequently, cellular immunology offers unique advantages for the exploration of the control of eukaryotic cell growth not to be found in any other biological system. Normal T cells can be isolated and cloned and appear to function physiologically in vitro. The critical determinant of T-cell mitosis has been identified and purified to homogeneity; the gene encoding the polypeptide has been molecularly cloned. Monoclonal antibodies reactive to the polypeptide and its receptor are available. The mecha-

Annual Reviews

nism of interaction of the peptide with responding T cells has been defined, with the result that detailed biochemical studies on the control of DNA replication are now possible. The appearance of two new diseases, the acquired immunodeficiency syndrome and adult T-cell leukemia, may well be examples of defects manifested by loss of the regulatory controls of cells that produce and respond to interleukin 2. Future experiments in this field will undoubtedly provide new insights into questions fundamental to the understanding of eukaryotic gene regulation, cell growth and differentiation, the regulation of the immune response, immunodeficiency, and leukemia.

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Literature Cited

1. Morgan, D. A., Ruscetti, F. W., Gallo, R. C. 1976. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 193:1007
2. Gillis, S., Smith, K. A. 1977. Long-term culture of cytotoxic T-lymphocytes. *Nature* 268:154
3. Smith, K. A. 1980. T-cell growth factor. *Immunol. Rev.* 51:337
4. Tanaguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R., Hamuro, J. 1983. Structure and expression of a cloned cDNA for human interleukin-2. *Nature* 302:305
5. Holbrook, N. J., Smith, K. A., Fornace, A. J., Comeau, C. C., Wiskocil, R. L., Crabtree, G. R. 1984. T-cell growth factor: complete nucleotide sequence and organization of the gene in normal and malignant cells. *Proc. Natl. Acad. Sci. USA*. In press
6. Smith, K. A., Favata, M. F., Oroszlan, S. 1983. Production and characterization of monoclonal antibodies to human interleukin-2: Strategy and tactics. *J. Immunol.* 131:1808
7. Copeland, T. D., Smith, K. A., Oroszlan, S. 1984. Characterization of immunoaffinity purified human T-cell growth factor from JURKAT cells. In *Thymic Hormones and Lymphokines*, ed. A. L. Goldstein. NY: Plenum. In press
8. Robb, R. J., Smith, K. A. 1981. Heterogeneity of human T-cell growth factor due to glycosylation. *Mol. Immunol.* 18:1087
9. Robb, R. J., Munck, A., Smith, K. A. 1981. T-cell growth factor receptors: quantitation, specificity and biologica[l] relevance. *J. Exp. Med.* 154:1455
10. Smith, K. A. 1983. T-cell growth factor, a lymphocytotrophic hormone. In *Genetics of the Immune Response*, ed. E. Moller, G. Moller, p. 151. NY: Plenum
11. Mier, J. W., Gallo, R. C. 1980. Purification and some characteristics of human T-cell growth factor from phytohemagglutinin-stimulated lymphocyte-conditioned media. *Proc. Natl. Acad. Sci. USA* 77:6134
12. Gillis, S., Ferm, M. M., Ou, W., Smith K. A. 1978. T-cell growth factor: parameters of production and a quantitative microassay for activity. *J. Immunol* 120:2027
13. Gillis, S., Henney, C. S. 1981. The biochemical and biological characterization of lymphocyte regulatory molecules. VI Generation of a B cell hybridoma whose antibody product inhibits interleukin 2 activity. *J. Immunol.* 126:1978
14. Stadler, B. M., Berenstein, E. H., Siraganian, R. P., Oppenheim, J. J. 198[2] Monoclonal antibody against human interleukin 2 (IL 2). I. Purification of IL 2 for the production of monoclonal antibodies. *J. Immunol.* 128:1620
15. Neufeld, E., Ashwell, G. 1979. Carbohydrate recognition systems for receptor mediated pinocytsis. In *Biochemistry of Glycoproteins and Proteoglycans*, ed. W Lennarz, p. 241. NY: Plenum
16. Goldwasser, E., Kung, C. K. H., Eliason, J. 1974. On the mechanism of erythropoietin-induced differentiation. XIII The role of sialic acid in erythropoietin action. *J. Biol. Chem.* 249:4202

Annual Reviews

17. Smith, K. A., Gillis, S., Baker, P. E., McKenzie, D., Ruscetti, F. W. 1979. T-cell growth factor mediated T-cell proliferation. *Ann. NY Acad. Sci.* 332:423
18. Bonnard, G. D., Yasaka, K., Jacobson, D. 1979. Ligand-activated T cell growth factor-induced proliferation: absorption of T cell growth factor by activated T cells. *J. Immunol.* 123:2704
19. Coutinho, A., Larsson, E.-L., Gronvik, K.-O., Andersson, J. 1979. Studies on T lymphocyte activation II. The target cells for concanavalin A-induced growth factors. *Eur. J. Immunol.* 9:587
20. Uchiyama, T., Broder, S., Waldmann, T. A. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J. Immunol.* 126:1393
21. Uchiyama, T., Nelson, D. L., Fleisher, T. A., Waldmann, T. A. 1981. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells, and on one of two types of helper cells. *J. Immunol.* 126:1398
22. Leonard, W. J., Depper, J. M., Uchiyama, T., Smith, K. A., Waldmann, T. A., Greene, W. C. 1982. A monoclonal antibody, anti-Tac, blocks the membrane binding and action of human T-cell growth factor. *Nature* 300:267
23. Smith, K. A., Wang, H. M., Cantrell, D. A. 1984. The variables regulating T-cell growth. *Prog. Immunol.* 5: In press
24. Osawa, H., Diamanstein, T. 1983. The characteristics of a monoclonal antibody that binds specifically to rat T lymphoblasts and inhibits IL 2 receptor functions. *J. Immunol.* 30:51
25. Cantrell, D. A., Smith, K. A. 1983. Transient expression of interleukin 2 receptors: Consequences for T-cell growth. *J. Exp. Med.* 158:1895
26. Pardee, A. B., Shilo, B.-Z., Koch, A. L. 1979. Variability of the cell cycle. In *Hormones and Cell Culture*, ed. G. H. Sato, R. Ross, p. 373. Cold Spring Harbor, NY: Cold Spring Harbor Lab.
27. Smith, K. A. 1982. T-cell growth factor-dependent leukemic cell growth: therapeutic implications. In *Adult Leukemias*, ed. C. D. Bloomfield, 1:43. The Hague: Martinus Nijhoff
28. Ceredig, R., Dialynas, D. P., Fitch, F. W., MacDonald, H. R. 1983. Precursors of T cell growth factor producing cells in the thymus: Ontogeny, frequency, and quantitative recovery in a subpopulation of phenotypically mature thymocytes defined by monoclonal antibody GK-1.5. *J. Exp. Med.* 158:1654
29. Yodoi, J., Takatsuki, K., Masuda, T. 1974. Two cases of T-cell chronic leukemia in Japan. *N. Engl. J. Med.* 290:572
30. Gazdar, A. F., Carney, D. N., Bunn, P. A., Russell, E. K., Jaffe, E. S., Schechter, G. P., Guccion, J. G. 1980. Mitogen requirements for the in vitro propagation of cutaneous T-cell lymphomas. *Blood* 55:409
31. Poiesz, B. J., Ruscetti, F. W., Mier, J. W., Woods, A. M., Gallo, R. C. 1980. T-cell lines established from human T-lymphocytic neoplasias by direct response to T-cell growth factor. *Proc. Natl. Acad. Sci. USA* 77:6815
32. Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., Gallo, R. C. 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* 77:7415
33. Gootenberg, J. E., Ruscetti, R. W., Mier, J. W., Gazdar, A., Gallo, R. C. 1981. Human cutaneous T cell lymphoma and leukemia cell lines produce and respond to T cell growth factor. *J. Exp. Med.* 154:1403
34. Seike, M., Hattori, S., Hirayama, Y., Yoshida, M. 1983. Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc. Natl. Acad. Sci. USA* 80:3618

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION

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INTRODUCTION

Arachidonic acid oxygenation products were for many years held to be synonymous with prostaglandins, but more recently they have been shown to include thromboxane A_2 and a multitude of lipoxygenase pathway products, most notably the leukotrienes. In their review of inflammatory mediators in the first volume of this series, Larsen & Henson (1) provided a broad perspective on how prostaglandins and leukotrienes play a central, often synergistic role with other mediators, both in the expression of the efferent limb of immune-based inflammatory responses and in acute inflammatory responses where specific immunity does not have a primary role.

Prostaglandins and leukotrienes are produced by most, if not all cells, participating in the afferent and efferent limbs of the immune system during host defense and inflammatory responses. Their formation depends on the deacylation of arachidonic acid from cellular phospholipids catalyzed by the activity

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of various phospholipases (2) and its subsequent utilization by cyclooxygenase and various lipoxygenase enzymes. Different cell types have widely varying capacities to synthesize arachidonic acid oxygenation products, both in terms of the nature of the products and the extent of their synthesis. The responsiveness of cells to prostaglandins and leukotrienes is also ubiquitous. The literature abounds with documentation of such pharmacological responses, and a major challenge facing investigators is whether such findings are relevant to the function of the responding cell in physiological and pathological situations. This question recurs repeatedly in this review. Here we focus on selected aspects of the effects and function of arachidonic acid oxygenation products in phenomena associated with inflammatory processes. We begin by summarizing the enzymes involved in, and the products formed during, arachidonic oxygenation. We discuss studies on the role of these products in the expression of classical signs of inflammation—namely erythema, increased vascular permeability, edema, pain, and fever. We provide a brief historical perspective and a summary of some recent studies, particularly with leukotrienes. We then describe the potent activities of leukotriene B_4 (LTB_4) on phagocytic cells and summarize some recent studies on the effects of arachidonic acid oxygenation products on various functions of lymphocytes.

THE ENZYMES AND PRODUCTS OF ARACHIDONIC ACID METABOLISM

Arachidonic acid is a polyunsaturated fatty acid present in large amounts within phospholipids of cell membranes. Little arachidonic acid occurs in the unesterified form; its liberation, however, may result in extensive metabolism to prostaglandins and leukotrienes by the pathways summarized below and shown in Figure 1. Free arachidonic acid may be liberated either directly by phospholipase A_2 or indirectly through the sequential action of phospholipase C and by diacylglycerol lipase (2). Metabolism via the cyclooxygenase pathway involves a bis-dioxygenation reaction with arachidonic acid to form the unstable endoperoxide intermediate prostaglandin G_2 (PGG_2), which is subsequently reduced by a hydroperoxidase to a second unstable endoperoxide intermediate, prostaglandin H_2 (PGH_2). These two activities are exhibited by one protein of molecular weight 70,000, which can be precipitated by a monoclonal antibody to the cyclooxygenase component (3,4). Immunocytochemical studies have shown the enzyme to be localized in the endoplasmic reticulum and nuclear envelope of 3T3 fibroblasts (5). PGH_2 breaks down nonenzymatically to produce prostaglandin E_2 (PGE_2) and prostaglandin D_2 (PGD_2). However, the ratios of PGE_2 to PGD_2 may be influenced by the presence of either a PGH-PGE isomerase or a PGH-PGD isomerase, and these enzymes can selectively lad to the production of either product. Unlike other enzymes

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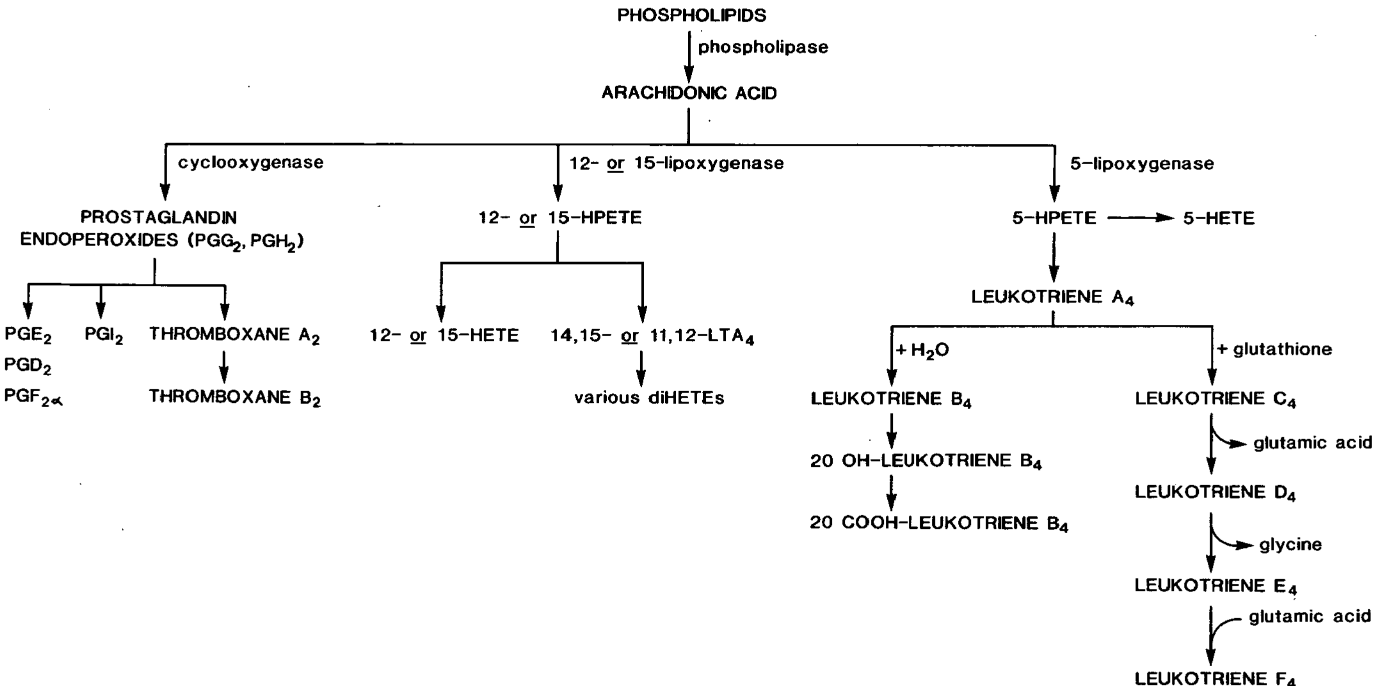

Figure 1 The pathways and products of arachidonic acid oxygenation.

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involved in the synthesis of prostaglandins, prostaglandin endoperoxide E isomerase is a soluble enzyme (6).

In addition, prostaglandin endoperoxides may be metabolized either by prostaglandin I_2 synthase to produce prostaglandin I_2 (PGI_2) or by thromboxane A_2 (TXA_2) synthase to produce TXA_2. PGI_2 and TXA_2 are unstable intermediates with important biological properties. PGI_2 is a vasodilator, inhibits platelet aggregation, and inhibits brochoconstriction whereas TXA_2 is a vasoconstrictor, promotes platelet aggregation, and causes bronchoconstriction. Both PGI_2 and TXA_2 break down rapidly through non-enzymatic reactions to produce respectively 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 (TXB_2), both compounds being devoid of biological activity. PGI_2 synthase has been purified as a 52,000-dalton hemoprotein from bovine aorta (7), and immunocytochemical studies have shown it to be present in both vascular and nonvascular smooth muscle with a characteristic pericellular localization in some cell types (8).

Arachidonic acid may be metabolized by lipoxygenase enzymes to produce corresponding hydroperoxyeicosatetraenoic acids (HPETEs) (9). These HPETEs may then be metabolized through the glutathione peroxidase system to produce the corresponding monohydroxyeicosatetraenoic acids (HETEs). Products of the 5-lipoxygenase pathway are of particular importance due to the production of biologically active leukotrienes. 5-HPETE may be converted by a dehydrase step to an unstable epoxide intermediate, leukotriene A_4 (LTA_4). Subsequent enzymic metabolism of LTA_4 involves either the addition of water by an epoxide hydrolase to produce a 5-12-dihydroxyeicosatetraenoic acid with a precise stereochemical configuration (LTB_4) or may involve conjugation with glutathione to produce a peptido-lipid conjugate, leukotriene C_4 (LTC_4). LTC_4 may be subsequently metabolized in a stepwise fashion through the loss of glutamic acid and glycine and readdition of glutamic acid to produce, respectively, leukotrienes D_4 (LTD_4), E_4 (LTE_4), and F_4 (LTF_4). LTC_4 and LTD_4, and to a lesser extent LTE_4, account for the biological activity known as slow-reacting substance of anaphylaxis. These biologically active leukotrienes are rapidly metabolized. LTB_4 may be oxidized to less biologically active compounds through ω-oxidation (10), and peptido-lipid leukotrienes may be oxidized to sulfoxides followed by cleavage of the peptide portion to produce 5,12-diHETEs (11). Further metabolism of leukotrienes probably involves extensive β-oxidation. In addition to the 5-lipoxygenase pathway, 12- and 15-lipoxygenase enzymes have been described that may lead to the production of leukotriene epoxides (11,12-LTA_4 and 14,15-LTA_4) which may be converted to a variety of dihydroxyeicosatetraenoic acids (12). No biological functions have been described to these alternative leukotrienes, and until such functions are described these compounds must be considered as mere chemical curiosities.

Little progress has been made with the purification of lipoxygenases and

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other enzymes involved in leukotriene synthesis (13). The 5-lipoxygenase enzyme is an easily solubilized, calcium-dependent enzyme that has not yet been fully purified. The enzyme has been characterized in rat basophilic leukemia cells (13) and guinea-pig peritoneal granulocytes (14). It has a specific requirement for calcium ions and is stimulated by several nucleotides, ATP being most effective (14). In a rat basophilic leukemic cell line both LTA_4 synthase and LTA_4 hydrolase are soluble while glutathione-S-transferase and γ-glutamyl transpeptidase are membrane bound (13). A 12-lipoxygenase has been characterized in rat lung (15) and a 15-lipoxygenase has been partially purified from rabbit peritoneal granulocytes (16).

ARACHIDONIC ACID OXYGENATION PRODUCTS AS MEDIATORS OF PAIN

Cyclooxygenase Products

Pain, one of the cardinal signs of inflammation, is a complex response involving the interaction of multiple inflammatory mediators released at sites of inflammation and tissue injury. The role of prostaglandins in pain following mechanical or chemical injury is well established (17). Prostaglandins have been detected in inflammatory exudates (18–20); and their interaction with other inflammatory mediators, such as histamine, serotonin, bradykinin, and substance P, is generally accepted as eliciting a hyperalgesic response, or an increased sensitivity to touch. There are many indications that the primary role of prostaglandins is the sensitization of pain receptors to other mediators. These mediators are derived from several sources, including cells (prostaglandins, leukotrienes, histamine, serotonin), nerve terminals (substance P), and humoral precursors (kinins) (21–26). The release of histamine and serotonin from mast cells and platelets can be stimulated by mechanical trauma, heat, radiation, enzymes such as lysosomal hydrolases or thrombin, collagen, arachidonic acid oxygenation products, or by specific immunological triggering (27–29). Bradykinin is formed upon activation of the coagulation system (30). When applied locally these mediators activate small-diameter afferent fibers to produce pain (31–33). In addition, when histamine, bradykinin, or slow-reacting substance of anaphylaxis (SRS-A, a mixture of LTC_4, LTD_4, and LTE_4) are applied to a cantharidin-induced blister or injected into the skin, a painful response is evoked (34–36).

The intradermal injection of prostaglandins alone at low doses or the application of E-type prostaglandins to a blister base did not cause overt pain (37,38). However, the edematous region induced by the injection of PGE_1 and PGE_2 became hyperalgesic (39). Extremely high doses of prostaglandins were needed to cause overt pain (38).

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In humans, intradermal injection of PGE_1 or PGE_2 produced hyperalgesia of longer duration than histamine or bradykinin (40). Subdermal infusions of PGE_1 and PGE_2 in humans, used to mimic the continuous release of an endogenous mediator of pain, revealed that prostaglandins increased the pain sensitivity to chemical and mechanical stimulation (40). In addition, the effects of the two prostaglandins were cumulative and dependent not only on the doses given but also on the time of exposure (40). Infusion of low doses of bradykinin or histamine did cause a painful sensation; but, if mixtures of PGE_1 and bradykinin or histamine were given, a strong pain response resulted. Bradykinin and histamine, infused together, cause no pain. Skin made hyperalgesic with a PGE_1 infusion demonstrated a greater degree of pain intensity upon a second infusion of bradykinin or histamine (40). No such sensitization occurred at the site at which bradykinin or histamine had been previously infused. The hydroperoxides of linoleic, linolenic, and arachidonic acid, when given intradermally in humans, caused a more intense, immediate hyperalgesia of short duration (40). Thus, prostaglandins, in the amounts released during inflammation, may produce hyperalgesia by sensitization of pain receptors (40).

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Consistent with this idea, evidence has been obtained that prostaglandins mediate painful responses induced by other mediators. Evidence implicates prostaglandins in the mediation of bradykinin-induced writhing in mice (41), reflex pressor responses in dogs following intraarterial administration of bradykinin to the spleen (42–44), and pain responses in the dog knee following intraarticular injection of bradykinin (26). These results have been interpreted to support the concept that prostaglandin release does not directly mediate the pain-producing action of bradykinin but that prostaglandins, such as PGE_2 and PGI_2, sensitize the pain receptors through an action on afferent nerve endings. Prostaglandins sensitize pain receptors in the rat paw, (45) the effect of PGI_2 being more potent, of more rapid onset, and of shorter duration than that of PGE_2 (46). Prostaglandins, probably PGI_2, have been implicated in rat-paw hyperalgesia induced by carrageenan (46).

Prostaglandins of the E series elevate cyclic AMP levels in nervous tissue (47). Intraplantar injection of dibutyryl cyclic AMP (Db-cAMP) in the rat caused a dose-related hyperalgesia whose onset was faster than that induced by PGE_2 (48). The phosphodiesterase inhibitory methylxanthines, caffeine and theophylline, potentiated the hyperalgesic effect of Db-cAMP and that of PGE_2 while the Ca^{2+} ionophore, A23187, and $BaCl_2$ induced a dose-dependent hyperalgesia in the rat paw (48). Since verapamil blocks prostaglandin hyperalgesia (48), these findings suggested that an increase in intracellular cyclic AMP and Ca^{2+} concentrations resulted in hyperalgesia (48). It is of interest that an intravenous injection of Db-cAMP in humans caused abdominal pain, headache, myalgia, and other types of pain (49). Cycloheximide, an inhibitor of protein synthesis, blocked the hyperalgesic effect of PGE_2 and

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PGI_2 in the rat paw but not that induced by Db-cAMP (50). Thus, it appeared that cycloheximide affected prostaglandin-induced hyperalgesia by interfering with some metabolic process and not the ionic events directly related to the evocation of generator potentials.

Lipoxygenase Products

Certain fatty acid hydroperoxides, formed as lipoxygenase products, caused pain in humans when infused intradermally (40). The intensity of pain caused by the hydroperoxides was greater than that induced by the parent fatty acid and other peripherally acting hyperalgesic agents, including PGE_1. LTB_4 caused a hyperalgesic response 1–5 hr after subplantar administration in the hindpaw of rats (51). Combinations of PGE_1 and LTB_4 elicited painful responses that did not differ from those observed with either agent used alone. Under the same conditions LTD_4 caused a hyperalgesic response of delayed onset. However, LTD_4 combined with PGE_1 elicited an immediate hyperalgesic response (51). In human skin, LTD_4 and LTC_4 caused a slight sensation of pain (52).

ARACHIDONIC ACID OXYGENATION PRODUCTS AS MEDIATORS OF FEVER

Fever results from the release of endogenous pyrogen from mononuclear phagocytes by a number of inflammatory stimuli, including gram-negative bacterial polysaccharides, immune complexes, and lymphokines. Upon its release pyrogen is thought to reach the hypothalamus where it exerts its pyrogenic activity via a protein synthesis–dependent, prostaglandin-mediated step (53,54). Pyrogen is now considered to be closely related, if not identical, to interleukin 1 (55,56). Indeed Duff & Durum (57) recently showed that a single preparation of interleukin 1 was both pyrogenic in mice and stimulatory of T-lymphocyte proliferation. This latter response was greatly enhanced when lymphocytes were cultured at elevated temperatures characteristic of fever. It was suggested that this exaggerated T lymphocyte response may reflect one reason for the retention of pyrogenic responses in contemporary phyla (57).

The capacity of exogenous prostaglandins to cause fever is well established, and the involvement of endogenous prostaglandins in fever was proposed by Vane upon the discovery that antiinflammatory and antipyretic drugs inhibited prostaglandin synthesis (58). The symptoms of fever were reproduced when low concentrations of PGE_1 (3×10^{-11}M) were injected directly into the central nervous system of a conscious cat (59). The site of action of PGE_1 is thought to be the preoptic area of the anterior hypothalamus, the postulated site of the thermoregulatory center. PGE_2 is equiactive with PGE_1 in causing fever whereas $PGF_{2\alpha}$, $PGF_{1\alpha}$, and PGA_1 are only slightly active (59). PGE_2 is one of the most potent pyretic agents known, and elevated levels of PGE_2

Annual Reviews

were found in cerebrospinal fluids (CSF) taken from pyrexic patients suffering from infections of viral and bacterial origin, viral encephalitis, pyrogenic meningitis, or fevers of unknown origin (60). No prostaglandin-like activity was present in the CSF of afebrile patients (60). The febrile response to PGE_2 has been confirmed in many mammalian species, including monkey, sheep, mouse, rabbit, and rat. Fever was induced in women undergoing abortion induction with an infusion of 200 μg/min of $PGF_{2\alpha}$ (61). The role of leukotrienes, if any, in fever is unknown at present.

ARACHIDONIC ACID OXYGENATION PRODUCTS AS REGULATORS OF BLOOD FLOW AND MEDIATORS OF INCREASED VASCULAR PERMEABILITY AND EDEMA

Cyclooxygenase Products

Increased blood flow and vascular permeability with resultant edema are two further cardinal features of tissue injury caused by trauma or more specific immunological events.

Exogenous prostaglandins have been shown to be poor inducers of edema formation in some species, such as guinea pigs (62) and rabbits (63), while in other species, such as the rat (37) and human (37,64), PGE_2 did cause edema. Regardless of the species, prostaglandins of the E series have been shown to synergize with other mediators, such as histamine and bradykinin, to cause increased vascular permeability and edema (63,64). These observations led Williams and his colleagues to analyze separately the effects of prostaglandins on blood flow and vascular permeability in the rabbit (63,65). They concluded that whereas PGE_2 (63) and PGI_2 (65) are primarily inducers of increased blood flow in rabbit skin, they also synergize with other mediators to promote increases in vascular permeability (66,67).

Subsequently Williams and his colleagues have demonstrated the role of endogenously synthesized cyclooxygenase products in inflammatory edema. Increased vascular permeability in rabbit skin caused by zymosan was inhibited by the cyclooxygenase inhibitor indomethacin (66); the response was reconstituted by exogenous PGE_1. Further experiments showed that the mediator synergizing with prostaglandins was C5a (68), presumably generated by activation of the alternate pathway of complement by zymosan. C5a alone did not increase vascular permeability, indicating the synergistic requirement for prostaglandin E_1. The function of C5a was shown to be dependent on the presence of granulocytes because rabbits depleted of these cells by treatment with nitrogen mustard did not show increases in vascular permeability induced by C5a and PGE_1 (67). Other evidence indicating that the granulocyte may mediate increased vascular permeability was indicated by the synergistic activ

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ity of PGE_1 with other chemotactic factors such as formyl methionyl leucyl phenylalanine (FMLP) and LTB_4 (67,69). The observation that granulocytes recruited and/or activated by C5a at sites of inflammation mediate increased vascular permeability provides a clear indication that these cells are critical for this function in immune-based inflammation where complement activation occurs following antigen-antibody complex formation. The precise mechanism by which the granulocyte mediates increased vascular permeability remains to be established. Issekutz and his colleagues have induced vascular permeability and tissue injury in rabbit skin with zymosan-activated plasma (70,71) without the addition of exogenous vasodilator prostaglandin. In addition, it has been shown that indomethacin treatment reduced granulocyte accumulation but failed to affect the wet weight of reverse-passive Arthus lesions in rats (72). This suggests (but does not prove) that prostaglandins increase blood flow without necessarily contributing to edema formation under these conditions. The increased vascular permeability response in acute inflammatory reactions is, therefore, a multi-component system in which a primary role of PGE_2 and PGI_2 is to increase blood flow. The source of these endogenous prostaglandins remains a matter of conjecture. The most prolific source of PGE_2 and PGI_2 is the vascular endothelium (73); but other cells, such as tissue macrophages, present in the perivascular connective tissue are also potential sources of these mediators.

In contrast to the proinflammatory effects of endogenous prostaglandins at sites of inflammation discussed above, reports have appeared documenting the antiinflammatory activity of administered prostaglandins of the E series in a number of animal models. In the rat, PGE_1 suppressed increased vascular permeability caused by histamine, bradykinin, serotonin, and C3a (74); it also suppressed adjuvant arthritis in the rat (75), glomerulonephritis in the NZB/W mouse (76), and immune complex vasculitis (77).

Some recent in vivo and in vitro studies on the effect of PGE_1 on granulocyte function may shed some light on the mechanism(s) of its in vivo antiinflammatory activity. Fantone and his colleagues (78) have found that treatment of rats with the stable prostaglandin analog 15-(S)-15-methyl prostaglandin E_1 reduced the capacity of peritoneal granulocytes to secrete the lysosomal enzyme marker *N*-acetyl-β-D-glucosaminidase and to synthesize superoxide anion in response to FMLP. Also a diminished affinity of cellular receptors for FMLP was seen in granulocytes of treated animals. These studies raise the possibility that the antiinflammatory activity of prostaglandin E may be mediated by inhibiting granulocyte responses to chemotactic stimuli. This possibility requires further investigation with other chemotactic stimuli, such as C5a, as well as other cells participating in inflammatory responses.

A number of in vitro studies have shown that pharmacological concentrations of prostaglandin inhibit the release of lysosomal enzymes and the syn-

Annual Reviews

thesis of superoxide anions by granulocytes (79). Ham et al (80) have recently shown that both PGE_1 and PGE_2 at low concentrations (in the region of 10^{-8}–10^{-7}M) inhibited the FMLP-stimulated synthesis of LTB_4 by rat peritoneal and human peripheral blood granulocytes. The authors suggest that this could be of significance in vivo where the LTB_4-mediated aggregation and adhesion of granulocytes to endothelium/basement membrane could be modulated by prostaglandin E_2 or I_2 produced by the vascular endothelium.

Lipoxygenase Products

Products of the lipoxygenase pathway have widely differing effects on blood flow and vascular permeability, with considerable species-to-species variation. Exogenous LTC_4 and LTD_4 caused vasoconstriction in guinea-pig skin (81,82), LTC_4 being more potent than LTD_4. LTD_4 also caused a clear increase in vascular permeability, an effect potentiated by PGE_2. Indeed the combination of LTD_4 with PGE_2 or PGE_1 was approximately 400 times more potent than histamine in promoting plasma leakage. In contrast, little exudation was induced by a combination of LTC_4 and PGE_1, although significant plasma exudation was seen when LTC_4 was combined with the more potent vasodilator PGE_2.

When applied topically to the vascular network of the hamster cheek pouch (83), LTC_4 and LTD_4 caused an intense constriction of capillaries and were as potent as angiotensin in this respect. The vasoconstriction was brief and was followed by a concentration-dependent extravasation of protein from postcapillary venules. Granulocyte depletion failed to abolish LTC_4-induced plasma leakage indicating a direct action of LTC_4 on the endothelial lining of the blood vessel wall. In marked contrast, LTC_4 and LTD_4 cause vasodilation in the human forearm (52,84), and the increased blood flow observed was equal to that caused by equimolar amounts of histamine.

LTC_4 administered subcutaneously in a rat also caused a marked increase in vascular permeability (85), although in contrast to the guinea pig, PGE_2 had no potentiating effect on the increased vascular permeability induced by LTC_4. Woodward et al (86) have shown that the intratracheal injection of LTC_4, LTD_4, and LTE_4 caused a dose-dependent increase in the permeability of guinea-pig lung microvasculature. This was antagonized by the leukotriene antagonist FPL 55712, but not by mepyramine or indomethacin, again suggesting a direct effect on the capillary wall. In a comparative study in several species, Ueno et al (87) have established further the variations discussed above. In rabbits LTC_4 or LTD_4 failed to cause increased vascular permeability, in contrast to the significant effect seen in guinea pigs and rats.

As will be discussed below, LTB_4 is noted mainly for its effects as a potent chemoattractant for granulocytes. Bray et al (69,88) have shown it to be a potent stimulator of vascular permeability in rats, rabbits, guinea pigs, and mice in the presence of a vasodilator such as PGE_2. In the hamster cheek

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Table 1 Prostaglandins and leukotrienes as mediators of increased blood and vascular permeability

Vascular bed	Mediator	Biological effect	Reference
Rabbit skin	PGE_1, PGE_2, PGI_2	vasodilation	63,65
	LTB_4	vascular permeability	67,69
Guinea-pig skin	LTC_4, LTD_4	vasoconstriction	81,82
	LTD_4	vascular permeability	87
	LTB_4	vascular permeability	67,69
Hamster cheek pouch	LTC_4, LTD_4	vasoconstriction	83
	LTC_4, LTD_4	vascular permeability	83
	LTB_4	vascular permeability	83
Human skin	PGE_1	vascular permeability	64
	LTC_4, LTD_4	vasodilation	52,84
Rat skin	PGE_1	vascular permeability	37
	LTC_4	vascular permeability	85
	LTB_4	vascular permeability	88

pouch preparation, LTB_4 was found to be 100 times less potent than the cysteinyl-containing leukotrienes in causing macromolecular leakage (83). In this study a correlation was noted between the number of leakage spots and the number of granulocytes accumulating in the vessels and sticking to the endothelial lining. This dependence on granulocytes of LTB_4 in inducing plasma leakage has been discussed above in relation to studies in the rabbit by Wedmore & Williams (67), who demonstrated that LTB_4 alone produces little increase in vascular permeability but is potent in this respect in combination with PGE_2. It is clear therefore that prostaglandins and leukotrienes have potent effects on blood flow and vascular permeability. It is equally apparent that these effects are not consistent from species to species. Some of these differences are summarized in Table 1.

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THE EFFECT OF ARACHDIONIC ACID METABOLITES ON PHAGOCYTIC CELL FUNCTION

Effect on Phagocytic Cells In Vitro

The first evidence that an arachidonic acid metabolite could be important in eliciting the chemotaxis of granulocytes came in 1971 when Kaley & Weiner demonstrated that PGE_1 stimulates the chemotaxis of rabbit peritoneal granulocytes (89). Subsequent studies, however, have shown that PGE_1 is only chemotactic for this particular cell population and has no effect on peripheral granulocytes from humans, rabbits, or rats (90). Other studies have suggested that another cyclooxygenase product, TXB_2, is chemotactic for granulocytes

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(91); but these results have not been confirmed using synthetic TXB_2 (92). The most potent chemotactic product of the cyclooxygenase pathway was shown to be 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (92), but even this compound was a weak chemotactic agent when compared with lipoxygenase products of arachidonic acid metabolism.

The first suggestion that a lipoxygenase product possessed chemotactic activity was that of Turner et al, who demonstrated that 12-HETE, derived from platelets, was chemotactic for granulocytes at high concentrations (93). Subsequent studies have shown that a number of mono-HETEs possess chemotactic activity for granulocytes, the most potent being 5-HETE (94). Subsequently, it has been shown that LTB_4 is a potent chemokinetic and chemotactic agent for granulocytes (95–97). This compound is an order of magnitude more potent than the mono-HETEs and shows comparable activity to other known chemotactic factors such as the complement-derived peptide C5a and FMLP (98). LTB_4 has many of the properties associated with these other chemotactic factors and stimulates the chemotaxis, chemokinesis, and aggregation of leukocytes (95–97); stimulates ion fluxes (99) and the expression of C3b receptor sites (100); and, in the presence of cytochalasin B, is a weak inducer of enzyme release (97,101) and superoxide anion production (102). These and other properties of LTB_4 are summarized in Table 2. LTB_4 also has effects on other cell types, including eosinophils and macrophages. It is a potent chemotactic factor for eosinophils and enhances C3b receptor site expression on these cells (100). It is probable that LTB_4 was first identified as the biological activity known as eosinophil chemotactic factor of anaphylaxis (ECF-A) (105). LTB_4 has been reported to stimulate the chemokinesis of human monocytes and rat macrophages (96), and enhanced responsiveness to LTB_4 has been observed in monocytes from patients with various skin diseases (106).

It has been suggested on the basis of model studies using artificial membrane systems (liposomes) that LTB_4 may exert its biological actions by acting as a calcium ionophore (107). Indeed biological activation of the granulocyte by LTB_4 is associated with an influx of calcium ions like that demonstrated for other chemotactic factors (99). While the putative ionophore properties may account for some of the actions of LTB_4, such as contraction of the guinea pig parenchymal strip (108), it is unlikely that all the chemotactic actions of the compound can be explained through this mechanism. It is more probable that LTB_4 interacts with specific receptor sites on the granulocyte plasma membrane, as has been reported for C5a and FMLP (109). In support of this hypothesis, specific binding of LTB_4 to high-affinity receptor sites on the granulocyte surface has been reported (110).

Other lipoxygenase products may also have a role in modifying leukocyte function. Granulocytes inactivate LTB_4 through ω-oxidation to produce 20

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Table 2 In vitro biological activities of leukotriene B_4

Target cell	Biological activity	Reference
Polymorphonuclear leukocyte. eosinophil	chemotaxis	96,97
Polymorphonuclear leukocyte, eosinophil, macrophage, monocyte	chemokinesis	95,96
Polymorphonuclear leukocyte	aggregation	95
Polymorphonuclear leukocyte	adherence	114
Polymorphonuclear leukocyte	enhancement of lysosomal enzyme release	97,101
Polymorphonuclear leukocyte	superoxide anion production	102
Polymorphonuclear leukocyte	stimulation of Ca^{2+} and rat influx	99
Polymorphonuclear leukocyte	elevation of intracellular cyclic AMP levels	103
Polymorphonuclear leukocyte. eosinophil	enhancement of C3b receptor site expression	100
Polymorphonuclear leukocyte. eosinophil	enhancement of complement dependent cytotoxicity reactions	104
T lymphocytes	suppression of proliferation and leukocyte inhibitory factor production	143
T lymphocytes	induction of suppressor cell activity	145

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OH LTB_4 and 20-COOH LTB_4 (111). This inactivation is associated with loss of biological activity (108,111). Mono-HETEs, in particular 5-HETE, may also have a role in granulocyte function, either through their weak chemotactic activity (94) or through their ability to modulate lysosomal enzyme release. In connection with the latter property, 5-HETE has been shown to potentiate the granulocyte degranulating actions of platelet activating factor, LTB_4, and phorbol myristate acetate (112,113). LTC_4 and D_4 have no effect on the chemotaxis or degranulation of granulocytes (97). However, they have been reported to enhance the adherence of granulocytes, possibly through a mechanism involving generation of TXA_2 (114).

Effects on Phagocytic Cell Accumulation In Vivo

Consonant with their relative inactivity as chemotactic factors in vitro, prostaglandins and hydroxy- and hydroperoxy-eicosatetraenoic acids have little or no effect on granulocyte migration in vivo (115). LTB_4, on the other hand, has been demonstrated to be a potent inducer of leukocyte infiltration in a variety of models. It has been shown to induce granulocyte and macrophage

Annual Reviews

infiltration into the guinea-pig peritoneal cavity (96), granulocyte migration into rabbit skin and hamster cheek pouch (116), granulocyte migration into the rabbit eye (117), and granulocyte migration into human skin (118). In addition, topical application of LTB_4 to human skin results in the production of intraepidermal microabscesses that are histologically similar to lesions observed in pustular psoriasis (119). Together with the discovery of elevated levels of LTB_4 in psoriatic lesions (120), it has been suggested that this leukotriene may be an important mediator of leukocyte accumulation in this disease (121). LTB_4 has also been reported to be present in other granulocyte-rich inflammatory exudates, including sponge exudates in the rat (122), gastrointestinal mucosa from patients with inflammatory bowel disease (123), and synovial fluid from patients with either rheumatoid arthritis or gout (124,125). These results suggest that LTB_4 may be an important mediator of leukocyte accumulation in pathological situations.

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THE EFFECTS OF ARACHIDONIC ACID OXYGENATION PRODUCTS ON LYMPHOCYTE FUNCTION

The effectiveness of prostaglandins as pharmacological modulators of lymphocyte function became apparent more than a decade ago with many observations indicating that PGE_1 or PGE_2 suppressed various responses through a mechanism presumed to involve stimulation of adenyl cyclase and subsequent increases in the levels of cellular cyclic AMP (126). One of the first indications that endogenous, leukocyte-derived prostaglandins had immunoregulatory functions came from Morley and his colleagues (127), who suggested that monocyte/macrophage-derived prostaglandins have important immunoregulatory functions. Subsequently studies from many laboratories have documented that mononuclear phagocytes from various sources produce a variety of arachidonic acid oxygenation products, both prostaglandins and leukotrienes. In some instances these products are synthesized on a constitutive basis; in addition, stimuli and mediators associated with inflammatory responses greatly enhance the synthesis of one or more of these products. This information has been reviewed in detail elsewhere (128–130). Many studies have indicated that arachidonic acid oxygenation products synthesized by monocytes suppress various functions of lymphocytes with which they are cocultured and that such inhibitory effects are more pronounced when the leukocytes are obtained from patients with overt immunosuppression. These extensive studies have been thoroughly reviewed elsewhere (131,132). In this section we discuss two distinct areas concerning the role of arachidonic acid oxygenation products and lymphocyte function: first, the altered sensitivity of lym-

Annual Reviews

phocyte populations from patients with inflammatory disease to prostaglandins; and second, the pharmacological effects of lipoxygenase products upon lymphocytes.

Increased Sensitivity of Lymphocytes to Exogenous Prostaglandins in Inflammatory Diseases

Several sources indicate that lymphocytes from patients suffering from immune-based chronic inflammatory diseases have an increased sensitivity to exogenous prostaglandins. In multiple sclerosis the adherence of lymphocytes was significantly more sensitive to inhibition by added PGE (133,134). In rheumatoid arthritis a subpopulation of E rosetting lymphocytes was identified that was sensitive to modulation by low concentrations of PGE_1 and PGE_2 in the region of 10^{-11}M (133). Phytohemagglutinin-stimulated lymphocytes from individuals of greater than 70 years of age were also shown to possess greater sensitivity to inhibition of their responses by PGE_2 (135).

Recent studies have suggested that the defective inhibition of EBV-virus-induced transformation of B lymphocytes in rheumatoid arthritis patients (136,137) depends on a prostaglandin-sensitive T lymphocyte (138). The function of this cell may involve the production of γ-interferon produced by autologous and allogeneic mixed-leukocyte reactions (139). T lymphocytes from rheumatoid arthritis patients fail to produce γ-interferon in an autologus mixed-lymphocyte response (138). This defect was found to be dependent on adherent cells, possibly monocytes, and it was reversed by indomethacin, indicating a role for cyclooxygenase products. This possibility was not supported by direct measurement of prostaglandin E levels in 72-hr autologous mixed-lymphocyte response supernatants from normal subjects and rheumatoid arthritis patients, no significant differences being found. These observations contrast with other findings (140) that peripheral-blood mononuclear cells from rheumatoid arthritis patients produced more PGE_2 than normal age- and sex-matched individuals. That the cultures may produce other cyclooxygenase products, such as PGI_2 (141), in varying amounts could account for the differential regulation of interferon production. This study also shows that extraordinarily low concentrations of exogenous PGE_1, in the range of 10^{-13}M–10^{-12} M, inhibited production of suppressive supernatants for EBV-induced B-lymphocyte transformation by rheumatoid cells (136). These concentrations were several orders of magnitude below the reported K_D for PGE_2 for various cell types (142). This effect was readily reversed by indomethacin, suppression then only being seen with 10^{-7}–10^{-6}M prostaglandin E_1, levels that were also effective in suppressing the production by lymphocytes from normal individuals of supernatants suppressing EBV-induced B-lymphocyte transformation. This suggested an additive or synergistic effect between the very low levels of exog-

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enously added PGE_1 and an endogenous substance produced by an indomethacin-sensitive mechanism.

Effects of Lipoxygenase-Pathway Products on Lymphocyte Function

In a survey of the effects of leukotrienes on PHA-induced human T-lymphocyte responses, Payan & Goetzl (143) found that LTB_4 at a concentration of 1×10^{-7}M or greater significantly inhibited the production of soluble factors inhibiting leukocyte migration (LIF). This effect was accompanied by a slight inhibition of proliferation at these same concentrations. 12(S)LTB_4, LTC_4, LTD_4, and LTE_4 were inactive as inhibitors of LIF production and of proliferation. This contrasts with the findings that LTD_4 and LTE_4 may suppress murine lymphocyte transformation (144). It has also been reported that LTB_4 may induce human suppressor-cell activity at exceedingly low (1×10^{-10}M–1×10^{-14}M) levels in a non-concentration-related way (145). This activity was not exhibited by LTD_4 over a wide concentration range.

Gualde et al (146) have examined the effect of a series of HPETEs on several parameters of lymphocyte function. When tested at a single concentration of 3.7×10^{-6}M, 5-, 8-, 9-, 11- and 12-HPETE caused pronounced suppression of murine spleen lymphocyte responsiveness to PHA or Concanavalin A. Similar inhibitory effects of HPETEs were seen with mixed-lymphocyte reactions and the generation of cytotoxic splenocytes. The injection of 100 μg 15-L-HPETE on three successive days grossly reduced the responsiveness of murine splenocytes to Concanavalin A. The authors speculated that the mechanism of inhibition involves the incorporation of the HPETEs into lymphocyte membranes. The use of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene showed that Concanavalin A increases membrane fluidity; preliminary data showed that 15-HPETE reduces this increase to control levels (146). This is consistent with the incorporation of the 15-HPETE, or more probably the reduced form, 12-HETE, into cell membranes. Similar reincorporation of HETE has been reported to occur in neutrophils (147). The effects of 15-HPETE on lymphocyte responsiveness to mitogens has also been reported with human lymphocytes; T cells that are OKT8(+) are less susceptible to inhibition than OKT8(−) cells (146).

Early indications that lymphocytes produced lipoxygenase-pathway products were reported by Parker et al (148). Subsequently Goetzl (149) has shown that human peripheral lymphocytes synthesize a variety of lipoxygenase-pathway products, of which 5- and 15-HETE predominate. LTB_4 was also detected in lower amounts. The levels of these products were increased by calcium ionophore, exogenous arachidonic acid, and Concanavalin A. 15-HETE inhibited induced increases in 5-HETE and LTB_4 but was ineffective in altering 11-HETE production.

Annual Reviews

15-HETE is a potent and specific inhibitor of lipoxygenases in platelets and granulocytes (150). Bailey et al have shown that 15-HETE inhibits PHA-induced proliferation of murine splenocytes while lacking such activity on LPS-induced proliferation in the same system (151). Aspirin, presumed to act as a cyclooxygenase inhibitor, enhanced PHA-induced proliferation, an effect that was reversed in the presence of 15-HETE. Based on these observations it was speculated that 15-HETE inhibits the production of certain lipoxygenase pathway products, such as 5-HETE and 5,12-diHETE, shown to be produced by proliferating T cells (149). Goodman & Weigle (152) have also reported inhibitory effects of 15-HPETE acid on the response of immune splenocytes to mitogens.

These early observations point out that the diverse products of the lipoxygenase pathways may have major modulatory effects in lymphocyte function. Since other cells participating in inflammatory responses, including granulocytes, macrophages, mast cells, and also platelets, are rich sources of lipoxygenase-pathway products, they can also be expected to exert regulatory effects on lymphocyte function. Thus leukotrienes and other lipoxygenase-pathway products may well emerge as potent endogenous immunoregulant mediators, each with a characteristic profile of activity.

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Literature Cited

1. Larsen, G. L., Henson, P. M. 1983. Mediators of inflammation. *Ann. Rev. Immunol.* 1:335
2. Irvine, R. 1982. How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.* 204:3
3. Miyamoto, T., Ogino, N., Yamamoto, S., Hayaishi, O. 1976. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J. Biol. Chem.* 251:2629
4. Marnett, L. J., Dix, T. A., Sachs, R. J., Siedlick, P. H. 1983. Oxidation by fatty acid hydroperoxides and prostaglandin synthase. *Adv. Prostagl. Thrombox. Leukotr. Res.* 11:79
5. Rollins, T. E., Smith, W. L. 1980. Subcellular localization of prostaglandin-forming cyclooxygenase in Swiss mouse 3T3 fibroblasts by electron microscopic immunocytochemistry. *J. Biol. Chem.* 255:4872
6. Ogino, N., Miyamoto, T., Yamamoto, S., Hayaishi, O. 1977. Prostaglandin E endoperoxide isomerase from bovine vesicular gland microsomes, a glutathione-requiring enzyme. *J. Biol. Chem.* 252:890
7. Dewitt, D. L., Smith, W. L. 1983. Purification of prostacyclin synthase from bovine aorta by immunoaffinity chromatography. Evidence that the enzyme is a hemoprotein. *J. Biol. Chem.* 258:3285
8. Smith, W. L., Dewitt, D. L., Day, J. S. 1983. Purification, quantitation, and localization of PGI_2 synthase using monoclonal antibodies. *Adv. Prostagl. Thrombox. Leukotr. Res.* 11:87
9. Samuelsson, B. 1983. Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* 220:568
10. Jubiz, W., Radmark, O., Malmsten, C., Hansson, G., Lindgren, J. A., Palmblad, J., Uden, A., Samuelsson, B. 1982. A novel leukotriene produced by stimulation of leukocytes with formylmethionylleucylphenylalanine. *J. Biol. Chem.* 257:6106
11. Lee, C. W., Lewis, R. A., Corey, E. J., Barton, A., Oh, H., Tauber, A. I., Aus-

Annual Reviews

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org
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ten, F. K. 1982. Oxidative inactivation of leukotriene C_4 by stimulated human polymorphonuclear leucocytes. *Proc. Natl. Acad. Sci. USA* 79:4166

12. Maas, R. L., Brash, A. R. 1983. Evidence for a lipoxygenase mechanism in the biosynthesis of epoxide and dihydroxy leukotrienes for 15(S)-hydroperoxeicosatetraenoic acid by human platelets and porcine leukocytes. *Proc. Natl. Acad. Sci. USA* 80:2884
13. Jakschik, B., Kuo, C. G. 1983. Subcellular localization of leukotriene-forming enzymes. *Adv. Prostagl. Thrombox. Leukotr. Res.* 11:141
14. Ochi, K., Yashitomo, T., Yamamoto, S., Taniuguchi, K., Miyamoto, T. 1983. Arachidonate 5-lipoxygenase of guinea pig peritoneal polymorphonuclear leukocytes. Activation by adenosine 5'-triphosphate. *J. Biol. Chem.* 258:5754
15. Yokoyama, C., Mizuno, K., Mitachi, H., Yoshimoto, T., Yamomoto, S., Pace-Asciak, C. R. 1983. Partial purification and characterization of arachidonate 12-lipoxygenase from rat lung. *Biochem. Biophys. Acta* 750:237
16. Narumiya, S., Salmon, J. A., Cottee, F. H., Weatherley, B. C., Flower, R. J. 1981. Arachidonic acid 15-lipoxygenase from rabbit peritoneal polymorphonuclear leukocytes. Partial purification and properties. *J. Biol. Chem.* 256:9583
17. Ferreira, S. H., Vane, J. R. 1967. Prostaglandins: Their disappearance from and release into the circulation. *Nature* 216:868
18. Hamberg, M., Jonsson, C. E. 1973. Increased synthesis of prostaglandins in the guinea pig following scalding injury. *Acta Physiol. Scand.* 87:240
19. Jonsson, C. E., Schimizu, Y., Fredholm, B., Granstrom, E., Olivo, E. 1979. Effect of cyclic AMP, prostaglandin E_2, $F_2\alpha$, and thromboxane B_2 in leg lymph of rabbits after scalding injury. *Acta Physiol. Scand.* 107:377
20. Wiquist, N., Widholm, D., Nillius, S. J., Nilsson, B. 1978. Dysmenorrhea and prostaglandins. *Acta Obtset. Gynaecol. Scand. (Suppl.)* 87:5
21. Armstrong, D., Dry, R. M. L., Keele, C. A., Markham, J. W. 1952. Pain producing substances in blister fluid and in serum *J. Physiol. (Lond.)* 117:4P
22. Armstrong, D., Jepson, J. B., Keele, C. A., Stewart, J. W. 1957. Pain producing substance in human inflammatory exudate and plasma. *J. Physiol. (Lond.)* 135:350
23. Ferreira, S. H., Nakamura, M., Castro, M. S. A. 1978. The hyperalgesic effects of prostacyclin and prostaglandin E_2. *Prostaglandins* 16:31
24. Potter, G. D., Guzman, F., Lim, R. S. 1962. Visceral pain evoked by in arterial injection of substance P. *Nat.* 193:983
25. Melmon, K. L., Webster, M. E., Go, fine, S. E., Seegmiller, J. E. 1967. T. presence of kinin in inflammatory sy. ovial effusion from arthritides of varyin etiologies. *Arthr. Rheum.* 10:13
26. Moncada, S., Ferreira, S. H., Vane, J. R. 1975. Inhibition of prostaglandin biosynthesis as the mechanism of analgesia of aspirin-like drugs in the dog knee joint. *Eur. J. Pharmacol.* 31:250
27. Kaliner, M., Austen, K. F. 1975. Immunological release of chemical mediators from human tissues. *Ann. Rev. Pharmacol.* 15:177
28. Morrison, D. C., Henson, P. M. 1978. Release of mediators from mast cells and basophils induced by different stimuli. In *Immediate Hypersensitivity: Modern Concepts and Developments*, ed. M. K. Bach, p. 431. NY: Marcel Dekker
29. Sullivan, T. J., Parker, C. W. 1979. Possible role of arachidonic acid and its metabolites in mediator release from rat mast cells. *J. Immunol.* 122:431
30. Kaplan, A. P. 1983. Hageman factor-dependent pathways: mechanism of initiation and bradykinin formation. *Fed. Proc.* 42:3123
31. Beck, P. W., Handwerker, H. O. 1974. Bradykinin and serotonin effects on various types of cutaneous nerve fibers. *Pflügers Arch.* 374:209
32. Bessou, P., Perl, E. R. 1969. Response of cutaneous sensory units with unmyelinated fibers to noxious stimuli. *J. Neurophysiol.* 32:1025
33. Fock, S., Mense, S. 1976. Excitatory effects of 5-hydroxytryptamine, histamine and potassium ions on muscular group IV afferent units: a comparison with bradykinin. *Brain Res.* 105:459
34. Armstrong, D., Dry, R., Keele, C. A., Markham, J. W. 1953. Observations on chemical excitants of cutaneous pain in man. *J. Physiol. (Lond.)* 120:326
35. Elliot, D. E., Horton, E. W., Lewis, G. P. 1960. Actions of pure bradykinin. *J. Physiol. (Lond.)* 153:473
36. Keele, C. A., Armstrong, D. 1964. Substances producing pain and itch. In *Monographs of the Physiological Society*, ed. H. Barcroft, H. Davson, W. D. M. Paton, 12: p. 1. London: Edward Arnold
37. Crunkhorn, P., Willis, A. L. 1971. Cutaneous vascular reactions to intradermal prostaglandins. *Brit. J. Pharmacol.* 41:49
38. Horton, E. W. 1963. Action of prostaglandin E_1 on tissues which respond to bradykinin. *Nature (Lond.)* 200:892

Annual Reviews

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

39. Juhlin, S., Michaelsson, G. 1969. Cutaneous vascular reactions to prostaglandins in healthy subjects in patients with urticaria and atopic dermatitis. *Acta Derm. Venerol.* 49:251
40. Ferreira, S. H. 1972. Prostaglandins, aspirin-like drugs and analgesia. *Nature* 240:200
41. Collier, H. O. J., Schneider, C. 1972. Nociceptive response to prostaglandins and analgesic actions of aspirin and morphine. *Nature New Biol.* 236:141
42. Moncada, S., Ferreira, S. H., Vane, J. R. 1972. Does bradykinin cause pain through prostaglandin production. *Proceedings of the V International Congress of Pharmacology.* Oxford: Pergamon. 160
43. Ferreira, S. H., Moncada, S., Vane, J. R. 1973. Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. *Brit. J. Pharmacol.* 49:86
44. Guzman, F., Braun, C., Lim, R. K. S. 1962. Visceral pain and the pseudo-affective response to intra-arterial injections of bradykinin and other algesic agents. *Arch. Int. Pharmacodyn.* 136:353
45. Kuhn, D. C., Willis, A. L. 1973. Prostaglandin E_2, inflammation and pain threshold in rat paws. *Brit. J. Pharmacol.* 49:183P
46. Ferreira, S. H., Nakamura, M., Castro, M. S. A. 1978. The hyperalgesic effects of prostacyclin and prostaglandin E_2. *Prostaglandins* 16:31
47. Wellmann, W., Schwabe, U. 1973. Effects of prostaglandins E_1, E_2 and $F_2\alpha$ on cyclic AMP levels in brain *in vivo*. *Brain Res.* 59:371
48. Ferreira, S. H., Nakamura, M. 1979. Prostaglandin hyperalgesia. I. A cAMP/Ca^{2+} dependent process. *Prostaglandins* 18:179
49. Levine, R. A. 1969. Effects of exogenous adenosine 3′–5′-monophosphate in man. *Clin. Pharmacol. Ther.* 11:238
50. Ferreira, S. H., Lorenzetti, B. B. 1981. Prostaglandin hyperalgesia. IV: A metabolic process. *Prostaglandins* 21:789
51. Rackham, A., Ford-Hutchinson, A. W. 1983. Inflammation and pain sensitivity: Effects of leukotrienes D_4, B_4 and prostaglandin E_1 in the rat paw. *Prostaglandins* 25:193
52. Bisgaard, H., Kristensen, J., Sondergaard, J. 1982. The effect of leukotriene C_4 and D_4 on cutaneous blood flow in humans. *Prostaglandins* 23:797
53. Lachman, L. B. 1983. Human interleukin 1: purification and properties. *Fed. Proc.* 42:2639
54. Dinarello, C. A., Wolff, S. M. 1978. Pathogenesis of fever in man. *New Engl. J. Med.* 298:607
55. Rosenwasser, L. J., Dinarello, C. A., Rosenthal, A. S. 1979. Adherent cell function in murine thymphocyte antigen recognition. IV. Enhancement of murine T-cell antigen recognition by human leukocytic pyrogen. *J. Exp. Med.* 150:709
56. Murphy, P. A., Simon, P. L., Willoughby, W. F. 1980. Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte activating factors made by rabbit alveolar macrophages. *J. Immunol.* 124:2498
57. Duff, G. W., Durum, S. K. 1983. The pyrogenic and mitogenic actions of interleukin 1 are related. *Nature* 304:449
58. Vane, J. R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.* 231:232
59. Milton, A. S., Wendlandt, S. 1970. A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *J. Physiol. (Lond.)* 207:76P
60. Saxena, P. N., Beg, M. M. A., Singhal, K. C., Ahmad, M. 1979. Prostaglandin-like activity in the cerebrospinal fluid of febrile patients. *Indian J. Med. Res.* 70:495
61. Hendricks, C. H., Brenner, W. E., Ekbandh, L., Brotanek, V., Fisburne, J. I. 1973. Efficacy and tolerance of intravenous prostaglandins F_2 and E_2. *Am. J. Obstet. Gynecol.* 11:564
62. Williams, T. J., Morley, J. 1973. Prostaglandins as potentiators of increased vascular permeability in inflammation. *Nature* 246:215
63. Williams, T. J., Peck, M. J. 1977. Role of prostaglandin-mediated vasodilation in inflammation. *Nature* 270:530
64. Basran, G. S., Paul, W., Morley, J., Turner-Warwick, M. 1982. Evidence in man of synergistic interaction between putative mediators of acute inflammation and edema. *Lancet* 1:1935
65. Williams, T. J. 1979. Prostaglandin E_2, prostaglandin I_2 and the vascular changes of inflammation. *Brit. J. Pharmacol.* 65:517
66. Williams, T. J., Jose, P. J. 1981. Mediation of increased vascular permeability after complement activation. *J. Exp. Med.* 153:136
67. Wedmore, C. V., Williams, T. J. 1981. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature* 289:646
68. Jose, P. J., Forrest, M. J., Williams, T. J. 1981. Human C5a des arg increases vascular permeability. *J. Immunol.* 127:2376
69. Bray, M. A., Cunningham, F. M., Ford-

Annual Reviews

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Hutchinson, A. W., Smith, M. J. 1981. Leukotriene B_4: a mediator of vascular permeability. *Brit. J. Pharmacol.* 72:483

70. Issekutz, A. C., Movat, K. W., Movat, H. Z. 1980. Enhanced vascular permeability and haemorrhage-inducing activity of zymosan-activated plasma. *Clin. Exp. Immunol.* 41:505
71. Issekutz, A. C., Movat, K. W., Movat, H. Z. 1980. Enhanced vascular permeability and haemorrhage-inducing activity of rabbit C5a des arg: probable role of polymorphonuclear leucocyte lysosomes. *Clin. Exp. Immunol.* 41:512
72. Bailey, P. J., Sturm, A. 1983. Immune complexes and inflammation. A study of the activity of anti-inflammatory drugs in the reverse passive Arthus reaction in the rat. *Biochem. Pharmacol.* 32:475
73. Moncada, S., Herman, A. G., Higgs, E. A., Vane, J. R. 1977. Differential formation of prostacyclin (PGX or PGI_2) of layers of the arterial wall. An explanation for the anti-thrombotic properties of vascular endothelium. *Thromb. Res.* 11:323
74. Fantone, J. C., Kunkel, S. L., Ward, P. A., Zurier, R. B. 1980. Suppression by prostaglandin E_1 of vascular permeability induced by vasoactive inflammatory mediators. *J. Immunol.* 125:2591
75. Zurier, R. B., Quagliata, F. 1971. Effects of prostaglandin E_1 on adjuvant arthritis. *Nature* 234:304
76. Zurier, R. B., Damjanov, I., Sayadoff, D. M., Rothfield, N. 1977. Prostaglandin E_1 treatment of NZB1 NZW F_1 hybrid mice. 11. Prevention of glomerulonephritis. *Arthr. Rheum.* 20:1449
77. Kunkel, S. L., Thrall, R. T., Kunkel, R. G., Ward, P. A., Zurier, R. B. 1979. Suppression of immune complex vasculitis by prostaglandins. *J. Clin. Invest.* 64:1525
78. Fantone, J. C., Marasco, W. A., Elgas, L. J., Ward, P. A. 1983. Anti-inflammatory effects of prostaglandin E_1: *in vivo* modulation of the formyl peptide chemotactic receptor on the rat netrophil. *J. Immunol.* 130:1495
79. Smolen, J. E., Korchak, H. M., Weissmann, G. 1982. Stimulus-response coupling in neutrophils. *Trends Pharmacol. Sci.* 3:483
80. Ham, E. A., Soderman, D. D., Zanetti, M. E., Dougherty, H. W., McCauley, E., Kuehl, F. A., Jr. 1983. Inhibition by prostaglandins of leukotriene B_4 release from activated neutrophils. *Proc. Natl. Acad. Sci. USA* 80:4349
81. Williams, T. J., Piper, P. J. 1980. The action of chemically pure SRS-A on the microcirculation *in vivo*. *Prostaglandins* 19:779
82. Peck, M. J., Piper, P. J., Williams, T. J. 1981. The effect of leukotrienes C_4 and D_4 on the microvasculature of guinea pig skin. *Prostaglandins* 21:315
83. Dahlen, S. E., Bjork, J., Hedqvist, P., Arfors, K.-E., Hammerstrom, S., Lindgren, J. A., Samuelsson, B. 1981. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary venules. *In vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. USA* 78:3887
84. Bisgaard, H., Kristensen, J., Sondergaard, J. 1983. The effect of leukotriene C_4 and D_4 on microcirculatory flow in humans. *Brit. J. Dermatol.* 109 (Suppl. 25): 124
85. Gulati, O. P., Malmsten, C., Ponard, G., Gulati, N. 1983. The local edemogenic effects of leukotriene C_4 and prostaglandin E_2 in rats. *Prost. Leuk. Med.* 10:11
86. Woodward, D. F., Weichman, B. M., Gill, C. A., Wasserman, M. A. 1983. The effect of synthetic leukotrienes on tracheal microvascular permeability. *Prostaglandins* 25:131
87. Ueno, A., Tanaka, K., Katori, M., Hayashi, M., Arai, Y. 1981. Species difference in increased vascular permeability by synthetic leukotriene C_4 and D_4. *Prostaglandins* 21:637
88. Bray, M. A. 1982. Leukotriene B_4: an inflammatory mediator with vascular actions *in vivo*. *Agents Actions* 11 (Suppl. 1):51
89. Kaley, G., Weiner, R. 1971. Effect of prostaglandin E on leukocyte migration. *Nature New Biol.* 234:114
90. Walker, J. R., Smith, M. J., Ford-Hutchinson, A. W. 1976. Prostaglandins and leukotaxis. *J. Pharm. Pharmacol.* 28:745
91. Kitchen, E. A., Boot, J. R., Dawson, W. 1978. Chemotactic activity of thromboxane B_2, prostaglandins and their metabolites for polymorphonuclear leucocytes. *Prostaglandins* 16:239
92. Goetzl, E. J., Gorman, R. R. 1978. Chemotactic and chemokinetic stimulation of human eosinophil and neutrophil polymorphonuclear leukocytes by 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT). *J. Immunol.* 120:526
93. Turner, S. R., Tainer, J. A., Lynn, W. S. 1975. Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. *Nature* 257:680
94. Goetzl, E. J., Sun, F. F. 1979. Generation of unique mono-hydroxy eicosatetraenoic acids from arachidonic acid by human neutrophils. *J. Exp. Med.*, 150:406
95. Ford-Hutchison, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., Smith, M. J. H. 1980. Leukotriene B: a potent che-

Annual Reviews

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org
by HINARI on 08/27/07. For personal use only.

mokinetic and aggregating substance released from polymorphonuclear leucocytes. *Nature* 286:264

96. Smith, M. J. H., Ford-Hutchinson, A. W., Bray, M. A. 1980. Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmacol.* 32:517
97. Goetzl, E. J., Pickett, W. C. 1980. The human PMN leukocyte chemotactic activity of complex hydroxy-eicosatetraenoic acids (HETEs). *J. Immunol.* 125:1789
98. Ford-Hutchinson, A. W. 1983. The role of leukotriene B_4 as a mediator of leucocyte function. *Agents Actions* (Suppl.) 12:154
99. Molski, T. F. P., Naccahe, P. H., Borgeat, P., Shaafi, R. I. 1981. Similarities in the mechanisms by which formylmethionyl-leucyl-phenylalanine, arachidonic acid and leukotriene B_4 increase calcium and sodium influxes in rabbit neutrophils. *Biochem. Biophys. Res. Commun.* 103:227
100. Nagy, L., Lee, T. H., Goetzl, E. J., Pickett, W. C., Kay, A. B. 1982. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin. Exp. Immunol.* 47:541
101. Rollins, T. E., Zanolari, B., Springer, M. S., Guindon, Y., Zamboni, R., Lau, C.-K., Rokach, J. 1983. Synthetic leukotriene B_4 is a potent chemotaxin but a weak secretagogue for human PMN. *Prostaglandins* 25:281
102. Serhan, C. N., Radin, A., Smolen, J. E., Korechak, H., Samuelsson, B., Weissmann, G. 1982. Leukotriene B_4 is a complete secretagogue in human neutrophils: a kinetic analysis. *Biochem. Biophys. Res. Commun.* 107:1006
103. Claesson, H. 1982. Leukotrienes A_4 and B_4 stimulate the formation of cyclic AMP in human leukocytes. *FEBS Lett.* 139:305
104. Moqbel, R., Sass-Kuhn, S. P., Goetzl, E. J., Kay, A. B. 1983. Enhancement of neutrophil and eosinophil-mediated complement-dependent killing of schistosomula of *Schitosoma mansoni in vitro* by leukotriene B_4. *Clin. Exp. Immunol.* 52:519
105. Czarnetzki, B. M., Konig, W., Lichenstein, L. M. 1976. Eosinophil chemotactic factor (ECF). I. Release from polymorphonuclear leucocytes by the calcium ionophore A23187. *J. Immunol.* 117:229
106. Czarnetzki, B. 1983. Increased monocyte chemotaxis towards leukotriene B_4 and platelet activating factor in patients with inflammatory dermatoses. *Clin. Exp. Immunol.* 54:486
107. Serhan, C. N., Fridovich, J., Goetzl, E. J., Dunham, P. B., Weissmann, G. 1982. Leukotriene B_4 and phosphatidic acid are calcium ionophores. Studies employing arzenazo III in liposomes *J. Biol. Chem.* 257:4746
108. Ford-Hutchinson, A. W., Rackham, A., Zamboni, R., Rokach, J., Roy, S. 1983. Comparative biological activities of synthetic leukotriene B_4 and its ω-oxidation products. *Prostaglandins* 25:29
109. Snyderman, R., Goetzl, E. J. 1981. Molecular and cellar mechanisms of leukocyte chemotaxis. *Science* 213:830
110. Goldman, D. W., Goetzl, E. J. 1982. Specific binding of LTB_4 to receptors on human polymorphonuclear leucocytes. *J. Immunol.* 129:1600
111. Camp, R. D. R., Wollard, P. M., Mallet, A. J., Fincham, N. J., Ford-Hutchinson, A. W., Bray, M. A. 1982. Neutrophil aggregating and chemokinetic properties of 5,12,20-trihydroxy-6,8,10,14-eicosatetraenoic acid isolated from human leucocytes. *Prostaglandins* 23:631
112. O'Flaherty, J. T., Thomas, M. J., Hammett, M. J., Caroll, C., McCall, C. E., Wykle, R. L. 1983. 5-L-hydroxy-6,8,11,16-eicosatetraenoate potentiates the human neutrophil degranulating action of platelet-activating factor. *Biochem. Biophys. Res. Commun.* 111:1
113. O'Flaherty, J. T., Thomas, M. J., McCall, C. E., Wykle, R. 1983. Potentiating actions of hydroxyeicosatetraenoates on human neutrophil degranulation responses to leukotriene B_4 and phorbol myristate acetate. *Res. Commun. Chem. Pathol. and Pharmacol.* 40:475
114. Goetzl, E. J., Brindley, L. L., Goldman, D. W. 1983. Enhancement of human neutrophil adherence by synthetic leukotriene constituents of the slow-reacting substance of anaphylaxis. *Immunology* 50:35
115. Higgs, G. A., Salmon, J. A., Spayne, J. A. 1981. The inflammatory properties of hydroperoxy and hydroxy acid products of arachidonate lipoxygenase in rabbit skin. *Br. J. Pharmacol.* 74:429
116. Bray, M. A., Ford-Hutchinson, A. W., Smith, M. J. H. 1981. Leukotriene B_4 an inflammatory mediator in vivo. *Prostaglandins* 22:213
117. Bhattacherjee, P., Hammond, B., Salmon, J. A., Stepney, R., Eakins, K. E. 1981. Chemotactic response to some arachidonic acid lipoxygenase products in the rabbit eye. *Eur. J. Pharmacol.* 73:21
118. Camp, R. D. R., Coutts, A. A., Greaves, M. W., Kay, A. B., Walport, M. J. 1982. Responses on human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. *Brit. J. Pharmacol.* 95:168P

Annual Reviews

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org
by HINARI on 08/27/07. For personal use only.

119. Camp, R., Jones, R. R., Brain, S., Wollard, P., Greaves, M. 1984. Production of intraepidermal micro-abscesses by topical application of leukotriene B_4 a potential experimental model of psoriasis. *J. Invest. Dermatol.* In press
120. Brain, S. D., Camp, R. D. R., Dowd, R. M., Kobza-Black, A., Wollard, P. M., Mallet, A. I., Greaves, M. W. 1982. Psoriasis and leukotriene B_4. *Lancet* 2: 762
121. Greaves, M. W. 1983. Neutrophil polymorphonuclears, mediators and the pathogenesis of psoriasis. *Brit. J. Dermatol.* 109:115
122. Simmons, P. M., Salmon, J. A., Moncada, S. 1983. The release of leukotriene B_4 during experimental inflammation. *Biochem. Pharmacol.* 32:1353
123. Sharon, P., Stenson, W. F. 1983. Production of leukotrienes by colonic mucosa from patients with inflammatory bowel disease. *Gastroenterology* 84:1306
124. Klickstein, L. B., Shapleigh, C., Goetzl, E. J. 1980. Lipoxygenation of arachidonic acid as a source of polymorphonuclear leukocyte chemotactic factors in synovial fluid and tissue in rheumatoid arthritis and spondyloarthritis. *J. Clin. Invest.* 66:1166
125. Rae, S. A., Davidson, E. M., Smith, M. J. H. 1982. Leukotriene B_4, an inflammatory mediator in gout. *Lancet* 2:1122
126. Bourne, H. R., Lichtenstein, L. M., Melmon, K. L., Henney, C. S., Weinstein, Y., Shearer, G. M. 1974. Modulation of inflammation and immunity by cyclic nucleotides. *Science* 184:19
127. Gordon, D., Bray, M. A., Morley, J. 1976. Control of lymphokine secretion by prostaglandins. *Nature* 262:401
128. Davies, P., Bonney, R. J., Humes, J. L., Kuehl, F. A., Jr. 1980. The synthesis of arachidonic acid oxygenation products by various mononuclear phagocyte populations. In *Mononuclear Phagocytes: Functional Aspects*, ed. R. van Furth. The Hague: Martinus Nijhoff, 1317 pp.
129. Scott, W. A., Rouzer, C. A., Cohn, Z. A. 1983. Leukotriene C release by macrophages. *Fed. Proc.* 42:129
130. Davies, P., Bonney, R. J., Humes, J. L., Kuehl, F. A., Jr. 1984. The synthesis of arachidonic acid oxygenation products by macrophages. In *The Reticuloendothelial System. A Comprehensive Treatise*, ed. H. Friedman, M. Escobar, S. M. Reichard. NY: Plenum. In press
131. Goodwin, J. S., Webb, D. R. 1980. Regulation of the immune response by prostaglandins. *Clin. Immunol. Immunopathol.* 15:106
132. Goldyne, M. E., Stobo, J. D. 1981. Immunoregulatory role of prostaglandins and related lipids. *CRC. Crit. Rev. Immunol.* 2:189
133. Offner, H., Danneskiold-Samsoe, B., Dore-Duffy, P. 1982. Effects of prostaglandins and aspirin on active E-rosette formation in patients with multiple sclerosis. *Clin. Immunol. Immunopathol.* 22:159
134. Dore-Duffy, P., Zurier, R. B. 1981. Lymphocyte adherence in multiple sclerosis. Role of monocytes and increased sensitivity of MS lymphocytes to prostaglandin E. *Clin. Immunol. Immunopathol.* 19:303
135. Goodwin, J. S., Messner, R. P. 1979. Sensitivity of lymphocytes to prostaglandin E_2 increases in subjects over age 70. *J. Clin. Invest.* 64:434
136. Bardwick, P. A., Bluestein, H. A., Zvaifler, N. J., Depper, J. M., Seegmiller, J. E. 1980. Altered regulation of Epstein Barr virus induced lymphoblast proliferation in rheumatoid arthritis lymphoid cells. *Arthr. Rheum.* 23:626
137. Tosato, G., Steinberg, A. D., Blaese, R. M. 1981. Defective EBV-specific suppressor T cell function in rheumatoid arthritis. *New Engl. J. Med.* 305:1238
138. Hasler, F., Bluestein, H. G., Zvaifler, N. J., Epstein, L. 1983. Analysis of the defects responsible for impaired regulation of EBV-induced B cell proliferation by rheumatoid arthritis lymphocytes. II. Role of monocytes and increased sensitivity of rheumatoid arthritis lymphocytes to prostaglandin E. *J. Immunol.* 131:768
139. Hasler, F., Bluestein, H. G., Zvaifler, N. J., Epstein, L. B. 1983. Analysis of the defects responsible by rheumatoid arthritis lymphocytes. Diminished gamma interferon production in response to autologous stimulation. *J. Exp. Med.* 157:173
140. Panayi, G. S., Corrigall, V., Youlten, L. I. F. 1981. Immunoregulation in the rheumatoid diseases. *Scand. J. Rheumatol.* (Suppl.) 38:9
141. Pawlowski, N. A., Kaplan, G. A., Hamill, A. L., Cohn, Z. A., Scott, W. A. 1983. Arachidonic acid metabolism by human monocytes. Studies with platelet depleted cultures. *J. Exp. Med.* 158:393
142. Opmeer, F. A., Adolfs, M. J. P., Bonta, I. L. 1983. Direct evidence for the presence of selective binding sites for (^{3}H) prostaglandin E_2 on rat peritoneal macrophages. *Biochem. Biophys. Res. Commun.* 114:155
143. Payan, D. G., Goetzl, E. J. 1983. Specific suppression of human T lymphocyte function by leukotriene B_4. *J. Immunol.* 131:551

Annual Reviews

144. Webb, D. R., Nowowiejski, I., Healy, C., Rogers, T. J. 1982. Immunosuppressive properties of leukotriene D_4 and E_4 *in vitro*. *Biochem. Biophys. Res. Commun.* 108:1531
145. Rola-Pleszczynski, M., Borgeat, P., Sirois, P. 1982. Leukotriene B_4 induces human suppressor lymphocytes. *Biochem. Biophys. Res. Commun.* 108:1531
146. Gualde, N., Chable-Rabinovitch, H., Motta, C., Durand, J., Beneytout, J. L., Rigaud, M. 1983. Hydroperoxyeicosatetraenoic acids. Potent inhibitors of lymphocytes responses. *Biochem. Biophys. Acta* 750:429
147. Walsh, C. E., Waite, B. M., Thomas, M. J., DeChatelet, L. R. 1981. Release and metabolism of arachidonic acid in human neutrophils. *J. Biol. Chem.* 256:7228
148. Parker, C. W., Stenson, W. F., Huber, M. G., Kelly, J. P. 1979. Formation of thromboxane B_2 and hydroxyarachidonic acids in purified human lymphocytes in the presence and absence of PHA. *J. Immunol.* 122:1572
149. Goetzl, E. J. 1981. Selective feedback inhibition of the 5-lipoxygenation of arachidonic acid in human T-lymphocytes. *Biochem. Biophys. Res. Commun.* 101:344
150. Vanderhoek, J. Y., Bryant, R. W., Bailey, J. M. 1980. Inhibition of leukotriene biosynthesis by the leukocyte product 15-hydroxy-5,8,11,13-eicosatetraenoic acid. *J. Biol. Chem.* 255:10064
151. Bailey, J. M., Bryant, R. W., Low, C. E., Pupillo, M. B., Vanderhoek, J. Y. 1983. Regulation of T-lymphocyte mitogenesis by the leukocyte product 15-hydroxyeicosatetraenoic acid (15-HETE). *Cell Immunol.* 67:112
152. Goodman, M. G., Weigle, W. O. 1980. Inhibition of lymphocyte mitogenesis by an arachidonic acid hydroperoxide. *J. Supramol. Struc.* 13:373

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org
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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:335-357. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

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HETEROGENEITY OF NATURAL KILLER CELLS

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INTRODUCTION

Natural killer cells were discovered about ten years ago (1–5) during studies of cell-mediated cytotoxicity. Although investigators expected to find specific cytotoxic activity of tumor-bearing individuals against autologous tumor cells or against allogeneic tumors of similar or the same histologic type, appreciable cytotoxic activity was observed with lymphocytes from normal individuals. Since this time, the studies of natural killer cells have expanded into a broad and multifaceted research area, stimulated by the increasing indications that these cells may play important roles in natural host resistance against cancer and infectious diseases (6–24). With the wide array of recent studies related to natural cell–mediated cytotoxicity, there has been considerable diversity in the terminology related to the effector cells and consequently some confusion in the literature. However, at a recent workshop devoted to the study of natural killer cells, a consensus definition for these effector cells was developed (25). Natural killer (NK) cells were defined as effector cells with spontaneous cytotoxicity against various target cells; these effector cells lack the properties of classical macrophages, granulocytes, or cytotoxic T lymphocytes (CTL); and the observed cytotoxicity does not show restriction related to the major histocompatibility complex (MHC). This definition is sufficiently broad to include not only "classical" NK cells but also other natural effector cells such as natural cytotoxic (NC) cells. The workshop participants agreed that the observations relating to the development of cytotoxic cells in culture [e.g. lectin-activated killers (LAK), anomalous killers (AK)] remain difficult to interpret and that, for the moment, it is best to categorize separately cultured or activated cells with cytotoxic reactivity that cannot be classified as CTL. Such culture-

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Annual Reviews

related cytotoxic activities can provisionally be referred to as NK-like activity.

NK cells are not the sole effectors of natural immunity but rather have been recognized to be one of several cell types that play important roles in natural resistance. In addition to NK cells, macrophages, natural antibodies, and polymorphonuclear leukocytes are major effector mechanisms involved in natural resistance (25–59). Detailed reviews and comparisons of these effectors and their characteristics have been published (13,15,17).

Until recently, the cells responsible for NK activity could be defined only in a negative way—i.e. by distinguishing them from typical T cells, B cells, or macrophages. However, it is now possible to isolate highly enriched populations and show that the NK activity is closely associated with a subpopulation of lymphocytes, morphologically identified as large granular lymphocytes (LGL) (60-68), that comprise about 5% of peripheral blood lymphocytes and 1–3% of total mononuclear cells. LGL have been found in all vertebrates tested (44), i.e. human, mouse, hamster, rat, chicken, guinea pig, and miniature swine. LGL, which contain azurophilic cytoplasmic granules, can be isolated by discontinuous density gradient centrifugation on Percoll. LGL are nonphagocytic, nonadherent cells that lack surface immunoglobulin receptors for the third component of complement but contain cell-surface receptors for the Fc portion of IgG (60–68). This latter quality allows them to bind antibody-coated target cells and mediate the phenomenon termed antibody-dependent cellular cytotoxicity (ADCC) (70–77), a function previously attributed to the killer (lymphocyte) K cell. Hence, the same cells (i.e. NK/K cells) seem able to mediate both forms of cytotoxicity, with NK activity due to NK receptors discrete from the Fc_γ receptors that interact with target cell–bound antibody (19,61,64,73,77).

The levels of NK activity have a characteristic organ distribution. Studies first performed with mouse and rat cells (2,8,13,17), and more recently with human cells (2,13,17,61,63,78–87), have demonstrated high levels of NK activity in the peripheral blood and spleen, with intermediate to low levels of activity present in the lymph nodes, peritoneal cavity, and bone marrow, and undetectable levels present in the tonsil or thymus. Recently, studies in the rat (2,13,17,88) have demonstrated a high degree of association of LGL with mucosal epithelial tissues, especially with the bronchial-associated lymphoid tissue and epithelium of the gut. Mucosal LGL have been isolated from the small intestine of mice (64) and shown to possess intermediate to high levels of NK activity. In addition, precursors of NK cells have been shown by transplantation experiments to be derived from the bone marrow (89).

Initially many investigators considered the cytotoxic activity of NK cells to be nonselective and independent of specific recognition structures. NK cells lyse a wide range of targets (20,70,71,83–85,90,91), including a variety of

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tumors [leukemias (1,3,9), carcinomas (14,20,22,55,63,82), and other selected tumors (14,20,22,55,56,63,82,83,85,86)], including autologous as well as allogeneic and xenogeneic tumor cells (55,56,63); several types of normal cells (20,63,82); fetal fibroblasts, macrophages, and subpopulations of bone marrow and thymus cells (71); cells infected with viruses or other microbial agents (84) (e.g. herpes and influenza virus or mycoplasma-infected cells); and some microorganisms. However, despite this diverse array of NK-susceptible targets, increasing evidence has demonstrated selective patterns of reactivity, with NK cells able to recognize a variety of different specificities (17,70,91).

Recent data (92–106) have indicated that the activity of NK cells is subject to considerable regulation. A number of agents influence the levels of activity of NK cells. Interferon (α, β, γ) (63,66,82,92,93,94,97,101,102,103,105,106) and IL-2 (95,100,104) augment the cytolytic function of NK cells. These cytokines affect NK activity both in vivo (90,102) and in vitro (95,101). In addition to a wide variety of agents shown to augment NK activity, both soluble agents and cells clearly inhibit NK activity (13,15,17,80,96,99,107–126). Agents shown to depress NK function include cyclic nucleotides (116), prostaglandins (99,108,116,120), phorbol esters and other tumor promoters (96,113,114), carcinogens, cyclophosphamide, corticosteroids (96), and also inhibitors of phospholipase-A_2 (117) or of protein synthesis (122). Suppressor cells of both the adherent and nonadherent types (80,107,109) can depress NK activity. Rodent NK activity is negatively regulated by both adherent macrophages and by lymphocytes. In the human, adherent cells have been especially associated with suppression of NK activity in cancer patients, whereas in multiple sclerosis patients (13,15,126), nonadherent and more classical T suppressor cells have been associated with suppression of NK activity. In addition to cytotoxic effects, more recently a wide variety of noncytotoxic functions have been associated with NK cells (128–134). These other functions, particularly the ability to produce cytokines, have raised the possibility that NK cells are important immunoregulatory cells. NK cells are highly secretory cells that can secrete a variety of cytokines (128–134) in response to stimulation with lectins, viruses, bacteria, or NK-susceptible target cells. The cytokines associated with LGL include α and γ interferons (127,129,130), interleukin 1 (128,129), interleukin 2 (129,130,131), natural killer cytotoxic factor (NKCF) (129,132,133,134), lymphotoxin (LT) (129), colony-stimulating factor (129,130), and B-cell growth factor (129).

The salient features of the cytokine-producing capability of LGL are under active investigation. It will be of considerable importance to determine the levels of production of each cytokine by LGL relative to those produced by other cell types, particularly those considered the principal source of these

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factors. The evidence to date indicates that LGL can release considerable levels of various cytokines, often as high as those derived from the prototype secretory cells. It will also be crucial to determine the range of stimuli that will cause production and release of each cytokine. In addition to conventional stimuli, incubation of human LGL with NK-susceptible target cells has triggered release of several cytokines, including interferon, IL-2, and NKCF (129). Finally, in regard to the immunoregulatory implications of these findings, it will be important to identify the types of lymphoid cells primarily affected by the LGL-derived soluble factors.

In this review we focus mainly on the evidence for NK-cell heterogeneity, its extent, and its implications. We focus on heterogeneity of NK cells in regard to (*a*) their general characteristics; (*b*) their ability to lyse a wide variety of target cells; (*c*) their responsiveness to various cytokines; (*d*) their production of cytokines; and (*e*) the lymphoid cell lineage(s) from which they may be derived. Most of our examples use data on human NK cells, both because of space limitations and because more extensive studies have been possible with the human than with other effector cells.

We encourage the reader to keep in mind the possible interpretations of the observed heterogeneity in characteristics and function. Of primary concern is whether NK activity is a function shared by cells of diverse origin and characteristics. An analogy to this possibility would be the heterogeneity of phagocytic cells, with distinct cell types (monocytes and macrophages, granulocytes) exhibiting phagocytic function. Alternatively, NK cells may be a discrete subpopulation of lymphoid cells, which can be defined and separated according to a constellation of characteristics, in the same way that B cells, T cells, macrophages, etc can be distinguished and categorized. If indeed NK activity can be ascribed to a particular subset of lymphoid cells, one must then explain the heterogeneity within the population of NK cells.

Two main alternatives may be considered, as depicted in Figure 1. On the one hand, diversity of NK cells in regard to phenotype, specificity for target cells, and their array of functional capabilities may be due to true clonal heterogeneity, as has been demonstrated for T and B cells. According to this model for NK cell heterogeneity, each clone of NK cells would have a characteristic and distinct set of cell-surface markers and receptors for certain target cells; each would have a limited array of functional capabilities. Alternatively, however, the heterogeneity of NK cells may be due, completely or in part, to variations in the level of activation of differentiation. The heterogeneity among mononuclear phagocytes, for example, appears attributable to this model. According to this possibility, all of the subsets of NK cells may be interrelated, with a variety of factors or other signals responsible for transition from one stage to another.

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GENERAL CHARACTERISTICS OF NATURAL KILLER CELLS

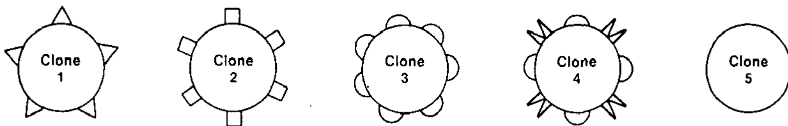
Morphology, Cytochemistry, and Receptors

Many of the characteristics of NK cells are summarized in Table 1. To place these features in a familiar frame of reference, we have indicated the various attributes shared by, or similar to, either T cells or macrophages (Mϕ). NK cells have been found to be mainly nonadherent, nonphagocytic, surface immunoglobulin-negative cells that are positive for β-glucuronidase and acid phosphatase and negative for nonspecific esterases (13,60–62,135). No diversity in the cytochemical features of these cells has been reported (13,17,60–62), with virtually all cells in the population showing the same pattern of cytoplasmic enzymes.

A high degree of correlation between cells with the morphology of large granular lymphocytes (LGL) and NK function has indicated that most, if not all, of the human, mouse, and rat NK activity is mediated by LGL (61–68). LGL represent approximately 5% of the peripheral-blood mononuclear cells and can be readily identified morphologically in Wright's-Giemsa stained cytocentrifuge preparations of low-density cells separable by Percoll density gradient centrifugation (13,15,17,60–63,65,67). LGLs are characteristically large lymphoid cells with slightly indented nuclei; they possess distinct azurophilic cytoplasmic granules and range from 16 to 20 μm in diameter.

Although LGL appear to account for most NK activity in humans, other

1. True clonal heterogeneity (stable genetic determination)



2. Differentiation and/or activation-dependent heterogeneity

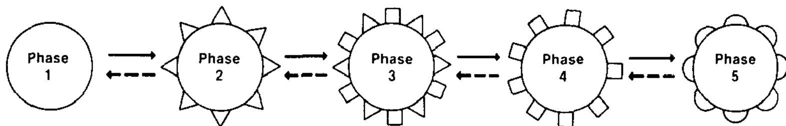


Figure 1 Alternative models for heterogeneity among NK cells. The various distinct surface features shown might represent either cell surface antigens, receptors for various target cells, or even different functions of the cells.

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Table 1 Comparison of LGL with T cells and Mϕ: general and functional characteristics

Characteristic	LGL property	Similar to T	Similar to Mϕ
Size	16–20 nm		+
Cytoplasmic nuclear ratio	High ratio		+
Nuclear shape	Lobed or indented		+
Adherence	Nonadherent	+	
Phagocytes	Nonphagocytic	+	
Nonspecific esterase	Absent	+	
Acid phosphatase	Present	+	+
β-Glucuronidase	In granules		+
Surface antigens	Several shared with other cell types	+	+
Fc receptors for IgG	Shared with PMN		
Spontaneous reactivity	Present in vivo		+
Period to develop augmented effector activity	Short (min–hrs)		+
Memory response	None		+
ADCC	Very effective		+
Activating factors:	IFN (α, β, γ)	+	+
	IL-2	+	+
	Bacterial products		+
Inhibiting factors:	PGE	+	+
	Phorbol esters		+

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primates, and rodents, not all LGL possess measurable NK activity (13,15,17,60–63,65,67). One possible explanation for the lack of detectable cytotoxic activity in some LGL is that the array of target cells tested has not been sufficient to reflect the entire repertoire and that some LGL may recognize and lyse only a limited variety of target cells. Despite this potential limitation, tests of human LGL against several NK-susceptible target cell lines in a single-cell cytotoxicity assay enable the estimate that in most normal individuals after activation of the cells with interferon, 75–85% (63) of the LGL are capable of killing at least one NK-susceptible target cell line. The nature of the other 15 or 20% of the LGL, with no detectable cytolytic activity, is unclear. They may represent a distinct subset of cells that inherently lack this functional capability or they may simply be at a noncytolytic stage of differentiation or activation. The concept that LGL may vary in their levels of activation is consistent with a variety of reports (13,15,17,63,67,82,83,92,106) of cells that lack spontaneous NK activity but can be activated by agents such as interferon or IL-2 to develop the ability to bind to NK-susceptible target cells or to lyse bound target cells. Thus it is currently unclear whether all LGL have the capacity to mediate NK activity when sufficiently activated and pre-

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sented with the appropriate target cells or whether a small proportion of LGL are not NK cells.

In regard to the converse issue, of whether all NK cells are LGL, the available data are not conclusive. On the one hand, as discussed above, LGL account for a high proportion of detectable NK activity. In the conventional assays for cytotoxic activity, cell populations depleted of LGL show little if any NK activity. However, a recent study, using a single-cell binding and cytotoxicity assay (136), has indicated that some human NK cells are not LGL but rather small T lymphocytes, bearing OKT3 and OKT4. It will be important to reconcile these findings with the extensive evidence for the restriction of NK activity to the LGL subpopulation, and to determine the extent of the possible contribution of these non-LGL to the total detectable NK activity.

Most LGL have surface receptors for the Fc portion of IgG, and both K cell–mediated antibody-dependent cellular cytotoxicity (ADCC) and NK activity have been closely associated with LGL (13,15,17,61,65,117,137,138). Approximately one half of human NK cells and LGL express detectable receptors for sheep erythrocyte (13,15,17,87,139), as measured by rosette formation at 4°C. However, some monoclonal antibodies to the sheep erythrocyte receptor react with a considerably higher proportion of LGL (128,139). Analogously, a proportion of mouse NK cells express Thy 1 antigens, and most rat NK cells express OX-8 and some other T cell–associated markers (65). Thus, although NK cells are clearly not thymus-dependent [since high levels of activity have been detected in athymic nude mice or in neonatally thymectomized mice (13,15,17,71,140–148)], they share many characteristics associated with T cells (see the section on augmenting agents, below).

In both rat and human spleens, and to a lesser extent in other organs, large agranular lymphocytes (LAL) have been detected that possess morphological characteristics similar to those of LGL but that lack detectable azurophilic granules (13,15,17). Aside from their lack of cytoplasmic granules, LAL have been indistinguishable from LGL. They copurify in the lower density fractions of Percoll density gradients, they have the same nuclear morphology, and their cytoplasm has the same appearance with Wright's-Giemsa stain. Also, no cell surface markers have been found to distinguish between LGL and LAL. In studies with purified populations of human LGL plus LAL, a high proportion of both LAL and LGL binds to NK-susceptible target cells. However, because we cannot yet separate these cells or distinguish them adequately in a cytolytic assay, it has not as yet been documented whether LAL have NK activity similar to that of LGL. Thus, the precise relationship of LAL with LGL or with NK cells remains unclear. One possibility is that LGL and LAL are directly related and differ only in their stages of granule maturation. A range of granulopoiesis has been reported in LGL, detectable by electron microscopy (13,15,135). It has been suggested that the earliest forms of LGL are in the bone marrow,

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with immature granules, and that differentiation of this cell population is reflected by increasing development of mature, typical granules. According to this hypothesis, LGL in the peripheral blood, with high NK activity and usually few cells without readily detectable granules, would represent the most differentiated cells in this lineage. Cells in the spleen and lymph nodes, with lower NK activity and less prominent granules, might be at an intermediate stage of differentiation. It is possible that the cytoplasmic granules are more directly related to cytolytic capability and that LAL are pre-NK cells, with the ability to bind but not lyse NK-susceptible targets. This hypothesis is consistent with a body of evidence for the existence of pre-NK cells (63,66,84,94,102,106), which can be induced to develop NK activity after treatment with interferon or other activating stimuli.

Overall, the results to date indicate that a discrete, small subpopulation of lymphoid cells—i.e. LGL—are responsible for most NK activity (at least 90%). These findings seem to rule out the possibility that diverse cell types share the NK function. Rather, the observed heterogeneity remains mainly within the LGL and related populations.

Cell-Surface Markers on NK Cells

Some cell surface antigens, particularly those detected by monoclonal antibodies, have been found on virtually all NK cells. They therefore help to characterize the phenotype of these effector cells. For example, most human NK cells react with the following antibodies (Figure 2): (*a*) several monoclonal antibodies (B73.1, 3G8, Leu 11) (150–152) reactive with Fc receptors for IgG on LGL and 3G8 and Leu 11 are strongly expressed on granulocytes (PMN); (*b*) rabbit antisera to the glycolipid asialo GM_1 which also reacts with monocytes and granulocytes; (*c*) OKT10, which also reacts with most thymocytes and activated lymphocytes (139,153,154); and (*d*) OKM1, which also reacts with monocytes/macrophages, polymorphonuclear leukocytes, and platelets (13,15,137–139,153,155). Removal of cells bearing any of these markers, either by treatment with antibody plus complement or by negative selection immunoaffinity procedures, results in a depletion of most or all detectable NK activity.

In the rat, a monoclonal antibody, OX-8, which also reacts with the subpopulation of T cells with suppressor activity [similar to the human T8 subpopulation of T cells with cytotoxic and suppressor activities (13,15,65,66,156)] reacts with most NK cells and LGL. Antisera to asialo GM_1 also react with virtually all rat and mouse NK cells (15,65,68).

NK cells can also be characterized by a lack of expression of certain cell surface markers. For example, human NK cells have no detectable surface

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reactivity with monoclonal antibodies to pan-T-cell antigens such as Leu 1, OKT3, or to T-helper antigens as defined by OKT4 or Leu 3 (13,15,137–139,152). Human NK cells also do not express surface antigens detected by a number of monocyte-specific reagents such as MO2 and Leu-M1 (13,15,139).

In contrast to a pattern of some phenotypic features common to most or all NK cells, these effector cells and also LGL in general are rather heterogeneous with respect to other monoclonal antibody–defined markers. Human NK cells react to a variable extent with monoclonal antibodies (MoAb) directed against the sheep erythrocyte receptors (Lyt3, OKT11, Leu 5) (135,139), with only about half of the NK cells in some experiments giving positive results. Only a portion of human NK cells have been shown to react with a variety of other monoclonals, including 3A1 (on most T cells and 50–60% of LGL) (139), HNK1 (on 40–60% of NK cells) (165), OKT8 (on the suppressor/cytotoxic T lymphocytes and 10–30% of LGL) (139,153), and about 25% of the LGL react with MoAb against Ia framework (HLA-DR) determinants (139).

Similarly in the mouse, only about half of the NK cells (as detected by abrogation of cytolytic function) express Thy 1, and only 20% express readily detectable Lyt 1 (13,15,160,162). In addition, the allelic markers NK1.1 and NK2.1 (13,15,160) are expressed on about 50–60% of mouse NK cells.

In contrast to NK, NC cells appear to be devoid of most lymphoid surface markers, being Lyt 1^-, Thy 1^-, asialo GM_1^-, H_2^{K-D-} (13,15,56,57,75,157–162). All attempts to phenotype NC cells have failed to define a characteristic marker on these cells. However, despite such indications that NC cells might be completely distinct from typical NK cells, NC cells and typical mouse NK

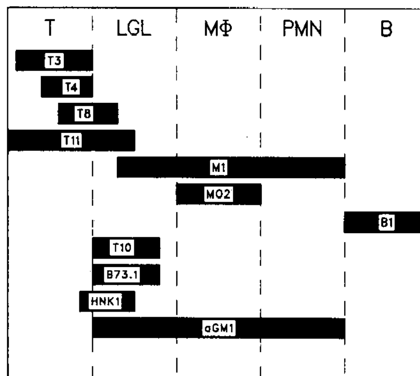

Figure 2 Pattern of reactivity of various monoclonal antibodies with human LGL and other leukocyte subsets.

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cells copurify in Percoll density gradients (164). Thus NC cells may also be LGL—either a subset of NK cells or at a stage of differentiation associated with poor expression of cell-surface markers and altered receptors for target cells.

Although most NK cells and LGL are nonadherent to plastic or nylon wool, a subset of these cells shows some adherence. For instance, when isolating human myelomonocytic cells by means of their adherence to plastic, the small percentage of contaminating cells is disproportionately comprised of LGL (163). In addition, after in vivo stimulation of NK cells with microbial agents such as *C. parvum* or with interferon after in vitro stimulation with allogeneic cells or lectins, a substantial proportion of NK cells adhere either to plastic or to nylon wool columns (13,123,166). Although this subpopulation of cells shares the adherence property with myelomonocytic cells, it retains the morphology and cell-surface characteristics of LGL. The phenotype of human adherent NK cells has been shown to be OKT3$^-$, OKT10$^+$, OKT11$^+$, OKM1$^+$, Leu-M1$^-$, B73.1$^+$ (163). Such cells thus contrast with typical adherent monocytes, which only react with OKM1 and Leu M1.

In summary, NK cells have a characteristic phenotype. For example, most human NK cells and LGL can be described as OKT3$^-$, OKT4$^-$, OKT10$^+$, B73.1$^+$, OKM1$^+$, Leu-M1$^-$. Thus, these cells have a readily definable and general phenotype, which sets them apart from all other lymphoid cell types. The heterogeneity in cell-surface phenotype extends to only a few markers—e.g. with human NK cells: OKT8, HNK1, Ia, and OKT11.

The observed heterogeneity in surface-marker expression on NK cells has not been explained. However, recent studies using a new method to examine intracellular expression of MoAb-defined markers in NK and T cells have indicated the need for caution in conclusions about the ability of lymphoid-cell subpopulations to express particular antigens (167,168). By means of this new procedure, all human T cells have been shown to react with OKT4 (only on the surface of the helper–T cell subset), B_2 (considered to be B cell–associated), and MO2 (expressed on the surface of only myelomonocytic cells). Most LGL were also found to have internal MO2 and B_2 and to contain Leu 1 (pan-T) antigen. Although the mechanisms responsible for expression of markers intracellularly and/or on the cell surface remain to be determined, expression of a variety of markers may depend on many factors. One must be cautious in using marker data to draw conclusions about the degree of divergence among subsets of NK cells or other lymphoid cells.

Taken together, the above data indicate that there may be discrete subsets of NK cells that vary in their cell-surface markers and adherence properties. However, an alternative explanation for such data is that cell-surface markers and adherence properties of NK cells vary with the stage of activation or maturation of the cells. In regard to this possibility, one notes that most of

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these markers do not change after stimulation with interferon (84,127,129) or short-term incubation with agents such as IL-2 (95,100) or *C. parvum* (127). In contrast, such treatments induce substantial changes in the levels of expression of Fc receptors and β_2-microglobulin on a variety of lymphoid cells.

Tissue Distribution of NK Cells

The proportions of LGL in various lymphoid and other tissues vary; in general, the levels of NK activity follow a similar distribution. Generally, the highest reactivity is in the peripheral blood, and spleen cells have intermediate levels of activity (3,5,18,23). Low levels of reactivity are found in other sites (9,23,64), including lymph nodes, bone marrow, lung, and gut epithelium. As noted above, spleens contain a higher proportion of LAL than does peripheral blood (15,64). However, it is as yet unclear whether human or rat NK cells at various sites differ substantially in other characteristics. However, studies in mice have indicated that the NC and NK in subsets of effector cells have characteristic tissue distributions distinct from that of typical NK cells (55–57), with high activity in lymph nodes and bone marrow. Further, it has been suggested that the NK cells in bone marrow may represent a particular subset, $NK_{(M)}$ (169), which shares several features with NC cells.

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NK Cells in Short-Term Culture

NK cells can be maintained in culture (90,170,171). Studies involve either short-term cultures (i.e. generally 1–10 days in culture) or long-term cultures (i.e. 2–6 weeks or longer), with IL-2 as the only clearly identified growth factor. Cytolytic activity can be maintained and NK-like activity can be generated during in vitro culture of subpopulations of cells devoid of reactivity at the initiation of culture (13,15,172–174). Since the relationship of the generated cytotoxic effector cells to NK cells remains somewhat uncertain, it has been decided to refer to such activities as NK-like (see Introduction).

Short-term cultures of mononuclear cells with lymphokine supernatants containing IL-2 (170,171), with lectins (171,174), with fetal bovine serum alone (172), and with allogeneic cells (173,174) result in considerable NK-like activity. After mixed lymphocyte cultures or incubations with other stimuli, substantial levels of NK-like activity were present (13,15,172–174). In addition, if NK cells were removed prior to culture, similar levels and patterns of NK-like activity were generated within 3–4 days in vitro (172). The nature of this reactivity was distinct from that of cytotoxic T lymphocytes (13,15,69,70). Instead, the cytotoxic activity closely resembled NK activity in regard to its specificity, the physical characteristics of the effector cells, and some of their phenotypic markers (172–174).

Recently, lectin-activated killer (LAK) cells have been described (175) that share many of the characteristics of the previously described culture-activated

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NK-like cells (13,15,69,70,100,176). LAK cells have been activated after a short period of culture in vitro with highly purified IL-2 and display cytotoxic activity against a variety of autologous, allogeneic, and xenogeneic tumors. These cells lack markers typical of fresh NK cells (i.e. they have been reported to be $OKM1^-$, $OKT10^-$, $OKT11^-$) and appear devoid of cytolytic activity prior to culture. The exact relationship between NK cells and LAK cells requires further investigation. However, since LAK cells seem to be derived from low-density Percoll fractions highly enriched in LGL and NK activity, it seems likely that they are related.

Comparative examination of the phenotypes of NK cells and cultured NK-like cells reveals several differences (13,15,69,70,100,170,171,175,176). In contrast to NK cells ($OKT3^-$, $OKM1^+$, and mainly Ia^-), the major phenotype of cultured NK-like cells is $OKT3^+$, $OKM1^-$, Ia^+. In cultures the presence of IL-2, the progenitors of the effector cells appeared different from NK cells, being $B73^-$, $OKT11^+$, and $OKM1^-$ (176). It should be noted, however, that the limiting dilution assays used for these studies (104,176–178) identify the most common progenitors (those that grow fastest and kill best), and only 5% of LGL grow in this assay. These studies do not indicate that other subsets of cells cannot be progenitors. In addition, since IL-2 is used as the growth factor, the information obtained pertains only to the subset of cells able to grow in response to this lymphokine. Recent studies with nude mouse spleen cells (15,104) suggest that other growth factors may also play an important role in the development of some NK cells. Nude mouse cells, despite their high NK activity, are deficient in IL-2 production and have a low frequency of detectable IL-2-dependent progenitors of NK-like activity. Thus the main population of NK cells in nude mice may be an IL-2-independent subset that responds to a different set of proliferative and activating signals.

When similar short-term culture experiments were performed with cells having low or undetectable cytolytic activity (thymus, bone marrow, lymph node), substantial NK-like activity usually developed (15,177–179). The cytotoxic cells are likely derived from pre-NK cells in these tissues, but it is not possible to exclude the possibility that cells from quite divergent origins develop into NK-like effector cells in vitro.

NK Cells in Long-Term Culture

Human LGL have been found to proliferate in vitro in the presence of IL-2 and to maintain high levels of cytolytic activity (15,180–186). Since cytotoxic cells can be cloned, it has been possible to begin to test the hypothesis of clonal heterogeneity of NK cells (Figure 1).

Long-term cultures derived from highly purified human LGL have several consistent characteristics: They retain the morphology of LGL and the same

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pattern of selective cytotoxicity against NK-susceptible targets. In a single-cell assay, most of the cultured LGL were capable of binding and lysing NK-susceptible targets. They show a decrease or loss of reactivity with the monoclonal antibodies OKM1 and OKT10, and they form rosettes with antibody-coated ox erythrocytes. However, they maintain Fc_γ receptor-dependent cytolytic capacity against antibody-coated mouse lymphoma cells (RL♂1), and continue to react with a MoAb (B73.1) directed against Fc_γ receptors. Most of the cultured LGL acquire reactivity with T cell–specific MoAb (OKT3, Leu 1) and with MoAb against Ia (HLA-DR) antigens.

Clones derived from cultured human LGL have most of the features described above for bulk cultures of human LGL. However, considerable heterogeneity in several features has been observed among the clones.

Table 2 summarizes selected results from studies with over 200 LGL clones (180,183). These results indicate heterogeneity among the clones in regard to patterns of cytotoxic reactivity and MoAb-defined phenotype. Phenotype and cytolytic pattern showed no apparent correlation, although most of the cytolytic clones were B73.1$^+$, OKT8$^+$. A considerable number of OKT4$^+$ LGL clones were found (180,183). OKT4$^+$ LGL have not been detected, and therefore it seems likely that these clones were derived from OKT4$^-$ progenitors. Similarly, clones derived from LGL positively or negatively selected for certain cell-surface markers frequently had phenotypes different from their progenitors. Thus, the expression of some cell-surface antigens is not a stable characteristic but can vary substantially upon the major environmental change when the cells are placed in culture.

Do the results with the LGL clones support the clonal heterogeneity hypothesis for NK cells? The possible interpretation of the specificity data are discussed below in the section on specificity of natural killer cells. Regarding phenotype, we note that once clones were established, the array of cell-surface markers usually remained stable over a period of 1–3 weeks (Table 3). Thus, heterogeneity in marker expression among NK cells may, at least in part, be clonally distributed. However, the available data are insufficient to support a contention of true genetically determined clonal heterogeneity.

Mouse NC cells have been maintained in culture with another lymphokine, interleukin 3 (IL-3) (187). These effector cell lines have lytic activity against NC-susceptible but not NK-susceptible targets. In addition, IL-2-dependent cultures have been reported from bone-marrow cells, termed $NK_{(M)}$ (169), and are considered NC-like cultures because of their reactivity mainly against NC-susceptible targets (188). To date, the characteristics of these cultured cells and their progenitors have not been examined sufficiently to allow adequate interpretation of the relationship of these findings to the heterogeneity of fresh NK cells.

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Table 2 Heterogeneity among human LGL clones in regard to specificity and phenotype[a]

	Cytotoxicity against:					Reactivity with *monoclonal antibodies*[a]					
Clone	K562	ADCC	MOLT-4	Alab	Daudi	OKT3	OKT4	OKT8	OKT10	OKM1	B73.1
H-77	0	0	6[b]	0	15	+	NT[c]	+	+	+	NT
H-49	0	22	NT	13	NT	+	NT	−	+	+	NT
H-66	0	17	NT	0	NT	+	NT	−	+	+	NT
H-45	11	0	NT	0	NT	+	NT	+	−	+	NT
RF-C	73	68	11	45	NT	−	NT	+	NT	+	+
L-9	69	51	81	52	NT	+	+	+	NT	NT	+
L-11	76	68	79	31	NT	+	+	+	NT	NT	+
L-12	72	79	82	44	NT	−	−	−	NT	NT	+
LGL uncultured[d]	69	70	68	55	60	−	−	+	+	+	+
LGL cultured[e]	65	60	55	49	45	+	−	+	+	+	+

[a]Selected clones from Allavena & Ortaldo (180).
[b]$p < 0.1$, all other values > 6 had a $p < 0.05$.
[c]Not tested.
[d]Whole LGL population after Percoll density centrifugation separation from the blood.
[e]Whole LGL population cultured in vitro for 30 days in IL-2 containing medium.

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Table 3 Cytotoxic activity, phenotype, and cytokine production of some LGL clones[a]

		Cytotoxic				Phenotype				Cytokine				
Clone	Day of culture	K562	ADCC	Daudi	Alab	OKT3	OKT8	OKM1	B73.1	IFN	IL-1	IL-2	CSF	BCGF
A8	21	–	–	–	–	+	–	+	+/–	+	–	+	–	NT[b]
	28	–	–	–	+/–	+	+	+	+	NT	–	–	NT	–
D1	21	–	–	–	–	+	+	+	–	+	–	+	–	NT
	28	–	–	–	–	+	+	+	–	NT	+	–	NT	–
	45	–	–	–	–	+	+	+	–	NT	–	+	NT	NT
C4	21	–	–	–	+/–	+	+	+	–	NT	+	–	NT	–
	28	–	–	–	+/–	+	+	+	–	NT	+	–	NT	–
E	21	+	+	+	+	–	+	+	+	+	+	+	NT	NT

[a]LGL clones were selected from eight tested to indicate typical results. 75% (6/8) produced IL-1; 83% (5/6) produced IL-4; 80% (4/5) produced IFN (129,223).
[b]Not tested.

Annual Reviews

SPECIFICITY OF NATURAL KILLER CELLS

Introduction

NK cells react against a wide variety of syngeneic, allogeneic, and xenogenei tumor cells. Susceptibility to cytotoxic activity is not restricted to malignan cells, with fetal cells, virus-infected cells, and subpopulations of normal lymphoi or hematopoietic stem cells (thymus cells, bone-marrow cells) susceptible t lysis by NK cells.

In contrast to cytolytic T lymphocytes, NK cells demonstrate no know MHC restriction (3,5,6–15,18,23,70,73,86,91,189,190). In fact, they hav strong reactivity against MHC-deficient targets (e.g. K562), and their activit is not inhibited by antibodies against MHC determinants (23,73,189). In addi tion, unlike CTL, the activity of NK cells does not appear to be dependent o antigenic sensitization (3,4,12,23,73), and no specific secondary memor response has been demonstrated (3,4,12,23). Differentiation antigens may b a major type of target-cell structure recognized by NK cells. Studies wit maturational agents (191) and with a wide variety of target cells (4,60,70 indicate that undifferentiated cells are generally more susceptible NK targets In further support of this possibility, normal lymphoid cells are totally insen sitive to NK lysis, whereas a subpopulation of relatively immature hemato poietic thymus (71,192) and bone-marrow cells (8,192) are susceptible t cytolysis.

A central issue in the study of the specificity of NK cells is whether on common target structure is recognized by all NK cells or whether subsets o NK cells recognize a variety of target-cell structures. If multiple structure exist, attention must be focused on the extent or size of the repertoire and o whether discrete subpopulations of NK cells each have restricted reactivit against one or a few of these target structures.

Nature of Target Structures

Knowledge of the biochemical nature of the target-cell structures recognize by NK cells would help to determine the extent of their diversity. Howeve few studies have examined this problem (193,194). Digestion of target cell with proteases has resulted in the loss of susceptibility to lysis with most NI targets. In a study of target structures recognized by mouse NK cells, Rode et al (193) reported that high-molecular-weight glycoproteins from NK-sus ceptible targets inhibit binding of NK cells to targets, but a more detaile characterization of these molecules has not been made. In order to identif target-cell molecules recognized by NK human cells, we (194) used a simila approach, examining the inhibition of binding of purified populations of huma LGL to NK-susceptible targets by detergent-solubilized plasma membrane

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from target cells. Such preparations were inhibitory, but only when incorporated into micelles of synthetic lipids. Reconstituted membranes from human and rat NK-susceptible targets inhibited specifically, and in a dose-dependent fashion, human or rat NK cell-target conjugates, respectively. The inhibitory target-cell material was a glycoprotein with a broad molecular weight range (30,000–165,000). These preliminary results suggest that a number of molecular moieties may be recognized by NK cells.

Heterogeneity in Specificity

CYTOTOXICITY STUDIES Early studies (3,5,12–15,23) indicated that in vitro cultured tumor cells and tumor cell lines were usually susceptible to lysis by NK cells, whereas fresh tumor cells were relatively resistant. However, recent studies with highly purified populations of LGL have demonstrated substantial levels of cytolytic activity against most primary autologous (83,85,195) as well as allogenic tumor cells (85,86,195). Studies with various virus-infected target cells (13,15,196–198) have demonstrated increased susceptibility to lysis compared to that of the uninfected control cell lines.

Rather than nonselective reactivity of each NK cell against the wide array of susceptible targets, distinct subsets of NK cells appear to possess different patterns of reactivity. LGL with receptors for sheep erythrocytes (as demonstrated by reactivity with the MoAb Lyt 3) demonstrated strong reactivity against virus-infected cells, whereas the receptor-negative LGL showed relatively low activity against these targets (13,15,199).

In addition to reactivity of NK cells against tumor or microbial targets, considerable evidence indicates that NK cells play an important role in hybrid resistance. The available data suggest that a subpopulation of NK cells can kill or inhibit the growth of bone-marrow progenitors on the basis of recognition of MHC-linked Hh antigens (15,192,200–202); this subset seems to differ from the subset of cells responsible for lysis of tumor targets.

Another heterogeneity within the LGL population is the divergence between NK and ADCC activities. Although some LGL appear to exhibit both types of reactivity (13,15,61), other cells seem to possess only one or the other type of cytotoxic function. (*a*) In miniature swine, ADCC develops earlier in life than NK activity, and other differences between the two effector cells have been noted (203); (*b*) LGL from the peritoneal cavity of rats have NK activity similar to that of LGL in peripheral blood, but these cells have little or no ADCC activity (204); (*c*) LGL leukemias often have only NK or ADCC activity (15; unpublished observation); and (*d*) some LGL clones may exhibit either NK or ADCC but not both (180,183). Such clones represent only a portion of the cytolytic clones examined; the other clones exhibited broader reactivity (Table 4).

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Table 4 Summary of the patterns of cytotoxic reactivity by human LGL clones[a]

	Number of Clones	% of cytotoxi clones
Tested for cytotoxicity	196	—
Cytotoxic	44	100
With broad reactivity	26	59
With restricted reactivity	18	41
Killing only K562	6	14
Killing only Daudi	2	5
Killing only Molt-4	1	2
Killing only in ADCC	3	7
Killing K562 + Molt-4	5	11
Killing in ADCC + Alab	1	2
Killing other targets but no ADCC	14	32

[a]Summary of data from (183).

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SPECIFICITY OF MOUSE NC CELLS The NC subset of mouse natural effectc cells has been defined mainly in terms of its specificity, with reactivity agains a limited series of target cells, mainly monolayer cultures of solid tumor cell such as Meth A, Meth 113, and WEHI 164, with no reactivity against th widely used NK-susceptible lymphoma target cells YAC-1 or RL♂1 (13,15,56- 57).

COLD-TARGET INHIBITION ASSAY The cold-target inhibition assay, in whic varying numbers of unlabeled NK-susceptible target cells are added to a mix ture of effector cells and labeled target cells, has provided some indication c which target cell structures are shared among different cell lines. This prc cedure (70,91) has been used extensively to analyze the specificity of huma and rodent NK cells (2,13,15,70,91). Optimal inhibition was generally prc duced by unlabeled cells that were the same as the labeled targets. In additior some NK-susceptible targets were unable to inhibit the lysis of other targets indicating either the recognition of separate target structures or heterogeneit among the effector cells. In a study with clones of mouse spleen cells wit NK-like activity (183), a lack of crossinhibition by some susceptible targel was observed, indicating that multiple receptors for target structures wer probably present on the same effector cells.

MONOLAYER ADSORPTION TECHNIQUES This procedure has provided anothe approach to determining the degree of heterogeneity among the target struc tures recognized by NK cells (205,206). This diversity could be due to he erogeneous clones of effector cells (*a*) with unique receptors, (*b*) with multipl receptors, or (*c*) with combinations of common and less frequently observe

Annual Reviews

target cell receptors (Figure 1). By first enabling interaction between a population of effector cells and a panel of immobilized NK-susceptible targets, it has been possible completely to remove NK activity against the absorbing target cell. Residual nonadherent effectors remained, which reacted against other NK-susceptible targets. Such results indicated multiple subsets of NK cells, with some effector cells recognizing a restricted range of target cells. One study with human NK cells and a panel of five target cells (205) indicated that at least seven specificities were recognized—direct evidence for heterogeneity in NK cell recognition.

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CLONES The availability of cytotoxic clones derived from human LGL has provided a powerful approach for analysis of the degree of heterogeneity of recognition by NK cells (180,183). A series (~ 200) of IL-2-maintained clones from fresh peripheral-blood LGL were tested against a panel of NK-susceptible target cells and against antibody-coated targets (to evaluate ADCC). Although most of the LGL clones tested demonstrated reactivity against most of the targets, many of the clones demonstrated selective reactivity against only some of the NK-susceptible targets (summarized in Table 4). In addition, some clones demonstrated a divergence between NK and ADCC activities. Only a small proportion of the clones tested had ADCC activity but no detectable NK activity (7%), whereas NK in the absence of ADCC was more frequent (32%).

The findings that some clones have restricted and divergent patterns of specificity seems to suggest the clonal heterogeneity of NK cells. However, an alternative explanation for such results is that each effector cell has multiple receptors, each recognizing separate specificities. The cold-target inhibition studies with mouse clones [(182); discussed above] support this possibility. In addition, in repeated tests of the specificity of some clones, the main source of variability in the cytotoxicity patterns has been the loss of reactivity against some targets upon continued culture in vitro (180,183). An additional type of alteration of specificity has been seen in studies with murine (207,208) or human (209–213) clones of CTL. Some clones developed NK-like activity upon continued culture in vitro with IL-2.

It seems that a small percentage of murine and human CTL clones can exhibit distinct receptors, with one type specifically recognizing MHC determinants and the other recognizing NK-susceptible targets (209,212,213). The relevance of such observations to the nature and heterogeneity of NK cells is not clear. On the one hand, one might suggest that NK cells and CTL are closely related and that it is possible to have transitions from CTL into NK cells (208). However, an equally likely alternative is that the ability to express receptors for NK-susceptible targets is not restricted to NK cells; under some circumstances, other cell types, including CTL, may be able to express such receptors (209,212).

Annual Reviews

REGULATION OF NK ACTIVITY

Augmenting Agents

Interferon (IFN) is the most widely studied agent that can potently augment reactivity of NK cells (13,15,63,198). The mechanism by which IFN augments is dependent on the effector cells and the target cell type employed during the assay. IFNs α and β increase the proportion of human LGL that bind to target cells with low levels of susceptibility to NK activity (especially carcinoma cell lines) (61,63). In studies with mouse NK cells, IFN converted noncytotoxic LY 5^- cells into Ly 5^+ active NK cells (149). Against most targets studied, IFN also increased both the proportion of lytically active LGL among those able to form conjugates with NK-susceptible targets and the rate of killing by lytically active cells (13,15,63). In addition to increasing the number of effector cells and their efficiency, IFN increased recycling of LGL [i.e. the ability to kill multiple targets during the course of the assay (63,214)]. Thus, these studies with IFN indicate another type of heterogeneity among the NK cells, with cells at different stages of activation (63,84,94,215). They also indicate the existence of pre-NK cells, which lack the recognition receptors for target cells and/or lack the lytic machinery to exert NK activity.

Recent studies in mice have suggested yet another mechanism by which NK activity may be augmented. *C. parvum,* interferon, or other boosting agents induced the appearance of NK blast cells, apparently with some proliferation of the effector cells (13,15,215,216). The basis for this proliferative stimulation is not clear; but it may be due to an increased sensitivity of NK cells to low levels of IL-2, since in vitro studies in both mouse and human have indicated that IFN increases the proliferative response of cells to IL-2 (104,177,178).

It seems that not all NK cells are responsive to the effects of IFN. In studies with mouse NK cells, Minato et al (149) distinguished between IFN-responsive and IFN-independent subsets of NK cells. Also, NC cells have been reported to be unresponsive to IFN (13,56). In contrast, the activity of NC cells has been augmented by treatment with IL-3 (187).

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Inhibitory Agents

A variety of agents inhibit the lytic activity of highly purified populations of human LGL. This inhibition of cytolysis may occur either at the level of recognition and binding to target cells (as determined by conjugate formation) or at a postbinding or lytic stage. Most inhibitory agents (e.g. cAMP, cholera toxin, PGE, and some phosphorylated sugars) do not appear to act at the level of target binding (13,15,217,218). The only inhibitory agents that blocked binding of LGL to target cells were EDTA, phorbol esters, and proteases (96,112,218). It is of interest that the postbinding inhibition of NK activity

Annual Reviews

by some sugars (e.g. mannose 6-PO4, fructose 6-PO4) was not paralleled by inhibition of LGL-mediated ADCC activity (217). This suggests some important difference in the lytic mechanism responsible for NK and ADCC.

CYTOKINE PRODUCTION AND OTHER NONCYTOTOXIC FUNCTIONS OF LGL

In addition to mediating NK activity, LGL are secretory cells, releasing a variety of cytokines in response to various stimuli. At least some of these cells can secrete not only soluble NK cytotoxic factors (NKCF) (129,132–134) and lymphotoxin (129) but also IFNs α, β, γ (127,129,130), interleukin 1 (IL-1) (128), interleukin 2 (IL-2) (128,130), colony-stimulating factor (CSF) (130), and B-cell growth factor (BCGF) (129).

Fresh LGL

Analysis of the phenotype of human LGL producing IL-1 in response to bacterial lipopolysaccharide or IL-2 in response to phytohemagglutinin indicates that distinct subpopulations of LGL are responsible for the release of each cytokine (Table 5). An Ia-positive subset seems to produce IL-1 (128,219), whereas both Ia-positive and Ia-negative LGL can produce IL-2 (128,220). Much of the IL-1 production depended also on the presence of OKT-11$^+$ cells, whereas the removal of OKT8 or Leu-7-positive cells led to an increase in IL-1 production. IL-2 production appeared to be due to OKT11$^+$ LGL with both OKM1$^-$ and OKM1$^+$ cells contributing to a similar extent. Most other cytokines have been associated with OKM1$^+$ cells. IFN$_\gamma$ production by LGL in response to stimuation by viruses has been reported to be due predominantly to a small subpopulation of noncytotoxic cells (129). However, another study, with stimulation of IFN$_\gamma$ by K562 cells, indicated that LGL subsets with or without reactivity to OKT11 or OKM1 were equally effective producers of IFN$_\gamma$.

It appears that LGL can either positively or negatively regulate B cells, suppress B-cell responses (221), and secrete BCGF (222). However, we do

Table 5 Phenotype of subsets of human LGL producing various cytokines[a]

Activity measured	Phenotype	Stimuli
IFN$_\gamma$	B73.1$^+$ OKT11$^+$ OKM1$^+$ DR$^+$	PHA, Con A, K562
IL-1	B73.1$^+$ OKT11$^{+/-}$OKM1$^+$ DR$^+$	LPS, silica
IL-2	B73.1$^+$ OKT11$^+$ OKM1$^-$ DR?	PHA, K562
BCGF	B73.1$^+$ OKT11$^+$ OKM1$^-$ DR$^-$	PHA, Con A
Cell-mediated cytotoxicity	B73.1$^+$ OKT11$^+$ OKM1$^+$ DR$^-$	—

[a]Summary of data from (129).

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not yet know whether the same or different subpopulations of cells are responsible for these divergent effects.

Cloned LGL

Recently a number of cytotoxic and noncytotoxic clones of human LGL were examined for their ability to produce cytokines (129,223). Table 3 summarizes the cytotoxic activity, the phenotype, and the cytokine-producing capabilities of several clones. The clones of LGL were tested for production of IFN (after lectin stimulation), IL-1 (after LPS stimulation), IL-2, CSF, and BCGF (after lectin stimulation). Production of IFN_{γ}, spontaneously or after stimulation with PHA, was seen with all clones tested. However, only a portion of the clones tested produced IL-1 or IL-2. Among the cytotoxic clones, 15% produced IL-2 and 36% produced IL-1. A similar proportion of the noncytotoxic clones produced these cytokines.

Some clones could produce both IL-1 and IL-2 (Table 4). In most cases, both cytokines were not simultaneously released. However, one clone (E) produced both IL-1 and IL-2 at the same time. In regard to the possible correlation of the phenotype of the clones and their ability to produce the various cytokines, expression of either OKT3 or B73.1 markers did not correlate with the ability to produce either IL-1 or IL-2. However, the clones secreting these cytokines were generally positive for both OKM1 and OKT8.

Thus far, no clones have been found to produce BCGF or CSF. However, since only eight clones have been examined, it is not yet clear whether the subset of LGL that can be cloned with IL-2 lack the ability to produce these factors.

These data on cytokine production indicate that LGL are functionally diverse with the ability not only to mediate NK activity but also to produce a variety of cytokines. However, several important issues are not yet resolved: (*a*) To what extent are the cytotoxic and cytokine-producing functions shared by the same cells? It will be necessary to test many more clones, particularly more clones with cytotoxic activity, in order to resolve this question. Further, it would seem important to determine whether individual cells within a clone that have NK-like activity also secrete cytokines at the same time. (*b*) Can a single cell simultaneously produce multiple cytokines? Although some clones released more than one cytokine, the frequent lack of simultaneous production of IL-1 and IL-2 suggests that each cell may be able to produce only one factor at a time. To answer this question, it will probably be necessary to analyze single cells (e.g. by two-color immunofluorescence with monoclonal antibodies to different cytokines). (*c*) What is the extent of association of cytokine production with discrete LGL subsets, and is this true clonal heterogeneity regarding cytokine production? Although the data obtained with fresh

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LGL suggest the association of production of particular cytokines with subsets of cells, the results with the clones have not tended to support a clear segregation of functions. It seems possible that, over a period of time and under the appropriate environmental conditions and stimuli, each clone of LGL may be able to produce the whole array of cytokines.

LINEAGE OF NK CELLS

Although most if not all NK activity is mediated by LGL, one might ask whether LGL represent a single lineage or multiple lineages with similar functions and morphology (13,15,225,226). Because of the sharing of several features with T cells and monocytes (Table 1), several alternatives must be considered: (*a*) NK cells may derive from the T-cell lineage, (*b*) NK cells may derive from the myelomonocyte lineage, (*c*) NK cells may be from separate lineages with a precursor stem cell common to lymphocytes and monocytes, or (*d*) some NK cells may derive from the T-cell lineage while others derive from the myelomonocytic or other lineages (Figure 3).

T-Cell Lineage

The possibility that NK cells are associated with the T-cell lineage is supported by evidence that both human and rodent NK cells share a number of characteristics with T cells. NK cells form rosettes with sheep erythrocytes, express T cell–associated antigens, respond to a variety of mitogens, and demonstrate continuous growth in the presence of IL-2. However, although the growth of human LGL in response to IL-2 suggests some relationship to the T-cell lineage, neither this nor the other available data conclusively indicate that NK cells derive from it. Although all previous evidence indicated that IL-2 was a selec-

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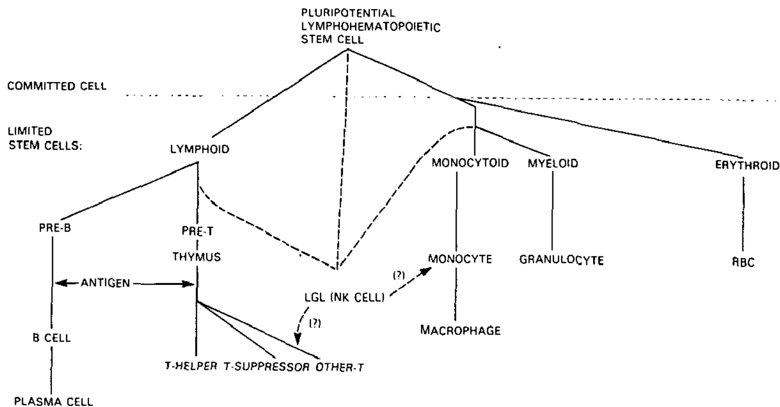

Figure 3 Possible alternative lineages of LGL.

Annual Reviews

tive growth factor only for T cells, IL-2 may in fact be able to promote the growth of a separate NK-cell lineage in addition to T cells.

The most unequivocal evidence for a direct relationship between NK cells and T cells may come from studies of expression of T cell–restricted or rearranged genes in NK cells. The recent report that two clones with NK-like activity have recognition structures homologous to the T-cell receptors (224) is intriguing; but, as discussed above, the development of NK-like activity in clones of T cells may not represent a true conversion from T cells to NK cells.

Monocyte Lineage

NK cells appear to be derived from stem cells in the bone marrow (89). Lohmann-Mattes et al (161) reported that mouse promonocytes cultured from bone marrow exhibit NK-like activity. This led to the suggestion that NK cells are derived from the myelomonocytic lineage. However, these results may have been due to contamination of promonocytes by NK-cell progenitors or to expression of NK-like activity by macrophages. As yet, no direct evidence exists that NK cells are derived from the same stem cell as promonocytes. In addition, preliminary studies indicate that rat LGL lack receptors for CSF and fail to grow in response to CSF (J. R. Ortaldo, R. B. Herberman, C. W. Reynolds, unpublished observation). Similarly, human bone-marrow NK-like progenitors appear to require at least both IL-2 and CSF for maximal growth and development of cytolytic function (176).

The expression of some myelomonocytic markers on LGL (i.e. OKM1, M102, MAC-1) has also raised the possibility of a relationship of NK cells to monocytes (139,155,161,162). However, these markers are not restricted to monocytes, reacting also with polymorphonuclear leukocytes and platelets (139). In contrast, MoAb with more selective reactivity with human monocyte lineage cells (e.g. Leu-M1, MO2) do not react with LGL.

Separate Lineage

NK cells may develop along a separate pathway from the pluripotent hematopoietic stem cell (Figure 3). The sharing of a common ancestor might account for the similarities between NK cells and both the T lymphocyte and myelomonocytic lineages. This possibility is attractive since the phenotypic characteristics of NK cells do not allow them to be readily placed into a niche in the main pathways of differentiation of either T cells or myelomonocytic cells.

Multiple Lineages

A final hypothesis to consider is that NK cells, despite their sharing of cytotoxic functions and LGL morphology, are comprised of separate subsets of cells, some derived from the T-cell lineage and others from the myelomonocytic lineage. The question of whether NK cells are derived from one or

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multiple lineages is difficult to answer. Since a low frequency of human LGL (1/70) grow in response to IL-2 (104,176–178), these cells may represent a subset that can respond to this specific cytokine. Other subsets of NK cells may be stimulated to grow by another set of factors. The paradoxical findings discussed above (see the section on NK cells in short-term culture), of high NK activity and an increased proportion of LGL in nude mice, despite the deficiency in production of IL-2 and the low frequency of IL-2-responsive progenitors of NK cells, are compatible with this hypothesis. Alternatively, the low frequency of IL-2-responsive progenitors of cytotoxic cells among peripheral-blood LGL may simply reflect the relative maturity and perhaps terminal differentiation of most cells in this population, with only a small proportion of cells being sufficiently immature to grow in response to IL-2. In support of this argument, only a small proportion of T cells can grow under identical conditions in response to IL-2 (177,178).

CONCLUSIONS

Most data indicate that NK cells are a distinct cell population that can be defined by a series of characteristics like those we use to define T cells, B cells, or macrophages. The existence of a discrete morphological counterpart of the NK cell—i.e. the LGL—has made it particularly convenient to categorize this effector and distinguish it from a variety of other effector cells. The heterogeneity seen with NK cells is really not greater than that seen with such other cell types as T cells and macrophages (40,46,153,154,227,228). For example, within the T-cell compartment there are separate subsets of cells that exhibit cytolytic activity, helper functions, or suppressor functions; like LGL, T cells can secrete a wide array of lymphokines. In addition, considerable diversity in phenotype exists among the subsets of T cells, with helper T cells and suppressor T cells expressing different cell-surface markers, and activated T cells expressing antigens not detectable on resting T cells (e.g. OKT10, Ia, Tac).

With T cells, much of the heterogeneity is clonally distributed. T cells have idiotypic surface receptors for antigens (229-235), which appear to be analogous to the clonally distributed idiotypic immunoglobulins associated with B cells. It remains unclear whether NK cells have analogous, genetically determined clonal heterogeneity. Although clones of human LGL vary in their patterns of cytotoxic activity, it does not appear that NK cells have an extensive repertoire that even approaches that of T or B cells. It remains possible that much or even all of the heterogeneity in phenotype and function that has been seen is due to differences in the phase of differentiation and/or activation of the cells, with clones tending to remain in essentially the same stage as the LGL from which they were derived.

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Such differentiation-dependent, nongenetically determined heterogeneity appears to account for the heterogeneity seen in subsets of monocytes and macrophages. Different subpopulations of these cells can vary markedly in their markers and functions. For example, expression of Ia antigen is not a stable characteristic of monocytes or macrophages but is associated with IFN activation of the cells (236–241), with concomitant expression of accessory functions and cytokine production. In addition, as with NK cells, the wide spectrum of cytolytic activity by monocytes and macrophages appears to be mediated by subsets of effector cells, each with more restricted specificity (242–245). Furthermore, as with NK cells and pre-NK cells, monocytes and macrophages can be categorized according to their level of activation for cytotoxic reactivity (242,245). Some macrophages are unable to bind tumor target cells, while others can bind but lack the ability to lyse the targets.

A major unresolved issue is whether non-NK cells mediate NK-like activity. Since recent studies have indicated T-cell clones can develop NK-like activity (207,210), and some populations of human monocytes have been reported to exert rapid cytolytic activity against NK-susceptible target cells (246), this possibility must be kept in mind. However, the definition of other cell types as having NK-like activity solely on the basis of their killing a few NK-susceptible target cells is problematic. To resolve this question definitively, it would be helpful to characterize the recognition receptors on NK cells and determine whether the same types of receptors are expressed on other cells with similar cytotoxic activities.

Although heterogeneity within NK cells exists, this function is likely mediated by a discrete population of lymphoid cells (LGL), separable from other lymphoid cells. It is no longer satisfactory to speak of ill-defined, nonspecific spontaneous cytotoxic reactivity, mediated by unrelated types of lymphoid cells.

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Literature Cited

1. Rosenberg, E. B., Herberman, R. B., Levine, P. H., Halterman, R. H., McCoy, J. L., Wunderlich, J. R. 1972. Lymphocyte cytotoxicity reactions to leukemia-associated antigens in identical twins. *Int. J. Cancer* 9:648
2. McCoy, J. L., Herberman, R. B., Rosenberg, E. B., Donnelly, F. C., Levine, P. H., Alford, C. 1973. ^{51}Chromium release assay for cell-mediated cytotoxicity of human leukemia and lymphoid tissue-culture cells. *Natl. Cancer Inst. Monogr.* 37:59
3. Kiessling, R., Klein, E., Wigzell, H. 1975a. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur. J. Immunol.* 5:112
4. Kiessling, R., Petranyi, G., Klein, G., Wigzell, H. 1975b. Genetic variation of *in vitro* cytolytic activity and *in vivo* rejection potential of nonimmunized semisyngeneic mice against a mouse lymphoma line. *Int. J. Cancer* 15:933
5. Kiessling, R., Petranyi, G., Klein, G., Wigzell, H. 1976. Non-T-cell resistance against a mouse Moloney lymphoma. *Int. J. Cancer* 17:275
6. Baldwin, R. W. 1976. Role of immunosurveillance against chemically induced rat tumors. *Transplant Rev.* 28:62
7. Oldham, R. K., Siewarski, D., McCoy, J. L., Plata, E. J., Herberman, R. B. 1973. Evaluation of a cell-mediated cytotoxicity assay utilizing ^{125}iododeoxyuridine labelled tissue culture target cells. *Natl. Cancer Inst. Monogr.* 37:49
8. Cudkowicz, G., Hochman, P. S. 1979. Do natural killer cells engage in regulated

Annual Reviews

Annu. Rev. Immunol. 1984.2:359-394. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

reactions against self to ensure homeostasis? *Immunol. Rev.* 44:13

9. Haller, O., Kiessling, R., Örn, A., Kärre, K., Nilsson, K., Wigzell, H. 1977. Natural cytotoxicity to human leukemia mediated by mouse non-T cells. *Int. J. Cancer* 20:93
10. Hanna, N., Burton, R., 1981. Definitive evidence that natural killer (NK) cells inhibit experimental tumor metastasis *in vivo*. *J. Immunol.* 127:1754
11. Hanna, N., Fidler, I. J. 1980. The role of natural killer cells in the destruction of circulating tumor emboli. *J. Natl. Cancer Inst.* 65:801.
12. Herberman, R. B. 1974. Cell-mediated immunity to tumor cells. *Adv. Cancer Res.* 19: 207–63
13. Herberman, R. B., ed. 1980. *Natural Cell-Mediated Immunity Against Tumors*. NY: Academic
14. Herberman, R. B., Holden, H. T. 1978. Natural cell-mediated immunity. *Adv. Cancer Res.* 27:305
15. Herberman, R. B., ed. 1982. *NK Cells and Other Natural Effector Cells*. NY: Academic
16. Herberman, R. B., Brunda, M. J., Djeu, J. Y., Domzig, W., Goldfarb, R. H., Holden, H. T., Ortaldo, J. R., Reynolds, C. W., Riccardi, C., Santoni, A., Stadler, B. M., Timonen, T. 1981c. Immunoregulation and natural killer cells. In *Natural Killer Cells. Human Cancer Immunology*, ed. B. Serrou, C. Rosenfeld, R. B. Herberman, 4:37–52. Amsterdam: Elsevier North-Holland
17. Herberman, R. B., Ortaldo, J. R. 1981. Natural killer cells: Their role in defenses against disease. *Science* 214:24
18. Nunn, M. E., Herberman, R. B., Holden, H. T. 1977. Natural cell-mediated cytotoxicity in mice against non-lymphoid tumor cells and some normal cells. *Int. J. Cancer* 20:381
19. Ojo, E., Wigzell, H. 1978. Natural killer cells may be the only cells in normal mouse lymphoid populations endowed with cytolytic ability for antibody-coated tumor target cells. *Scand. J. Immunol.* 7:297
20. Ortaldo, J. R., Oldham, R. K., Cannon, G. C., Herberman, R. B. 1977. Specificity of natural cytotoxic reactivity of normal human lymphocytes against a myeloid leukemia cell line. *J. Natl. Cancer Inst.* 59:77
21. Petrayni, G., Kiessling, R., Povey, S., Klein, G., Herzenberg, E., Wigzell, H. 1976. The genetic control of natural killer cell activity and its association with *in vivo* resistance against a Moloney lymphoma isograft. *Immunogenetics* 3:15
22. Pollack, S., Heppner, S., Brawn, R. J., Nelson, K. 1972. Specific killing of tumor cells *in vitro* in the presence of normal lymphoid cells and sera from hosts immune to the tumor antigens. *Int. J. Cancer* 9:316
23. Herberman, R. B., Nunn, M. E., Lavrin, D. H. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and aldogeneic tumors. I. Distribution of reactivity and specificity. *Int. J. Cancer* 16:216
24. Herberman, R. B., Djeu, J. Y., Kay, H. D., Ortaldo, J. R., Riccardi, C., Bonnard, G. D., Holden, H. T., Fagnani, R., Santoni, A., Puccetti, P. 1979. Natural killer cells: Characteristics and regulation of activity. *Immunol. Rev.* 44:43
25. Koren, H. S., Herberman, R. B. 1983. Natural killing—present and future (Summary of workshop on natural killer cells). *J. Natl. Cancer Inst.* 70:785
26. Adams, D. O., Synderman, R. 1979. Do macrophages destroy nascent tumors? *J. Natl. Cancer Inst.* 62:1341
27. Allison, A. C., Hammington, J. S., Birbeck, M. 1966. An examination of the cytotoxic effects of silica on macrophages. *J. Exp. Med.* 124:141
28. Blair, P. B., Lane, M. A., Mar, P. 1976. Antibody in the sera of tumor-bearing mice that mediates spleen cell cytotoxicity toward the autologous tumor. *J. Immunol.* 116:606
29. Chow, D. A., Greene, M. I., Greenberg, A. H. 1979. Macrophage-dependent, NK cell-independent natural surveillance of tumors in syngeneic mice. *Int. J. Cancer* 23:788
30. Chow, D. A., Wolosin, L. B., Greenberg, A. H. 1981. Immune natural antitumor antibodies. II. The contribution of natural antibodies to tumor surveillance. *Int. J. Cancer* 27:459
31. Collavo, D., Colombatti, A., Chieco-Bianchi, L., Davis, A. J. S. 1974. T lymphocyte requirement for MSV tumour prevention or regression. *Nature* 249:169
32. Doll, R., Kinlen, L. 1970. Immunosurveillance and cancer: Epidemiological evidence. *Br. Med. J.* 4:420
33. Eccles, S. A., Alexander, P. 1974. Macrophage content of tumors in relation to metastatic spread and host immune reaction. *Nature* 250:667
34. Evans, R. 1972. Macrophages in syngeneic animal tumors. *Transplantation* 14:468
35. Tagliabue, A., Mantovani, A., Kilgallen, M., Herberman, R. B., McCoy, J. L. 1979. Natural cytotoxicity of mouse monocytes and macrophages. *J. Immunol.* 122:2363

Annual Reviews

36. Evans, R. 1979. Host cells in transplanted murine tumors and their possible relevance to tumor growth. *J. Reticuloendothel. Soc.* 26:427
37. Fidler, I. J. 1974. Inhibition of pulmonary metastases by intravenous injection of specifically activated macrophages. *Cancer Res.* 34:1074
38. Hafeman, D. G., Lucas, Z. J. 1979. Polymorphonuclear leukocyte-mediated, antibody-dependent cellular cytotoxicity against tumor cells: dependence on oxygen and the respiratory burst. *J. Immunol.* 123:55
39. Haskill, J. S., Fett, J. W. 1976. Possible evidence for antibody-dependent macrophage-mediated cytotoxicity directed against murine adenocarcinoma cells *in vivo*. *J. Immunol.* 117:1992
40. Herberman, R. B., Holden, H. T., Djeu, J. Y., Jerrells, T. R., Varesio, L., Tagliabue, A., White, S. L., Oehler, J. R., Dean, J. H. 1980. Macrophages as regulators of immune responses against tumors. In *Macrophages and Lymphocytes*, Part B, ed. M. R. Escobar, H. Friedman, pp. 361–69. NY: Plenum
41. Mantovani, A., Tagliabue, A., Dean, J. H., Jerrells, T. R., Herberman, R. B. 1979. Cytolytic activity of circulating human monocytes on transformed and untransformed human fibroblasts. *Int. J. Cancer* 23:29
42. Hibbs, J. B. 1975. Activated macrophages as cytotoxic effector cells. I. Inhibition of specific and nonspecific tumor resistance by trypan blue. *Transplantation* 19:77
43. Hibbs, J. B., Jr., Lambert, C. H., Jr., Remington, J. S. 1972. Control of carcinogenesis: a possible role for the activated macrophage. *Science* 177:998
44. Herberman, R. B., Brunda, M. J., Domzig, W., Fagnani, R., Goldfarb, R. H., Holden, H. T., Ortaldo, J. R., Reynolds, C. W., Riccardi, C., Santoni, A., Stadler, B. M., Taramelli, D., Timonen, T., Varesio, L. 1982. In *The Biological Significance of Immune Regulation*, ed. M. E. Gershwin, L. N. Ruben, pp. 139–66. NY: Marcel Dekker
45. Hibbs, J. B., Jr., Chapman, H. A., Jr., Weinberg, J. B. 1978. The macrophage as an antineoplastic surveillance cell; biologic perspectives. *J. Reticuloendothel. Soc.* 24:549
46. Holden, H. T., Varesio, L., Taniyama, T., Puccetti, P. 1979. Functional heterogeneity and T cell–dependent activation of macrophages from murine sarcoma virus (MSV)-induced tumors. See Ref. 40, pp. 509–20
47. Houghton, A.
Ikeda, H., Wata
Old, L. J. 198
normal human
cell surface anti
Natl. Acad. Sci
48. Korec, S. 1980
in host defense
13, pp. 1301–
49. Landazuri, M.
1974. Antibody
toxicity to a
induced lymph
52:147
50. Levy, M. H., W
role of macrop
neoplastic dise
20:131
51. Levy, P. C., Yh
1979. Human m
granulocyte a
mediated cytoto
J. Immunol. 12
52. Mantovani, A.
J. H., Herberm
tic and cytostat
of circulating h
Cancer 23:18
53. Mantovani, A.
rutti, N., Spreaf
Divergent effe
on growth of
metastasis. *Int.*
54. Marcelleti, J.
Spontaneous re
induced erythr
macrophages i
120:1
55. Wiltrout, R. H.
H. T. 1982. V
tumor cell cyt
phages, macro
NK cells. *Int.*
56. Stutman, O., Fi
Lattime, E. C.
(NC) cells agai
general charact
natural killer (N
187–229
57. Stutman, O., F
1980. Natural
tumor-bearing
1073–79
58. Ting, C. C., I
Humor host de
tumors. In *I*
Experimental
Richter, M. A.
Academic
59. Wood, G. W.,
Studies on the
regulation of g

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Annual Reviews

murine chemically induced fibrosarcomas. *Int. J. Cancer* 16:1022
60. Timonen, T., Saksela, E. 1980. Isolation of human natural killer cells by discontinuous gradient centrifugation. *J. Immunol. Meth.* 36:285
61. Timonen, T., Ortaldo, J. R., Herberman, R. B. 1981. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J. Exp. Med.* 153:569
62. Timonen, T., Reynolds, C. W., Ortaldo, J. R., Herberman, R. B. 1982. Isolation of human and rat natural killer cells. *J. Immunol. Meth.* 41:269
63. Timonen, T., Ortaldo, J. R., Herberman, R. B. 1982. Analysis by a single cell cytotoxicity assay of natural killer (NK) cell frequencies among human large granular lymphocytes and of the effects of interferon on their activity. *J. Immunol.* 128:2514
64. Reynolds, C. W., Timonen, T., Herberman, R. B. 1981. Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cell. *J. Immunol.* 127:282
65. Reynolds, C. W., Sharrow, S. O., Ortaldo, J. R., Herberman, R. B. 1981. Natural killer activity in the rat. III. Analysis of surface antigens on LGL by flow cytometry. *J. Immunol.* 127:2204
66. Reynolds, C. W., Timonen, T., Holden, H. T., Hansen, C. T., Herberman, R. B. 1982. Natural killer (NK) cell activity in the rat. Analysis of effector cell morphology and effects of interferon on NK cell function in the athymic (nude) rat. *Eur. J. Immunol.* 12:577
67. Tagliabue, A., Luini, W., Soldaleschi, D., Boraschi, B. 1981. Natural killer activity in gut mucosal lymphoid cells in mice. *Eur. J. Immunol.* 11:919
68. Kumugai, K., Itoh, K., Suzuki, R., Hinuma, S., Saitoh, F. 1981. Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. *J. Immunol.* 127:282
69. Ortaldo, J. R. 1983. Heterogeneity of cytotoxicity of human cultured T cells. *Immunol. Clin. Speriment.* 2:20
70. Ortaldo, J. R. 1983. Specificity of human natural killer (NK) cells. *Immunol. Clin. Speriment.* 2:13
71. Axberg, I., Gidlund, M., Orn, A., Pattengale, P., Riesenfeld, I., Stern, P., Wigzell, H. 1980. Natural killer cells: notes on features and functions. In *Thymus, Thymic Hormones and T Lymphocytes*, ed. F. Aiuti, pp. 181–96. NY: Plenum
72. Mattes, M. J., Sharrow, S. O., Herberman, R. B., Holden, H. T. 1979. Identification and separation of Thy-1 positive mouse spleen cell active in natural cytotoxicity and antibody-dependent cell-mediated cytotoxicity. *J. Immunol.* 123:2851
73. Kay, H. D., Bonnard, G. D., West, W. H., Herberman, R. B., 1977. A functional comparison of human Fc-receptor-bearing lymphocytes active in natural cytotoxicity and antibody-dependent cellular cytotoxicity. *J. Immunol.* 118:2058
74. Natural cell-mediated cytotoxicity in rats. III. Effects of immunopharmacologic treatments on natural reactivity and on reactivity augmented by polyinosinic-polycytidylic acid. *Int. J. Cancer* 21:221
75. Koren, H. S., Williams, M. S. 1978. Natural killing and antibody-dependent cellular cytotoxicity are mediated by different mechanisms and by different cells. *J. Immunol.* 121:1956
76. Landazuri, M. O., Silva, A., Alvarez, J., Herberman, R. B. 1979. Evidence that natural cytotoxicity are mediated in humans by the same effector cell populations. *J. Immunol.* 123:252
77. Shin, H. S., Johnson, R. J., Pasternack, G. R., Economou, J. S. 1978. Mechanisms of tumor immunity: the role of antibody and nonimmune effectors. *Prog. Allergy* 25:163
78. Eremin, O. 1980. NK cell activity in the blood, tumour-draining lymph nodes and primary tumours of women with mammary carcinoma. See Ref. 13, pp. 1011–29
79. Gerson, J. M. 1980. Systemic and in situ natural killer activity in tumor-bearing mice and patients with cancer. See Ref. 13, pp. 1047–62
80. Gerson, J. M., Varesio, L., Herberman, R. B. 1981. Systemic and in situ natural killer and suppressor cell activities in mice bearing progressively growing murine sarcoma virus-induced tumors. *Int. J. Cancer* 27:243
81. Roder, J. C., Laing, L., Haliotis, T., Kozbor, D. 1981. Genetic control of human NK function. See Ref. 16, pp. 169–86
82. Landazuri, M. O., Lopez-Botet, M., Timonen, T., Ortaldo, J. R., Herberman, R. B. 1981. Human large granular lymphocytes: Spontaneous and interferon-boosted NK activity against adherent and nonadherent tumor cell lines. *J. Immunol.* 127:1380
83. Mantovani, A., Allavena, P., Biondi, A., Sessa, C., Introna, M. 1981. Natural killer activity in human ovarian carcinoma. In *NK Cells: Fundamental Aspects and Role*

Annu. Rev. Immunol. 1984.2:359-394. Downloaded from arjournals.annualreviews.org
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Annual Reviews

in Cancer, ed. B. Serrou, R. B. Herberman, 4:123–37. Amsterdam: North-Holland

84. Biron, C. A., Welsh, R. M. 1982. Activation and role of natural killer cells in virus infections. *Med. Microbiol. Immunol.* 170:155
85. Serrate, S. A., Vose, B. M., Timonen, T., Ortaldo, J. R., Herberman, R. B. 1982. Association of human natural killer cell activity against human primary tumors with large granular lymphocytes. See Ref. 15, pp. 1055–60
86. Vanky, F. T., Argov, S. A., Einhorn, S. A., Klein, E. 1980. Role of alloantigens in natural killing. Allogeneic but not autologous tumor biopsy cells are sensitive for interferon-induced cytotoxicity of human blood lymphocytes. *J. Exp. Med.* 151:1151
87. West, W. H., Cannon, G. B., Kay, H. D., Bonnard, G. D., Herberman, R. B. 1977. Natural cytotoxic reactivity of human lymphocytes against a myeloid cell line: characterization of effector cells. *J. Immunol.* 118:355
88. Reynolds, C. W., Ward, J. M., Wiltrout, R. H., Herberman, R. B. 1983. Tissue distribution and in vivo localization of rat LGL. In *Proceedings of the International Symposium on Natural Killer Activity and Its Regulation.* Tokyo: Excerpta Medica. In press
89. Haller, O., Kiessling, R., Örn, A., Wigzell, H. 1977. Generation of natural killer cells: an autonomous function of the bone marrow. *J. Exp. Med.* 145:1411
90. Kedar, E., Ikejiri, B. L., Timonen, T., Bonnard, G. D., Reid, J., Navarro, N. J., Sredni, B., Herberman, R. B. 1983. Antitumor reactivity in vitro and in vivo of lymphocytes from normal donors and cancer patients propagated in culture with T-cell growth factor (TCGF). *Eur. J. Cancer Clin. Oncol.* 19:757
91. Ortaldo, J. R., Herberman, R. B. 1981. Specificity of natural killer cells. See Ref. 16, pp. 17–36
92. Brunda, M. J., Holden, H. T., Herberman, R. B. 1980b. Augmentation of natural killer cell activity of beige mice by interferon and interferon inducers. See Ref. 13, pp. 411–15
93. Brunda, M. J., Varesio, L., Herberman, R. B., Holden, H. T. 1982. Interferon-independent, lectin-induced augmentation of murine natural killer cell activity. *Int. J. Cancer* 29:299
94. Djeu, J. Y., Timonen, T., Herberman, R. B. 1981. Augmentation of natural killer cell activity and induction of interferon by tumor cells and other biological response modifiers. In *Role of Natural Killer Cells, Macrophages and Antibody Dependent Cellular Cytotoxicity in Tumor Rejection and as Mediators of Biological Responses Modifiers Activity,* ed. M. Chirigos, pp. 215–25. NY: Raven
95. Domzig, W., Stadler, B. M., Herberman, R. B. 1983. Interleukin-2 dependence of human natural killer (NC) cell activity. *J. Immunol.* 130:1970
96. Goldfarb, R. H., Herberman, R. B. 1981. Natural killer cell reactivity: Regulatory interactions among phorbol ester, interferon, cholera toxin, and retinoic acid. *J. Immunol.* 126:2129
97. Herberman, R. B., Ortaldo, J. R., Djeu, B. Y., Holden, H. T., Jett, J., Lang, N. P., Pestka, S. 1980. Role of interferon in regulation of cytotoxicity by natural killer cells and macrophages. Ann. NY Acad. Sci. 350:63
98. Herberman, R. B., Brunda, M. J., Cannon, G. B., Djeu, J. Y., Nunn-Hargrove, M. E., Jett, J. R., Ortaldo, J. R., Reynolds, C., Riccardi, C., Santoni, A. 1981. Augmentation of natural killer (NK) cell activity by interferon-inducers. In *Augmenting Agents in Cancer Therapy. Current Status and Future Prospects,* ed. E. Hersh, M. Mastrangelo, pp. 253–65. NY: Raven
99. Lang, N. P., Ortaldo, J. R., Bonnard, G. D., Herberman, R. B. 1982. Interferon and prostaglandins: Effects on human natural and lectin-induced cytotoxicity. *J. Natl. Cancer Inst.* 69:339
100. Ortaldo, J. R., Gerard, J. P., Henderson, L. E., Neubauer, R. H., Rabin, H. 1983. Responsiveness of purified natural killer cells to pure interleukin-2 (IL-2). In *Interleukins, Lymphokines and Cytokines,* ed. Oppenheim, J. J., Rabin, H., pp. 63–68. NY: Academic
101. Ortaldo, J. R., Mantovani, A., Hobbs, D., Rubinstein, M., Pestka, D., Herberman, R. B. 1983. Effects of several species of human leukocyte interferon cytotoxic activity of NK cells and monocytes. *Int. J. Cancer* 31:285
102. Biron, C. A., Turgiss, L. R., Welsh, R. M. 1983. Increase in NK cell number and turnover rate during acute viral infection. *J. Immunol.* 131:1539
103. Ortaldo, J. R., Mason, A., Rehberg, E., Moschera, J., Kelder, B., Pestka, S., Herberman, R. B. 1983. Effects of recombinant and hybrid recombinant human leukocyte interferon on cytotoxic activity of natural killer cells. *J. Biol. Chem.* 258:15011
104. Riccardi, C., Vose, B. M., Herberman, R. B. 1983. Modulation of IL-2 depen-

Annu. Rev. Immunol. 1984.2:359-394. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

dent growth of mouse NK cells by interferon and T lymphocytes. *J. Immunol.* 130:228
105. Saksela, E., Timonen, T., Virtanen, I., Cantell, K. 1980. Regulation of human natural killer activity by interferon. See Ref. 13, pp. 645–53
106. Zarling, J. M., Eskra, L., Borden, E. C., Horoszewicz, J., Carter, W. A. 1979. Activation of human natural killer cells cytotoxic for human leukemia cells by purified interferon. *J. Immunol.* 123:63
107. Allavena, P., Introna, M., Mangioni, C., Mantovani, A. 1981. Inhibition of natural killer activity by tumor-associated lymphoid cells from ascitic ovarian carcinomas. *J. Natl. Cancer Inst.* 67:319
108. Brunda, M. J., Herberman, R. B., Holden, H. T. 1980. Inhibition of murine natural killer cell activity by prostaglandins. *J. Immunol.* 124:2682
109. Brunda, M. J., Taramelli, D., Holden, H. T., Varesio, L. 1983. Suppression of the in vitro maintenance and interferon mediated augmentation of natural killer cell activity by adherent peritoneal cells from normal mice. *J. Immunol.* 130:1974
110. Brunda, M. J., Wiltrout, R. H., Holden, H. T., Varesio, L. 1983. Selective inhibition by monosaccharides of tumor cell cytotoxicity mediated by mouse macrophages, macrophage-like cell lines, and natural killer cells. *Int. J. Cancer* 31:373
111. Djeu, J. Y., Heinbaugh, J. A., Holden, H. T., Herberman, R. B. 1979. Role of macrophages in the augmentation of mouse natural killer cell activity by poly I:C and interferon. *J. Immunol.* 122:182
112. Goldfarb, R. H., Herberman, R. B. 1982. Inhibition of natural killer cell cytotoxic reactivity by tumor promoters and cholera toxin. See Ref. 15, pp. 595–600
113. Gorelik, E., Herberman, R. B. 1981. Inhibition of the activity of mouse NK cells by urethane. *J. Natl. Cancer Inst.* 66:543
114. Gorelik, E., Herberman, R. B. 1981. Carcinogen-induced inhibition of NK activity in mice. Fed. Proc. 40:1093
115. Gorelik, E., Rosen, B., Herberman, R. B. 1982. Depression of NK reactivity in mice by leukemogenic doses of irradiation. See Ref. 15, pp. 1423–1430
116. Goto, T., Herberman, R. B., Maluish, A., Strong, D. M. 1983. Cyclic AMP as a mediator of prostaglandin E-induced suppression of human natural killer cell activity. *J. Immunol.* 130:1350
117. Hattori, T., Hirata, F., Hoffman, T., Hizuta, A., Herberman, R. B. 1983. Inhibition of human natural killer (NK) activity and antibody dependent cellular cytotoxicity (ADCC) by lipomodulin, a phospholipase inhibitory protein. *J. Immunol.* 131:662
118. Keller, R. 1979. Suppression of natural antitumor defence mechanisms by phorbol esters. Nature 282:729
119. Keller, R. 1980. Regulatory capacities of mononuclear phagocytes with particular reference to natural immunity against tumors. See Ref. 16, pp. 1219–69
120. Leung, K. H., Koren, H. S. 1982. Regulation of cytotoxic reactivity of NK cells by interferon and PGE_2. See Ref. 15, pp. 615–20
121. Parkinson, D. R., Brightman, R. P., Waksal, S. D. 1981. Altered natural killer cell biology in C57BL/6 mice after leukemogenic split-dose irradiation. *J. Immunol.* 126:1460
122. Santoni, A., Riccardi, C., Barlozzari, T., Herberman, R. B. 1980. Inhibition as well as augmentation of mouse NK activity by pyran copolymer and adriamycin. See Ref. 13, pp. 753–63
123. Schmidt, A., Ortaldo, J. R., Herberman, R. B. 1984. Inhibition of human natural killer cell reactivity by exogenous adenosine 5′-triphosphate. *J. Immunol.* 132(1):146–51
124. Sulica, A., Gherman, M., Galatiuc, C., Manchiulea, M., Herberman, R. B. 1982. Inhibition of human natural killer cell activity by cytophilic immunoglobulin G^1. *J. Immunol.* 128:1031
125. Timonen, T., Stenius-Aarniala, B. 1984. Natural killer activity in asthma. Clin. Exp. Immunol. In press
126. Benczur, M., Petrányi, G. G., Palffy, G., Varga, M., Talas, M., Kotsy, B., Földes, I., Hollán, S. R. 1980. Impaired NK function in multiple sclerosis and association with the HLA system. *Clin. Exp. Immunol.* 39:657
127. Djeu, J. Y., Timonen, T., Herberman, R. B. 1982. Production on interferon by human natural killer cells in response to mitogens, viruses and bacteria. See Ref. 15, pp. 669–74
128. Scala, G., Djeu, J., Allavena, P., Herberman, R. B., Ortaldo, J. R. 1983. Secretory and noncytotoxic functions of human large granular lymphocytes. *CRC Rev.* In press
129. Herberman, R. B., Allavena, P., Scala, G., Djeu, J., Kasahara, T., Domzig, W., Procopio, A., Blanca, I., Ortaldo, J., Oppenheim, J. J. 1983. Cytokine production by human large granular lymphocytes (LGL). See Ref. 88. In press
130. Kasahara, T., Djeu, J. Y., Dougherty, S. F., Oppenheim, J. J. 1983. Capacity of human large granular lymphocytes

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Annual Reviews

(LGL) to produce multiple lymphokines: interleukin 2, interferon and colony stimulating factor. *J. Immunol.* 131:2379–85
131. Domzig, W., Timonen, T. T., Stadler, B. M. 1981. Human natural killer (NK) cells produce interleukin-2 (IL-2). *Proc. Am. Assoc. Cancer Res.* 22:309
132. Wright, S., Bonavida, B. 1982. Studies on the mechanism of natural killer cell mediated cytotoxicity. I. Release of cytotoxic factors specific for NK-sensitive target cells (NKCF) during coculture of NK effectors with NK target cells. *J. Immunol.* 129:433
133. Farrum, E., Targan, S. R. 1983. Identification of human natural killer soluble cytotoxic factors (NKCF) derived from NK-enriched lymphocyte populations: specificity of generation and killing. *J. Immunol.* 130:1252
134. Blanca, I., Ortaldo, J. R., Herberman, R. B. 1983. Studies of soluble natural killer cytotoxic factor(s) released by human peripheral blood lymphocytes. See Ref. 88. In press
135. Ferrarini, M., Cadoni, A., Franzi, T., Ghigliotti, C., Leprini, A., Zicca, A., Grossi, C. E. 1980. Ultrastructural and cytochemical markers of human lymphocytes. See Ref. 71, pp. 39–47
136. Cortes, M., Helström, U., Perlmann, P. 1983. Surface markers of human natural killer cells as analyzed in a modified single cell cytotoxicity assay on poly-L-lysine coated cover slips. *J. Immunol. Meth.* 62:87
137. Breard, J., Reinherz, E. L., O'Brien, C., Schlossman, S. F. 1981. Delineation of an effector population responsible for natural killing and antibody-dependent cellular cytotoxicity in man. *Clin. Immunol. Immunopathol.* 18:145
138. Kay, H. D., Horwitz, D. A. 1980. Evidence by reactivity with hybridoma antibodies for a possible myeloid origin of peripheral blood cells active in natural cytotoxicity and antibody-dependent cell-mediated cytotoxicity. *J. Clin. Invest.* 66:847
139. Ortaldo, J. R., Sharrow, S. O., Timonen, T., Herberman, R. B. 1981. Analysis of surface antigens on highly purified human NK cells by flow cytometry with monoclonal antibodies. *J. Immunol.* 127:2401
140. Cantor, H., Kasai, M., Shen, H. W., LeClerc, J. C., Glimcher, L. 1979. Immunogenetic analysis of natural killer activity in the mouse. *Immunol. Rev.* 44:1
141. Hanna, N. 1980. Expression of metastatic potential of tumor cells in young nude mice is correlated with low levels of natural killer cell-mediated cytotoxicity. *Int. J. Cancer* 26:675
142. Hanna, N., Fidler, I. J. 1981. Expression of metastatic potential of allogeneic and xenogeneic neoplasms in young nude mice. *Cancer Res.* 41:438
143. Law, L. W. 1965. Neoplasms in thymectomized mice following room infection with polyoma virus. *Nature* 205:672
144. Law, L. W. 1966. Studies of thymic functions with emphasis on the role of the thymus in oncogenesis. *Cancer Res.* 26:551
145. Maguire, H., Jr., Outzen, H. C., Custer, R. P., Prehn, R. T. 1976. Invasion and metastasis of a xenogeneic tumor in nude mice. *J. Natl. Cancer Inst.* 57:439
146. Schmidt, M., Good, R. A. 1976. Cancer xenografts in nude mice. *Lancet* 1:39
147. Shin, H. S., Hayden, M. L., Langley, S., Kaliss, N., Smith, M. R. 1975. Antibody-mediated suppression of grafted lymphoma. III. Evaluation of the role of thymic function, non-thymus-derived lymphocytes, macrophages, platelets and polymorphonuclear leukocytes in syngeneic and allogeneic hosts. *J. Immunol.* 114:1255
148. Yunis, E. J., Martinez, C., Smith, J., Stutman, O., Good, R. A. 1969. Spontaneous mammary adenocarcinoma in mice: influence of thymectomy and reconstitution with thymus grafts or spleen cells. *Cancer Res.* 29:174
149. Minato, N., Reid, L., Cantor, H., Lengyel, P., Bloom, B. R. 1980. Mode of regulation of natural killer cell activity by interferon. *J. Exp. Med.* 152:124
150. Perussia, D., Acuto, D., Terhorst, C., Faust, J., Lazarus, R., Fanning, V., Trinchieri, G. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor function. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane. *J. Immunol.* 130:2142
151. Lanier, L. L., Le, A. M., Phillips, J. H., Warner, W. L., Babcock, G. F. 1983. Subpopulations of human natural killer cells defined by expression of the Leu-7 and Leu-11 antigens. *J. Immunol.* 131:1789
152. Fleit, H. B., Wright, S. D., Unkeless, J. C. 1982. Human neutrophil Fc gamma receptor distribution and structure. *Proc. Natl. Acad. Sci. USA* 79:3275
153. Reinherz, E. L., Kung, P. C., Goldstein, G., Levey, R. H., Schlossman, S. F. 1980. Discrete stages of human intrathymic differentiation: analysis of normal thymocytes and leukemic lymphoblasts of T-cell lineage. *Proc. Natl. Acad. Sci. USA* 77:1588
154. Reinherz, E. L., Moretta, L., Roper, M., Breard, J. M., Mingari, M. C., Cooper, M. D., Schlossman, S. F. 1980. Human

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T lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. A comparison. *J. Exp. Med.* 151:969

155. Zarling, J. M., Kung, P. C. 1980. Monoclonal antibodies which distinguish between human NK cells and cytotoxic T lymphocytes. *Nature* 288:394
156. Ward, J. M., Argilan, F., Reynolds, C. W. 1983. Immunoperoxidase localization of large granular lymphocytes in normal tissues of athymic nude rats. *J. Immunol.* In press
157. Ault, K. A., Springer, T. A. 1981. Cross-reaction of a rat-anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. *J. Immunol.* 126:359
158. Glimcher, L., Shen, F. W., Cantor, H. 1977. Identification of a cell surface antigen selectively expressed on the natural killer cell. *J. Exp. Med.* 145:1
159. Kasai, M., Iwamori, M., Nagai, Y., Okumura, K., Tada, T. 1980. A glycolipid on the surface of mouse natural killer cells. *Eur. J. Immunol.* 10:175
160. Koo, G. C., Hatzfeld, A. 1980. Antigenic phenotype of mouse natural killer cells. See Ref. 13, pp. 105–16.
161. Lohmann-Matthes, M-L., Domzig, W. 1980. Natural cytotoxicity of macrophage precursor cells and of mature macrophages. See Ref. 13, pp. 117–29
162. Tai, A., Warner, N. L. 1980. Biophysical and serological characterization of murine NK cells. See Ref. 13, pp. 241–55.
163. Chang, Z.-L., Hoffman, T., Bonvini, E., Stevenson, H. C., Herberman, R. B. 1984. Spontaneous cytotoxicity of human and mouse tumor cell lines by peripheral blood mononuclear cells: Contributions of adherent and nonadherent NK-like cells. *Scand. J. Immunol.* In press
164. Herberman, R. B., Mason, L., Ortaldo, J. R. 1983. Studies on the possible relationship of NC cells to mouse NK cells. See Ref. 88. In press
165. Abo, T., Balch, C. M. 1981. A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* 127:1024
166. Ortaldo, J. R., Timonen, T. T. 1981. Modification of antigen expression and surface receptors on human NK cells grown *in vitro*. In *Proceedings of the 14th International Leukocyte Culture Conference*. NY: Elsevier-North Holland
167. Schroff, R. W., Bucana, C. D., Klein, R. A., Farrell, M. M., Morgan, A. C. Jr. 1984. Detection of intracytoplasmic antigens by flow cytometry. *J. Immunol. Meth.* In press
168. Morgan, A. C. Jr., Schroff, R., Ortaldo, J., Herberman, R. B. 1984. Occult leukocyte antigens in human NK and other lymphoid populations. *Fed. Proc.* In press
169. Minato, N., Bloom, B. R. 1982. Heterogeneity and regulation of the natural killer cell system. See Ref. 15, pp. 131–38
170. Timonen, T., Ortaldo, J. R., Stadler, B. M., Bonnard, G. D., Sharrow, S. O., Herberman, R. B. 1982. Cultures of purified human natural killer cells: Growth in the presence of interleukin 2. *Cell. Immunol.* 72:178
171. Timonen, T., Ortaldo, J. R., Vose, B. M., Henkart, M., Alvarez, J., Herberman, R. B. 1983. Cultures of human natural killer cells (large granular lymphocytes) and T cells in the presence of interleukin-2-containing conditioned medium. *J. Reticuloendothel. Soc.* 33:67
172. Ortaldo, J. R., Bonnard, G. D., Kind, P. D., Herberman, R. B. 1979. Cytotoxicity by cultured human lymphocytes: Characteristics of effectors and specificity of cytotoxicity. *J. Immunol.* 122:1489
173. Seeley, J. K., Golub, S. H. 1978. Studies on cytotoxicity generated in human mixed lymphocyte cultures. *J. Immunol.* 120:1415
174. Bolhius, R. L., Ronteltap, C. P., de Rooy-Braam, M. A., Van Krimpen, B. A., Schellekens, H. 1983. Phytohaemagglutinin-induced susceptibility to lysis by natural and activated killer cells of autologous allogeneic and xenogeneic target cells. *Mol. Immunol.* 19:1347
175. Grimm, E. A., Mazumder, A., Zhang, H. Z., Rosenberg, S. A. 1982. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2–activated autologous human peripheral blood lymphocytes. *J. Exp. Med.* 155:1823
176. Ortaldo, J., Mason, A., Allavena, P. 1984. Studies of progenitors of human natural killer (NK) cells in blood and bone marrow. In *Thymic Factor Therapy*, ed. N. A. Byrom, J. R. Hobbs. London: Academic. In press
177. Vose, B. M., Bonnard, G. D. 1983. Limiting dilution analysis of the frequency of human T cells and large granular lymphocytes proliferating in response to interleukin-2.I. The effect of lectin on the proliferative frequency and cytotoxic activity of cultured lymphoid cells. *J. Immunol.* 130:687
178. Vose, B. M., Riccardi, C., Bonnard, G. D., Herberman, R. B. 1983. Limiting dilution analysis of the frequency of human T cells and large granular lymphocytes proliferating in response to interleukin-2. II. Regulatory role of interferon in proliferative and cytotoxic precursors. *J. Immunol.* 130:768
179. Grossman, Z., Herberman, R. B. 1982.

Annual Reviews

Annu. Rev. Immunol. 1984.2:359-394. Downloaded from arjournals.annualreviews.org
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Hypothesis on the development of natural killer cells and their relationship to T cells. See Ref. 15, pp. 229–38
180. Allavena, P., Ortaldo, J. R. 1983. Specificity and phenotype of IL-2 expanded clones of human large granular lymphocytes. *Diagnost. Immunol.* 1:162
181. Dennert, G. 1980. Cloned lines of natural killer cells. *Nature* 287:47
182. Kedar, E., Ikejiri, B. L., Sredni, B., Bonavida, B., Herberman, R. B. 1982. Propagation of mouse cytotoxic clones with characteristics of natural killer (NK) cells. *Cell. Immunol.* 69:305
183. Allavena, P., Ortaldo, J. R. 1984. Characteristics of human NK clones: Target specificity and phenotype. *J. Immunol.* In press
184. Nabel, G., Bucalo, L. R., Allard, J., Wigzell, H., Cantor, H. 1981. Multiple activities of a cloned cell line mediating natural killer cell function. *J. Exp. Med.* 153:1583
185. Moretta, L., Mingari, M. C., Sekaly, P. R., Moretta, A., Chapuis, B., Cerottini, J. C. 1981. Surface markers of cloned human T cells with various cytolytic activities. *J. Exp. Med.* 154:569
186. Brooks, C. G., Kuribayashi, K., Sale, G. E., Henney, C. 1982. Characterization of five cloned cell lines showing high cytolytic activity against YAC-1 cells. *J. Immunol.* 128:2326
187. Lattime, E. C., Pecoraro, G. A., Stutman, O. 1983. The activity of natural cytotoxic cells is augmented by interleukin 2 and interleukin 3. *J. Exp. Med.* 157:1070
188. Stutman, O., Paige, C. J., Figarella, E. F. 1978. Natural cytotoxic cells against solid tumors in mice. I. Strain and age distribution and target cell susceptibility. *J. Immunol.* 121:1819
189. Kaplan, J., Callewaert, D. M. 1980. Are natural killer cells germ line V-gene encoded prothymocytes specific for self and nonself histocompatibility antigens? See Ref. 13, pp. 893–907
190. Vanky, F. T., Stjernsward, J. 1979. Lymphocyte stimulation by autologous tumor biopsy cells. In *Immunodiagnosis of Cancer*, Part 2, ed. R. B. Herberman, K. R. McIntire, pp. 998–1032. NY: Marcel Dekker
191. Kabelitz, D., Kunkel, H. G. 1983. Phorbol ester-treated human lymphocytes are susceptible to natural killer cell-mediated cytolysis. *J. Immunol.* 130:2505
192. Hansson, M., Kiessling, R., Andersson, B. 1981. Human fetal thymus and bone marrow contain target cells for natural killer cells. *Eur. J. Immunol.* 11:8
193. Roder, J. C. 1979. Target effector interaction in the natural killer cell system: Isolation of target structures. *Proc. Natl. Acad. Sci. USA* 3:1405
194. Ortaldo, J. R., Lewis, J. T., Braatz, J., Mason, A., Henkart, P. 1983. Isolation of target antigens from NK-susceptible targets. In *Proceedings of the 15th Leukocyte Culture Conference*, pp. 551–54. Sussex: John Wiley
195. Uchida, A., Micksche, M. 1983. Lysis of fresh human tumor cells by autologous large granular lymphocytes from peripheral blood and pleural effusions. *Int. J. Cancer* 32:37
196. Lopez, C., Ryshke, R., Bennett, M. 1980. Marrow-dependent cells depleted by 898r mediate genetic resistance to herpes simplex virus type 1 infection in mice. *Infect. Immun.* 28:1028
197. Shope, T. C., Kaplan, J. 1979. Inhibition of the *in vitro* outgrowth of Epstein-Barr virus-infected lymphocytes by T_G lymphocytes. *J. Immunol.* 123:2150
198. Trinchieri, G., Santoli, D. 1978. Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. *J. Exp. Med.* 147:1314
199. Fitzgerald, P. A., Kirkpatrick, D., Lopes, C. 1982. Studies on cell surface markers on NK cells. Evidence for heterogeneity of human NK effector cells. See Ref. 15, pp. 23–78
200. Klein, G., Kiessling, R., Karie, K. 1978. Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. *Immunogenetics* 6:561
201. Carlson, G. A., Terres, G. 1976. Antibody-induced killing in vivo of L1210/MTX-R cells quantitated in passively immunized mice with ^{131}I-iododeoxyuridine-labeled cells and whole-body measurement of retained radioactivity. *J. Immunol.* 117:822
202. Bordignon, C., Daley, J. P., Nakamura, I. 1983. Natural killer cell-like effectors regulate hemopoietic colony formation *in vitro:* A model for hybrid resistance to bone marrow graft? *J. Reticuloendothel. Soc.* 34:75
203. Kim, Y. B., Huh, N. D. 1980. Natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) in specific pathogen-free (SPF) miniature swine and germ-free piglets. I. Comparison of NK and ADCC. *J. Immunol.* 125:755
204. Deleted in proof.
205. Phillips, W. H., Ortaldo, J. R., Herberman, R. B. 1980. Selective depletion of

Annual Reviews

human natural killer cells on monolayers of target cells. *J. Immunol.* 125:2322
206. Koren, H. S., Jensen, P. J. 1982. Natural killing and antibody-dependent cellular cytotoxicity: Independent mechanisms mediated by overlapping cell populations. See Ref. 13, pp. 347–53
207. Brooks, C. G., Urdal, D. L., Henney, C. S. 1983. Lymphokine-driven "differentiation" of cytotoxic T-cell clones into cells with NK-like specificity: correlations with display of membrane macromolecules. *Immunol. Rev.* 72:43
208. Brooks, C. G. 1983. Reversible induction of natural killer cell activity in cloned murine cytotoxic T lymphocytes. *Nature* 305:155
209. Binz, H., Fenner, M., Frei, D., Wigzell, H. 1983. Two independent receptors allow selective target lysis by T cell clones. *J. Exp. Med.* 157:1252
210. Pawelec, G. P., Hadam, M. R., Schneider, E. M., Wernet, P. 1982. Clonally restricted natural killer-like cytotoxicity displayed by cloned human T cell lines. *J. Immunol.* 128:2271
211. Piontek, G. E., Kiessling, R., Grönberg, A., Åhrland-Richter, L. 1982. Non-NK leukocytes demonstrate NK-patterned binding. See Ref. 15, pp. 705–713
212. Hengartner, H., Acha-Orbea, H., Lang, R., Stitz, L., Rosenthal, L. L., Groscurth, P., Keller, R. 1982. Permanently growing murine cell clones with NK-like activities. See Ref. 15, pp. 893–902
213. Neefe, J. R., Carpenter, R. 1982. Natural killer activity in cloned IL-2-dependent allospecific lymphoid populations. See Ref. 15, pp. 903–909
214. Ullberg, M., Jondal, M. 1982. Human NK cell activation with interferon and with target cell-specific IgG. See Ref. 15, pp. 361–69
215. Welsh, R. M. 1984. NK cells and interferon. *CRC Rev.* In press
216. Santoni, A., Riccardi, C., Barlozzari, T., Herberman, R. B. 1982. *C. parvum*-induced suppressor cells for mouse NK activity. See Ref. 15, pp. 519–26
217. Ortaldo, J. R., Timonen, T. T., Herberman, R. B. 1984. Inhibition of activity of human NK and K cells by simple sugars: Discrimination between binding and post-binding events. *Clin. Immunol. Immunopathol.* In press
218. Goldfarb, R. H., Herberman, R. B. 1982. Characteristics of natural killer cells and possible mechanisms for their cytotoxic activity. *Adv. Inflamm. Res.* 4:45–72
219. Scala, G., Allavena, P., Djeu, J. Y., Kasahara, T., Ortaldo, J. R., Herberman, R. B., Oppenheim, J. J. 1984. Human large granular lymphocytes (LGL) are potent producers of interleukin 1. *Nature.* In press
220. Scala, G., Djeu, J. Y., Allavena, P., Ortaldo, J. R., Herberman, R. B. 1984. Secretory and non cytotoxic functions of human large granular lymphocytes. In *Immunobiology of Natural Killer Cells*, ed. E. Lotzova, R. B. Herberman. Boca Raton: CRC Press. In press
221. Arai, S., Yamamoto, H., Itoh, K., Kumugai, K. 1983. Suppressive effect of human natural killer cells on pokeweed mitogen-induced proliferation of B cells. *J. Immunol.* 131:651
222. Procopio, A. D. C., Scala, G., Herberman, R. B., Oppenheim, J. J., Ortaldo, J. R. 1984. Production of B-cell growth factor (BCGF) by human large granular lymphocytes (LGL). *Fed. Proc.* In press
223. Allavena, P., Scala, G., Djeu, J., Procopio, A., Oppenheim, J. J., Herberman, R. B., Ortaldo, J. R. 1984. Cytokine production by clones of human large granular lymphocytes (LGL). *Fed. Proc.* In press
224. Meuer, S. C., Hodgdon, J. C., Hussey, R. E., Protentis, J. P., Schlossman, S. F., Reinherz, E. L. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988
225. Ortaldo, J. R. 1982. Natural killer cells: A separate lineage? See Ref. 15, pp. 265–73
226. Peter, H. H. 1983. The origin of human NK cells. An ontogenic model derived from studies in patients with immunodeficiencies. *Blut* 46(5):239
227. Fidler, I. J., Gerstein, D. M., Hart, I. R. 1978. The biology of cancer invasion and metastasis. *Adv. Cancer Res.* 28:149
228. Keller, R. 1978. Macrophage-mediated natural cytotoxicity against various target cells *in vitro*. I. Comparison of tissue macrophages from diverse anatomic sites and from different strains of rats and mice. *Br. J. Cancer* 37:732
229. Nagy, Z. A., Elliott, B. E., Carlow, D. A., Rubin, B. 1982. T cell idiotypes recognizing self-major histocompatibility complex molecules: H-2 specificity, allotype linkage, and expression on functional T cell populations. *J. Immunol.* 12:393
230. Meuer, S. C., Hodgdon, J. C., Hussey, R. E., Protentis, J. P., Schlossman, S. F., Reinherz, E. L. 1983. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J. Exp. Med.* 158:988
231. Binz, H., Frischknect, H., Wigzell, H. 1979. Some studies on idiotypes and anti-idiotypic reactions and receptors in anti-

Annu. Rev. Immunol. 1984.2:359-394. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

allo-MHC-T-cell immunity. *Ann. Immunol.* 130:273

232. Julius, M. H., Cosenza, H., Augustin, A. A. 1978. Evidence for the endogenous production of T cell receptors bearing idiotypic determinants. *Eur. J. Immunol.* 8:484
233. Rubin, B., Hertel-Wulff, B. 1978. Studies on the structure of T lymphocyte receptors using xenogeneic anti-idiotype antibodies. *Scand. J. Immunol.* 7:523
234. Braun, M., Saal, F. 1977. The T-cell receptor and cytotoxicity. An anti-idiotype antiserum that inhibits a graft-versus-host reaction does not inhibit cell-mediated cytotoxicity. *Cell. Immunol.* 30:254
235. Krawinkel, U., Cramer, M., Melchers, I., Imanishi-Kari, T., Rajewsky, I. 1978. Isolated hapten-binding receptors of sensitized lymphocytes. III. Evidence for idiotypic restriction of T-cell receptors. *J. Exp. Med.* 147:1341
236. Vogel, S. N., English, K. E., Fertsch, D., Fultz, M. J. 1983. Differential modulation of macrophage membrane markers by interferon: analysis of Fc and C3b receptors, Mac-1 and Ia antigen expression. *J. Interferon Res.* 3:153
237. Steeg, P. S., Moore, R. N., Johnson, H. M., Oppenheim, J. J. 1983. Regulation of murine macrophage Ia antigen expression by a lymphokine with immune interferon activity. *J. Exp. Med.* 156:1780
238. Steeg, P. S., Moore, R. N., Oppenheim, J. J. 1980. Regulation of murine macrophage Ia-antigen expression by products of activated spleen cells. *J. Exp. Med.* 152:1734
239. King, D. P., Jones, P. P. 1983. Induction of Ia and H-2 antigens on a macrophage cell line by immune interferon. *J. Immunol.* 131:315
240. Blumenthal, E. J., Roberts, W. K., Vasil, A., Talmadge, D. W. 1983. Macrophage activation: dissociation of cytotoxic activity from Ia-A antigen expression. *Proc. Natl. Acad. Sci. USA* 80:2031
241. Wong, G. H., Clark-Lewis, I., McKimm-Breschkin, L., Harris, A. W., Schrader, J. W. 1983. Interferon-gamma induces enhanced expression of Ia and H-2 antigens on B lymphoid, macrophage, and myeloid cell lines. *J. Immunol.* 131:788
242. Johnson, W. J., Marino, P. A., Schreiber, R. D., Adams, D. O. 1983. Sequential activation of murine mononuclear phagocytes for tumor cytolysis: differential expression of markers by macrophages in the several stages of development. *J. Immunol.* 131:1038
243. Adams, D. O., Johnson, W. J., Marino, P. A., Dean, J. H. 1983. Effect of pyran copolymer on activation of murine macrophages: evidence for incomplete activation by use of functional markers. *Cancer Res.* 43:3633
244. Adams, D. O., Johnson, W. J. 1982. Activation of murine mononuclear phagocyte Hor destroying tumor cells: analysis of effector mechanisms and development. *Adv. Exp. Med. Biol.* 155:707
245. Marino, P. A., Adams, D. O. 1982. The capacity of activated murine macrophages for augmented binding of neoplastic cells: analysis of induction by lymphokine containing MAF and kinetics of the reaction. *J. Immunol.* 128:2816
246. Leung, K. H., Fischer, D. G., Koren, H. S. 1983. Erythromyeloid tumor cells (K562) induce PGE synthesis in human peripheral blood monocytes. *J. Immunol.* 131:445

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE

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INTRODUCTION

In this review I deal with antigen presentation and focus primarily on the antigen-presenting function of the macrophage. The two main topics considered here are: (*a*) handling of antigen by the macrophage and the general concept of "antigen processing"; and (*b*) the modulation of antigen-presenting cell (APC) function. In the latter section, I discuss APC other than the macrophage. Because previous reviews (1,2) have considered in detail the interactions between the macrophage and the T cell, I emphasize more recent studies here. The overwhelming evidence at present supports the following statements: (*a*) helper T cells are not activated by direct interactions of their receptors with soluble antigen; (*b*) the clones of helper T cells are activated by their interaction with antigen presented by an APC that must bear the Class II glycoproteins of the Major Histocompatibility Gene Complex (MHC) of the species—the Ia antigens; and (*c*) under physiological conditions of immunization, the clones of T cells and APC share the same allelic form of their Class II molecules.

ANTIGEN PROCESSING

Three requirements are essential for a cell to function optimally in antigen presentation: the capacity to (*a*) express the Class II glycoproteins on its surface; (*b*) process antigen, as described in this section; and (*c*) synthesize and release interleukin 1 (IL-1). The first issue to be considered is the nature of

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Annual Reviews

the immunogen presented by the macrophage to the T cell. How does the macrophage interact with the antigen; what is the fate of the antigen molecule; in what form is the antigenic determinant presented; and where in the macrophage is the antigen presented, on the cell surface, as released or exocytosed molecules? Attempts have been made to answer these questions by tracing antigen in the macrophage and following the fate of the molecule while at the same time studying the response of the T cell. The results obtained using this approach must be integrated with two other major approaches to this issue—i.e. (*a*) studying the reactivity of T cells (or B cells) to the immunogen in its native, denatured, or fragmented form; and (*b*) studying the effect of chemical modification of the protein on the T-cell reactivity to haptenic determinants. I first briefly summarize the two latter approaches before directly reviewing macrophage handling of protein antigens.

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Determinants in Natural Proteins Presented to B or T Cells

Two major conclusions can be derived from a large number of studies employing diverse protein antigens (or synthetic polypeptides). First, most of the antibody made by conventional immunization with globular proteins is directed to antigenic determinants found in the native molecule and not represented in the denatured protein or in fragments of it. Second, a major portion of the T-cell reactivities is directed to determinants found on unfolded molecules or on fragments of it. Thus, there appears to be a major difference in how B or T cells recognize antigen. The B-cell clones were selected by protein antigens that appeared not to have suffered extensive conformational changes while, in contrast, the T-cell clones were selected by antigen molecules that had been chemically modified.

Gell & Benacerraf (3) first showed differences in reactivity to antigen between B and T cells in 1959, when there was no information about two classes of lymphocytes. Gell & Benacerraf examined guinea pigs immunized with proteins and challenged with the same protein in either its native or denatured state. Delayed hypersensitivity reactions in the skin were elicited by native as well as denatured antigen; in contrast, anaphylaxis (an indication of gamma-1 antibodies) was triggered only by the native molecule. Later, more sophisticated methodologies for analyzing antibodies or T-cell reactivities confirmed these findings. Immunization with globular proteins always resulted in a major population of serum antibodies that recognized only the native molecule and would not react with the unfolded, denatured molecule (4–18). A minor population, however, was found that would bind to the denatured molecule, to what Sela (8) termed "sequential determinants." Most investigators interpreted these findings to mean that B cells were interacting with protein antigens prior to major chemical changes by the host's catabolic processes; the minor populations of antibodies were attributed to denatured contaminants in the

Annual Reviews

preparation used for immunization. Some have speculated that the Freund's adjuvant in which the native protein is frequently incorporated for immunization is responsible for the appearance of these antibodies (14). Freund's induces a strong granulomatous reaction madè up of Ia-positive macrophages (unpublished experiments of K. Behbehani, D. I. Beller, and E. R. Unanue). Furthermore, macrophages have been shown to release partially fragmented protein molecules. Thus, the macrophage itself could be producing the denatured molecules later recognized by the B cells. The results described above have been obtained with immunoglobulins (5), lysozyme (7, 10, 13, 14, 16, 18), ribonuclease (4), albumins (11, 12, 17), ovalbumin (17), myoglobin (6), antigen E of ragweed (15), and synthetic polypeptides (9).

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Many of the aforementioned studies also examined T-cell reactivity and found the opposite results, as noted in the following selected examples. Ishizaka et al examined the response of mice to antigen E of ragweed, a thymus-dependent antigen (15). Mice primed with urea-denatured antigen E developed a secondary antibody response (accelerated primary?) when later challenged with the *native* molecule. The antibody directed to native antigen E did not bind to the urea-denatured antigen. Furthermore, the T cells provided helper activity to B cells challenged with DNP-antigen E. Schirrmacher & Wigzell reported that methylation of bovine serum albumin (BSA) resulted in a change in the molecule such that it no longer would bind to anti-BSA (11). However, the T cells from mice immunized with BSA could provide help upon challenge with the methylated-BSA. Several groups studied hen egg white lysozyme (HEL) and confirmed that the T-cell reactivity, in the form of lymphokine production, delayed sensitivity, or proliferation, was directed to the unfolded molecule or to a large fragment of it (13, 18). The study of Senyck et al (19), using peptide fragments of glucagon, indicated that T cells and B cells recognized distinctly different regions of the molecule. Finally, an important paper to note is that of Chesnut et al who studied the immune response to ovalbumin (OVA) and BSA (17). In agreement with the many previous reports, they found that most of the antibodies were directed only to conformational determinants, while T-cell proliferation was triggered by either thè native or denatured proteins. Their major contribution was to show directly that the set of T cells triggered by native OVA was the same responding to denatured OVA. Elimination using BuDR (and light) of T cells that proliferated to native OVA also resulted in the loss of T cells reacting with denatured OVA.

In the past several years, attention has been focused on T-cell recognition of natural proteins under Ir gene control. Here I don't analyze in depth the issue of whether defects in a genetic nonresponder individual are directly attributed to determinant selection by the macrophage (20) or to a lack of development, or loss, of a T-cell clone (21, 22). Instead, I review studies examining the nature of the antigenic determinant recognized by the T cell.

Annual Reviews

In attempts to indicate what portion of the protein molecule may be presented by the macrophage two general approaches have been used. One is to compare the T-cell reactivity from animals immunized to a natural protein with the same protein from other species where there are known differences at selected amino acids. This comparison gives information on what may be the critical region recognized by the T cell but obviously will not reveal how the protein is handled by the presenting cell. A second approach uses fragments or synthetic peptides representing portions of the molecule. The overall results (18, 20, 23–37) indicate that: (*a*) T-cell reactivity is usually directed to a small segment of the protein molecule, to an area that shows amino acid differences from similar host proteins; (*b*) fragments of the natural protein containing the critical amino acids can, by themselves, stimulate T cells in the presence of APC of the correct syngeneic haplotype; and (*c*) small differences in amino acids, sometimes just a single amino acid change, may result in the loss or acquisition of T-cell reactivity. The chemical basis of these subtle changes has not been elucidated. Comments on critical studies follow.

A. Rosenthal and associates established that the response to insulin was under Ir gene control and that the two major strains of inbred guinea pig differed in the segment of the molecule presented by its macrophages (20, 24, 33). This observation was the basis for formulation of the determinant selection hypothesis—i.e. that the macrophage selected the determinant to be presented to the T cell in the context of Class II molecules of a given haplotype. Based on responses to whole insulin molecules from various species, these investigators concluded that strain 2 guinea pigs responded to the A fragment, specifically to three critical amino acids, A8-10 (Thr-Ser-Ile). The amino acids are in an area of the molecules contained within a loop (alpha loop) formed by an intrachain disulfide bridge between Cys6 and Cys11. Destruction of this loop resulted in a loss of the T-cell response, strongly indicating a role for conformational determinants. Strain 13 guinea pigs, on the other hand, responded to isolated B chains; most of the T-cell reactivity was directed to a portion of the molecule contained in a synthetically produced fragment containing amino acids from positions 5 to 19 (His-Leu-Cys-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys). This fragment contained one amino acid at portion 10 (His) that was different from self-insulins and that was thought to be critical for immunogenicity. Smaller peptides of the 5–19 fragment were not presented by the macrophage (a 5–9 fragment or a 9–16 fragment containing the critical His). This implies a requirement for a minimal size peptide for presentation. It is noteworthy that fragment 5–9, which is identical between the immunogen (pork insulin) and self-insulin, is made mostly of nonpolar amino acids.

Studies using cytochrome c as antigen have shown similar results. A cyanogen bromide fragment of the molecule (81–103) could be presented by APC

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(26, 31, 35). However, synthetically produced peptides encompassing only a portion of this peptide, including the critical amino acids, were not presented. Schwartz and his associates have argued persuasively for an interaction between Ia and the immunogenic fragment, favoring a determinant selection hypothesis (36). (Basic was the demonstration that T cells from a mouse strain unresponsive to a foreign cytochrome nevertheless reacted with it in the context of Ia molecules of APC bearing the correct—i.e. "responder"—haplotype.) From their studies, Schwartz and co-workers concluded that the critical antigenic fragment presented by the APC contained two critical sites—one that interacted with the T-cell receptor for antigen, and a second that interacted directly with the Ia molecule. The latter was critical in defining whether a given Ia-antigen complex was to be immunogenic.

Studies using HEL have indicated that a substantial amount of the T-cell proliferative activity is directed to a small peptide fragment (74–96 in studies made in H-2^b mice) (37). Interestingly, Sercarz, Miller, and associates have strong experimental evidence that the N terminus of the molecule is critical in stimulating T suppressor cells in certain strains of mice (25). Cyanogen bromide fragments of HEL lacking the first twelve amino acids were able to immunize; in contrast, the entire molecule resulted in no immunity as a result of active T suppression. A difference in the presentation or handling of the macrophage by macrophages or APC of responder or nonresponder haplotype was proposed as the critical event.

The Use of Synthetic Polypeptides made up of L- or D-Amino Acids

Synthetic polypeptides made of D-amino acids are poorly immunogenic, even highly tolerogenic. Such peptides are poorly degraded and remain in the tissues of the host for prolonged periods [reviewed in (38)]. These results, therefore, suggest strongly that polypeptides must be degraded, at least to some extent, before T cells recognize them as immunogenic. The role of a carrier made of amino acids varying in their optical rotation has been examined by inserting small chemicals to which part of the T-cell reactivity is directed. The haptens used have been the azobenzearsonate (ABA) hapten pioneered by the laboratory of Sidney Leskowitz, and, to a lesser extent, the dinitrophenyl hapten (39–42). T cells recognize the hapten as well as part of the peptide to which the carrier is attached. Leskowitz and associates found that delayed sensitivity to ABA was elicited by ABA conjugated to polymers of Glu or Lys or Glu, Lys, and Ala made of L-amino acids but not of D-amino acids (39, 40). Recent studies examined this phenomenon using cell-culture methods (41). T cells from Lewis rats immunized with ABA-Tyr were cultured and challenged with ABA conjugated to the polymer Ficoll, which contained a spacer made up of L- or D-amino acids (i.e. ABA-Tyr-Ala-Ala-Ficoll). The T cells were activated

Annual Reviews

only if the peptide was made of L-amino acids. The very interesting point is that the same ABA peptides without the Ficoll backbone could stimulate T-cell growth regardless of the optical rotatory characteristics of their amino acids. (In light of subsequent studies, to be discussed next, I interpret this to mean that the conjugates containing Ficoll are internalized by the APC and require intracellular processing, while those without Ficoll can interact directly with membrane structures involved in presentation without need of further processing.)

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Direct Analysis of Antigen Processing

In a previous review, I considered in detail up to 1981 the handling of protein antigens by macrophages in the context of antigen presentation (1). After summarizing the conclusions drawn at that time, I will now review recent information. The major results indicated that radiolabeled protein molecules were, as expected, internalized by the macrophage and subjected to catabolism. However, proteins or their fragments were also found, albeit in small amounts, to be (*a*) associated with the cell surface and (*b*) released into the culture supernatant. The surface-bound molecules decreased in number with time of culture; they could be identified by either their interaction with antibodies or following their release by trypsin. It appeared that these surface molecules were being internalized and recycled to the membrane without funneling into lysosomes. The secreted proteins were soluble fragments or whole molecules that derived from intracellular sites following their internalization. The cells slowly pumped out, in an active process, a small amount of their internalized pool of protein.

Attempts were made to relate these parameters of handling with presentation. Trypsin treatment of the macrophage abolished the response but only in certain systems. In my early experience, I found that macrophages pulsed with hemocyanins and then trypsinized lost their capacity to present antigen to mixtures of T and B cells (43). My assay was not MHC restricted, indicating in retrospect that the macrophage-bound immunogen, which was trypsin-sensitive, could be transferred eventually into other APC in the T-B cell mixtures. These APC were then responsible for the actual direct presentation to T helper cells.

In contrast, Rosenthal's laboratory found that trypsin treatment, although removing antigen at the cell surface, did not affect the capacity of the macrophage to induce T-cell proliferation (44, 45). At face value, trypsin treatment identifies two immunogenic molecules. One molecule is not associated with MHC-restricted presentation—these are easily accessible molecules that can be processed eventually and used for T-cell stimulation, either by the same macrophage or by other APC. (How the molecules were transferred from the macrophage to other APC was not established.) The second molecule is rep-

Annual Reviews

resented by a protease-resistant moiety that is directly involved in the MHC-restricted presentation (Table 1). Little or no information was obtained on the nature of this material. This molecule involved in the MHC-restricted presentation appeared not to react with antibodies: Antigen presentation was not blocked. One explanation is that antibodies were not directed to what may be a small fragment of the antigen. Thomas & Shevach argued that another factor could be the density of the fragment containing the determinant (46). They showed, using the hapten TNP bound to macrophage surface proteins, that antibodies blocked presentation only under critical conditions. Finally, the linkage between Ia and antigen was difficult to demonstrate. Two laboratories claimed to have found material released by macrophages containing Ia and antigen (47, 48). The nature of this association was not determined but may well involve fragments of membrane containing the various molecules. Evidence for release of antigen bound loosely to membranes released by cultured macrophages has now been obtained (49).

Significant advances have taken place recently with the direct demonstration of an antigen-processing step. This demonstration depended upon two techniques: one using drugs that alkalinize acid intracellular vesicles (the lysosomotropic drugs), the other using macrophages fixed lightly in formaldehyde.

Kirk Ziegler and I carried out a number of studies examining the presentation

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Table 1 Immunogenic molecules associated with macrophages[a]

	MHC-restricted fragment	MHC-unrestricted molecules	
		Surface bound	Soluble
Cellular localization	surface membrane	surface membrane	extracellular fluid
Assay	indirectly—by antigen presentation	directly, using radiolabeled proteins	directly, using radiolabled proteins
Effects of trypsin	no effect	sensitive	no effect
Effects of chloroquine	inhibits	no effect	no effect
Presentation by allogenic or Ia-negative macrophages	no presentation	positive (can be detected and re-presented)	positive
Major role	fragment functionally linked to Ia molecules for T-cell recognition	source of molecules available for B cells or other APC	source of molecules that can be taken up and processed

[a]Modified from (1). The explanation is in the text.

Annual Reviews

of the intracellular pathogen *Listeria monocytogenes* (50, 51). We used an assay that rapidly tested the interaction between the T cell and the macrophage. It was a modification of the studies of Lipsky & Rosenthal (52) in which T cells were shown to bind directly to antigen-pulsed macrophages. The assay was modified so as to establish quantitatively the degree of antigen-specific binding (53, 54). First, the T cells were spun onto the antigen-pulsed macrophages and incubated briefly; then the cultures were shaken, and nonadherent cells were removed; finally, the nonadherent cells were tested in a regular bioassay for growth or mediator secretion upon interaction, in a second culture, with fresh antigen-pulsed macrophages. If the antigen was effectively presented by the first macrophages, the T cells would remain adherent and be absent in the nonadherent cells. This assay proved to be quantitative and to reflect interactions between the T cell and the Ia-positive macrophages containing antigen. T cells would bind only to antigen associated with the macrophages and not to free antigen (53, 55). An excess of free antigen did not compete for the binding of T cells to macrophages.

The binding assay had the great advantage that macrophages could be pulsed briefly with antigen, and thus the interrelationship between handling and presentation could be critically examined. We first found that T cells would not bind to macrophages immediately following the binding of *Listeria*; a lag period was required, the duration of which depended on amounts of antigen and temperature. Furthermore, macrophages fixed in paraformaldehyde were still able to bind the T cells (Figure 1). The fixation, however, had to be made

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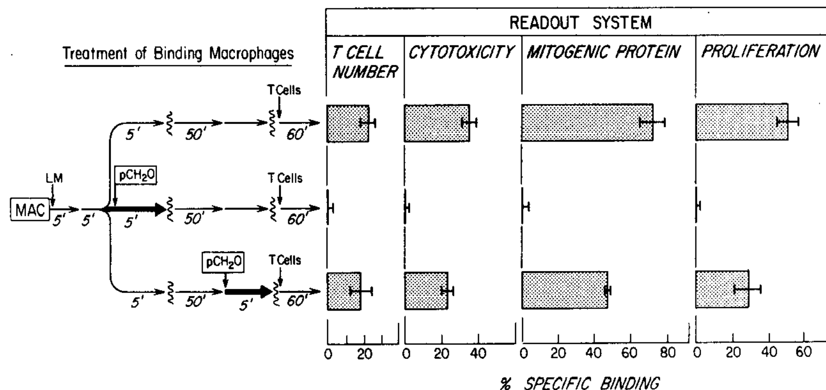

Figure 1 Macrophages fixed in paraformaldehyde can present the antigen (the bacterium *Listeria monocytogenes*) (line 1 versus line 3) (51). Fixation must be done after a period of handling (line 3 versus line 2). The specific binding of T cells to macrophages was calculated according to protocols described in the text (53). The nonadherent T cells are assayed by adding them to new cultures of macrophages and measuring activation of the macrophage for killing tumors (cytotoxicity), or the amounts of IL-1 by the macrophages (mitogenic protein), or T-cell proliferation.

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following a lag period of handling. The importance of this result is that it clearly establishes that the macrophage plasma membrane is the substrate for T-cell recognition of antigen. There is no need to postulate, therefore, a mechanism whereby T cells bind nonspecifically to macrophages as a first step to signal the macrophage to export a hidden intracellular immunogen to the membrane. Our result also suggests that soluble released materials are not operative in this form of presentation.

Because we had identified a short period required for handling the antigen before the immunogen became available, it was then possible to carry out pharmacological manipulations during that period. The drugs or chemicals that we selected were lysosomotropic agents like chloroquine or ammonium chloride. These weak bases cross the plasma membrane and accumulate in their protonated form on vesicles of acid pH. This results in an increase in the pH of the vesicles. Raising the pH of lysosomes, one of the targets of these compounds, results in inhibition of proteolytic activity. Cell biologists have shown directly that the lysosomotropic agents increase the pH of lysosomes and also inhibit lysosomal catabolism (56). Evidence also suggests that the lysosomotropic drugs may inhibit other cellular functions such as recycling of intracellular vesicles (57) or fusion of endosomes (58).

Treatment of macrophages immediately following internalization of *Listeria* resulted in the inhibition of antigen-presenting function (51). In contrast, macrophages treated with the drug following one hour of handling of *Listeria* minimally impaired presentation (Figure 2). These results indicated that the drug affected the macrophage and not the lymphocyte. Furthermore, there was a direct relationship between the degree of impairment of presentation and the inhibition of catabolism of ^{125}I-labeled organisms. We concluded that the *Listeria* had to be internalized (proof of which was obtained by direct microscopy) into an acid vesicle in order for the immunogen to be recycled to the surface to be presented.

We have extended these studies by examining directly the handling of radioactive *Listeria* (59). The macrophages were fed labeled bacteria for a brief period, after which the *Listeria* remaining on the membrane was removed by treatment with 10 mM EDTA. *Listeria* binds to a macrophage plasma membrane protein that requires Ca^{2+}; chelating the Ca^{2+} results in the release of any surface-bound bacteria. The removal of the *Listeria* allows one to study the fate of the intracellular material without any further endocytosis of new antigen. We found the expected catabolism, with time, of a large amount of the radiolabeled bacteria. However, we also found *Listeria* peptides that remained membrane associated for long periods as well as peptides that were released in soluble form into the culture medium (Figure 3). The physical properties of the membrane-bound and released peptides were distinct.

The membrane-bound peptides (which were identified either by directly

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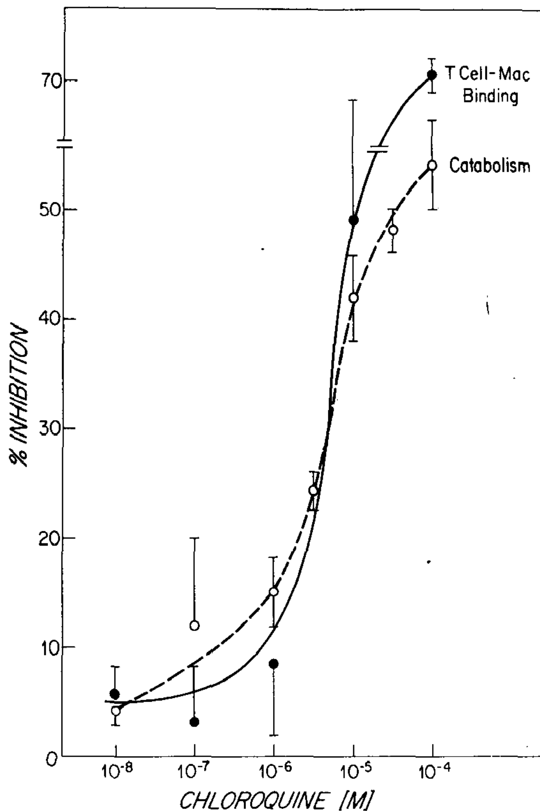

Figure 2 Using the assays depicted in Figure 1, it can be shown that treatment of macrophages with chloroquine inhibits antigen presentation (T cell–Mac binding). The catabolism of ^{125}I-labeled *Listeria* was also reduced by the drug (catabolism). Taken from (51).

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isolating plasma membrane or by their release by trypsinization) were soluble only after detergent treatment of the membranes. In contrast, the released materials were soluble in regular media and were not membrane-associated. The membrane peptides were of molecular weight 10,000 or smaller; the released peptides comprised molecular species of mol wt from 67,000 to 34,000. None of them was associated with Ia molecules: (*a*) Precipitation with anti-Ia antibodies did not co-precipitate labeled material, and (*b*) capping of membrane Ia in live macrophages did not affect the number of trypsin-bound molecules. We believe that the membrane-bound peptides are linked to membrane structures and may cycle back and forth between the surface and the cell interior. This material, if removed by trypsin, will not affect directly MHC presentation of *Listeria*; i.e. these are the molecules previously discussed, represented in the MHC-independent presentation (Table 1). Although trypsin treatment did not affect the presentation, the plasma membrane mate-

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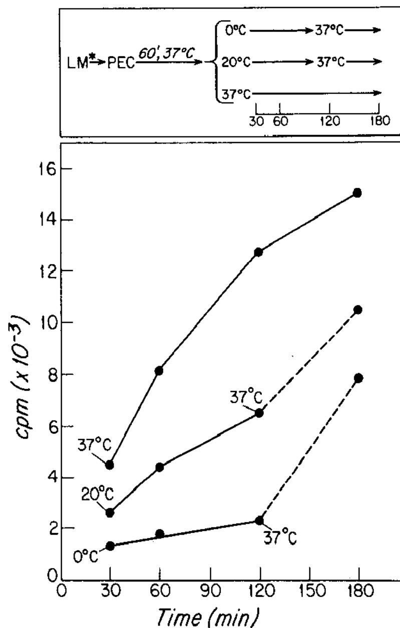

Figure 3 The release of peptides from macrophages. The macrophages were pulsed with radiolabeled *Listeria* for 1 hr, washed to remove all surface-bound *Listeria*, and then incubated at 37°C for 1 hr (upper box). At this time, the culture was split into three: Dishes were incubated at 0°C, 20°C, or 37°C for 120 min, and aliquots of culture fluid were collected. After 120 min, cell cultures were taken to 37°C for 1 hr. The graph depicts the release of peptides measured in the culture fluid. The release is temperature-dependent. The rate of release at 37°C is about 4% of cell-associated radioactivity per hour during the first 5 hr of culture. Taken from (59).

rial could be shown to be potentially immunogenic: Macrophages fed plasma membrane containing the antigen re-presented it to T cells in an MHC-restricted way. The membrane could be from allogenic macrophages; the macrophages re-presenting the membranes, however, had to be syngeneic.

The released peptides were released continuously, the rate decreasing progressively with time in a temperature-dependent and active process. The release was *not* affected by chloroquine which, moreover, *enhanced* the process slightly. The released molecules were weakly immunogenic.

We concluded from these studies that, following endocytosis, potentially immunogenic material can be identified: (*a*) at the membrane in a form that needs further handling in order to be presented in an MHC-restricted way; (*b*) by indirect evidence, in a trypsin-resistant form, also at the membrane; and (*c*) as soluble material released by the live cell (Table 1).

The studies with *Listeria* immediately raise the question of whether these phenomena also apply to soluble protein antigens. Grey, Chesnut, and their associates vigorously pursued this issue using OVA and keyhole limpet hemo-

cyanin (KLH) as antigen molecules and either peritoneal macrophages or B-cell tumors as antigen-presenting cells (60, 61). They used as assay the secretion of IL-2 by T-cell hybridomas when interacting with OVA- or KLH-containing APC. Their experiments, in essence, confirmed the results discussed above using *Listeria*: B-cell tumors or macrophages pulsed with antigen required a period of 45–60 min for handling the antigens. After this period, the cells could be fixed and were able to stimulate IL-2 production. Treatment with the drugs chloroquine or monensin during this period abrogated catabolism as well as antigen presentation. One experimental manipulation used a high dose of pronase. Treatment of the antigen-pulsed cells with pronase, followed by culture or fixation, had no effect; in contrast, pronase treatment followed immediately by fixation resulted in a loss of presentation. Chesnut et al argued that this showed the presence of antigen on the membrane (60). I believe that these results do not rule out a significant loss of Ia as a possible cause of the effect. In my own experience, pronase markedly affects the amounts of Ia on the membrane (59).

The inhibitory effects of chloroquine on antigen presentation have now been confirmed using other soluble antigens (62, 63). Likewise, metabolic studies on macrophages or B-cell tumors have also brought confirmatory evidence for the release of both intact and partially degraded molecules (64) as well as the presence of protein retained on the plasma membrane (65).

A further advance in our understanding of processing results from the recent study of Shimonkevitz et al (66). Extending the studies with OVA (60, 61), they found that macrophages fixed lightly in glutaraldehyde were not able to present the native or denatured OVA. However, the surprising finding was that such cells were able to present proteolytic fragments or CNBr-derived fragments of OVA. This implies that fragmentation of the antigen was essential. As expected, the pre-fixed cells were shown not to synthesize proteins nor to be capable of catabolizing the protein. The pattern of reactivity of the various hybridomas varied depending on the proteolytic fragment. In fact, some were able to react only with trypsin fragments but not to chymotryptic digests, for example, implying that the antigenic determinants could vary in different portions of the molecule.

Recent studies with the technologies described above have been informative in characterizing the actual region of the protein HEL presented by the macrophage. We examined the requirements for processing of HEL using two T-cell hybridomas (Table 2). Native HEL, carboxymethylated HEL (CM-HEL), and a tryptic digest were all immunogenic when presented by a live macrophage (67). Such live macrophages treated with chloroquine did not present native HEL. Likewise, native HEL was not presented by pre-fixed macrophages (i.e. macrophages fixed first in paraformaldehyde and then incubated with the antigen). The tryptic digest, in contrast, was presented by live mac-

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Table 2 Summary of the reactivity patterns of two lysozyme-reactive T-cell clones[a]

Presenting cells	Antigen	2A11	3A9
Live macrophages	HEL	+	+
	CM-HEL	+	+
	TD	+	+
Live macrophages +chloroquine	HEL	−	−
	CM-HEL	−	+
	TD	+	+
Prefixed macrophages	HEL	−	−
	CM-HEL	−	+
	TD	+	+

[a]These data were taken from (67). Two T-cell hybridomas were tested for the secretion of IL-2 after presentation of HEL by peritoneal macrophages (I-A^k restricted). HEL refers to the natural protein; CM-HEL refers to carboxymethylated HEL; and TD is a tryptic digest. Live macrophages: Macrophages were given the compounds for a time and then fixed. Live macrophages + chloroquine: The antigens were given in the presence of the drug, after which the macrophages were fixed. Prefixed macrophages: The macrophages were fixed and then pulsed with antigen. Native HEL required a processing event, while TD did not. One clone (2A11) recognized CM-HEL after processing, while the other (3A9) did not.

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rophages treated with chloroquine or pre-fixed. We concluded, therefore, that HEL had to be processed in an acid-sensitive, intracellular vesicle and there fragmented. Once fragmented, it was then recycled to the cell surface. A fragment of HEL would not require such a step through an intracellular compartment. The results with CM-HEL were of particular interest. One clone required processing of CM-HEL: we found a chloroquine-sensitive step with live macrophages and no presentation by fixed macrophages. A second clone, however, reacted to CM-HEL presented by live macrophages treated with chloroquine or pre-fixed. That is to say, the T cell was able to recognize the determinants in the unfolded molecule without any apparent need for fragmentation of the molecule.

We have now identified the tryptic peptide presented by the fixed macrophages (68). It is represented by a single peptide containing 16 amino acids from position 46 to 61 of the molecule. The peptide has the sequence Asn-Thr-Asp-Gly-Ser-Thr-Tyr-Gly-Ile-Leu-Gln-Ile-Asn-Ser-Arg. When this sequence was compared to two other lysozymes that were not recognized by our two clones, we found that human and mouse (self) were identical from positions 55 to 61, except for amino acid 56 (a Phe instead of Leu). At positions 47 to 79, however, there were different amino acids among the three proteins, suggesting that here lies the critical antigenic determinant recognized by the T cell. We have recently found that cleavage of the peptide with chymotrypsin resulted in two fragments (from position 46 to 52 and from 53 to 61), both of which were not presented by the pre-fixed macrophages. The two fragments differ considerably in their composition. Fragment 46–52 is made up of polar

amino acid and most likely contains the antigenic determinant. In contrast, fragment 53–61 is made of nonpolar amino acids; in fact, it is one of the most hydrophobic peptides of the digested lysozyme. We suggest that the fragment must contain an immunogenic determinant—recognized by the T cell—associated with a hydrophobic stretch made of nonpolar amino acids. This hydrophobic stretch anchors the peptides to a structure on the plasma membrane of the APC.

Summary

The three approaches discussed previously offer a persuasive argument in favor of an antigen-handling and antigen-processing step during antigen presentation to T cells. The scenario we favor is that protein antigens taken up by the macrophage (or other APC) undergo a series of changes, aside from their complete degradation to amino acids. Antigen presentation is the best example that protein molecules remain cell-associated for relatively long periods without extensive degradation. Table 1 indicates that the fate of protein antigen is complex and that, in fact, several states can be identified—from molecules that remain bound to the cell surface but are not presented directly to T cell, to molecules that are released in a soluble form, to molecules that have undergone an intracellular processing and are directly involved in the MHC-restricted presentation. The exact mechanisms involved in the biochemistry of the protein degradation and in the recycling of the products have yet to be determined. Presumably, proteins enter the cell either by fluid-phase pinocytosis or following their interaction with a plasma-membrane structure. Subsequently, the protein must enter an acid vesicle to enable the generation of the immunogenic fragment involved in the MHC-restricted presentation. The acid compartment has not yet been identified. There are likely several low-pH vesicles where proteins suffer various alterations. Claims have been made that the cell has an acid vesicle where ligand-receptor complexes that are internalized are dissociated and sorted. Direct ultrastructural proof of such vesicles has been obtained in liver cells (69). The same kind of argument can be made with regard to internalized proteins: Some molecules recycle to the membrane, while most funnel into the lysosome. Our own studies further indicate different but simultaneous modes of handling the proteins, which can be dissociated—i.e. chloroquine stopped the processing of the molecule destined to be presented to T cells but did not affect the exocytosis of protein (59).

It seems most globular proteins (even relatively small ones) or proteins associated with microbes must go through the processing stage. We interpret this to mean that the protein must be altered so that it can recycle and/or become anchored to the membrane. Analysis of the smallest peptides involved in presentation favors the argument that a basic structure is required, composed of a segment of nonpolar amino acids next to a stretch of polar amino acids

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that differ from those of self-proteins. Admittedly, studies supporting this claim are few, and the matter requires much further analysis.

The relationship between the immunogenic peptide and the Ia molecules is central to our understanding of this process. The immunogenic fragments complex either directly with Ia molecules, as favored by many (24, 36, 47, 48, 69, 70), or, instead, with an anchoring molecule that, by random collision, associates closely with Ia. Because of the small size of the immunogenic fragment, as surmised from the approach using fixed macrophages (66, 67), it may well be that there is a direct interaction with the Ia molecules. The polymorphic domains on the Ia molecule may interact with some degree of specificity with the antigen fragment; alternatively, the fragment may complex through its hydrophobic portion with nonpolymorphic domains of Ia. Regardless, a complex would eventually be formed among the structures comprising the T-cell receptor, the antigen fragment, and the Ia of the macrophage.

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MODULATION OF ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE

Extensive studies with macrophages have now indicated that antigen presentation is under critical regulation and that it can be modulated to a great extent by controlling the synthesis and expression of Ia molecules. The capacity of macrophages to synthesize and express Ia antigens on their surface is not constitutive. Furthermore, it varies depending on the state of maturation of the macrophage, the age of the individual, and the balance of stimulatory and inhibitory conditions. The best species to show Ia regulation of the macrophage are the mouse and rat, where fluctuations in the ratio of Ia-positive/Ia-negative macrophages are readily apparent. The studies summarized below refer to these species, except as noted. In all cases, the Ia molecules expressed on the surface of murine macrophages are represented by the A and E polypeptides, encoded in the two major I subregions. The term Ia molecules or antigens refers, therefore, to all I region–encoded molecules.

Ia-Positive Macrophages in Different Tissues

The first studies analyzing antigen presentation and Ia expression indicated that the percentage of Ia-positive macrophages varied extensively from tissue to tissue. Cowing et al called attention to the fact that, while spleen macrophages contained a high percentage (~50%) of Ia-positive cells, the peritoneal macrophage had relatively few (10–20%) (71). The results analyzing various tissues are summarized in Table 3. Comments on these studies follow.

Typical mononuclear phagocytes could be identified in suspensions of thymus cells (72). These displayed a high ratio of Ia-positive/Ia-negative. Histological studies showed a high concentration of typical phagocytes in the

Annual Reviews

Table 3 Ia-positive macrophages in various tissues[a]

	Stimuli	% Ia +
Peritoneum	resident	< 20
	peptone-oil	< 10
	T-cell activation	> 50
Spleen	none	50
	T-cell stimulants	> 75
Liver	none	25–50
Thymus	none	75
Lung	none	10
Blood	none	< 5

[a]References are given in the text.

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boundary between the cortex and the medulla as well as in the medullary area (73). The thymic macrophages derived from stem cells found in bone marrow (74, 75) were positive for Fc receptors and low in C3 receptors, and were capable of phagocytosis of latex particles and bacteria (72). Tissue culture studies showed that part of the program of thymus cell differentiation was modulated by the thymic macrophages or factors obtained from them (76, 77). [Ia-positive macrophages could also be involved in the interactions that might result in the development of T-cell receptors for MHC gene products. To be stressed is that macrophages are not the only Ia-positive cells in the thymus. Ia-positive stromal cells, most likely of epithelial origin, have been identified in the cortex in rats, mice, and humans (78–81). Dendritic-type cells most likely akin to Langerhans cells have also been identified (72, 78, 82).]

Studies on spleen macrophages have also brought out the relevance of different microenvironments in modulating the function of the macrophage. The laboratory of John H. Humphrey examined macrophages obtained from spleen following the intravenous injection of labeled polysaccharides (83). Trapping of the labeled material was examined on frozen tissue sections and on macrophages isolated with or without the use of proteolytic enzymes. The red pulp macrophages were isolated by dispersing the spleen cells, while the marginal-zone macrophages required a treatment of the spleen with collagenase and dispase in order to detach the macrophages from the stroma. Humphrey & Grennan distinguished macrophages of the red pulp and those of the marginal zone by means of four features. The red pulp macrophages were rich in Ia-positive cells and small; they could trap pneumococcal polysaccharides and had no lymphocytes attached to them when isolated. In contrast, marginal-zone macrophages were poor in Ia, were large, showed morphological indications of activation, and selectively trapped intravenously injected Ficoll and starch. The marginal-zone macrophages also contained B cells attached to

Annual Reviews

their surface. Such B cells could be detached by treatment with EDTA. Humphrey & Grennan called attention to such differences in macrophages and attributed them to the microenvironment of the spleen. They speculated that the marginal-zone macrophages could be involved in the interaction of B cells with thymic-independent antigens.

The alveolar macrophages also showed differences from peritoneal or other tissue macrophages. Two striking differences were the paucity of C3 receptors (84) and the lack of a binding protein required for the interaction with some bacteria such as *Listeria monocytogenes* (85). This surface protein was highly sensitive to treatment with proteases. The percentage of Ia-positive macrophages among the alveolar macrophage populations was about 5% [in the guinea pig, the figures were much higher (86)]. We found (85) that Ia-positive alveolar macrophages would not present *Listeria* to T cells unless the bacteria was opsonized (alveolar macrophages had a normal content of Fc receptors). The failure of alveolar macrophages to take up bacteria may have resulted from their exposure to proteases found in the alveolar fluid.

Macrophages have also been found in the renal glomerulus, particularly in the mesangial region where they can trap circulating immune complexes (87, 88). The mesangial macrophages have a high ratio of Ia-positive/Ia-negative macrophages. George Schreiner in our laboratory showed that the mesangial phagocytes were distinct from true contractile mesangial cells. The mesangial phagocytes derived from Ia-negative monocytes that entered the mesangium and resided in it for several days. Entrance into the mesangium resulted in a process that led to the expression of Ia by these cells. Inflammation of the glomerulus resulted also in fluctuations in the number of Ia-positive macrophages. Glomerular Ia-positive macrophages are important because they can stimulate T cells, which may induce inflammation of the delayed hypersensitivity type. Finally, the liver macrophages, the Kuppfer cells, have a relatively high percentage of Ia-positive cells (25 to 50%) and, when isolated, were shown to present antigen (89, 90).

The differences in basal number of Ia-positive macrophages in tissues was not related to the presence or absence of T cells. Nude athymic mice, or mice thymectomized at birth, had a normal basal level of Ia-positive macrophages (91). Their macrophages could present antigen to T cells as well as those macrophages from euthymic mice. These athymic mice lacked demonstrable T-cell activity in a number of assays. Moreover, the increase in the number of Ia-positive macrophages that followed a normal infection with bacteria (see below), an increase mediated by T cells, was not found in athymic mice. One laboratory found low antigen presentation by macrophage from nude mice (92). The experimental protocol used in those studies probably did not reflect directly the Ia-positive presentation of antigen but other properties of the macrophage.

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The studies in athymic mice indicate that mechanisms other than the level of T cells operate in different tissues to affect the ratio of Ia-positive/Ia-negative macrophages. Such mechanisms have yet to be identified. Regardless, T cells do control the level of Ia-positive macrophages in what is a clear amplification loop of cellular interactions.

The first studies showing T-cell regulation of macrophage Ia were done by Beller et al when examining the peritoneal macrophages following injection of a number of stimulants (93). Two distinct responses were found: Exudates induced by injection of oil, thioglycollate, or peptone were formed mostly by Ia-negative macrophages—i.e. the ratio of Ia-positive/Ia-negative was the same as in normal mice (about 0.9); in contrast, infection with the intracellular pathogen *Listeria monocytogenes* resulted in a marked increase in Ia-positive macrophages. By 3–5 days, 50–90% of the peritoneal exudates comprised Ia-positive cells. This increase in Ia was the result of T-cell activation since it could be transferred in vivo to normal mice by the immune T cells. Furthermore, a conditioned media (obtained from cultures of T cells with macrophages and antigen) contained a molecule able to reproduce the effects of live immune T cells, inducing exudates rich in Ia-positive macrophages, both in vivo (94) and in culture (95–97). The T-cell lymphokine behaved as a nonspecific lymphokine in not being restricted by the MHC haplotype of the responding cell. The increase in Ia-positive cells took place rapidly in all conditions that resulted in T-cell activation. Most notable were infections with intracellular pathogens like *Listeria monocytogenes* (93), *Mycobacterium tuberculosis* (93, 98), or *Trypanosoma cruzi* (99). Granulomas induced by schistosome eggs were rich in Ia-positive macrophages (100, 101). The increase in Ia was also found following conventional immunization with protein antigens, particularly with strong immunogens, or following a secondary challenge (93). In fact, the extent of secondary antibody responses was correlated with the percentage of Ia-positive macrophages at the site of the reaction.

Regardless of whether Ia-positive macrophages were derived from normal tissues or from T cell–mediated reactions, the period of expression of membrane Ia was relatively short—on the order of 12–48 hr (102). For example in cell cultures, Ia-positive macrophages synthesized Ia briefly, rapidly becoming Ia-negative and losing all their antigen-presenting function and their capacity to stimulate in a mixed-lymphocyte reaction (97). Other membrane proteins of the macrophage (e.g. the Fc receptors or the Class I MHC proteins) were maintained for long periods of culture. Similar results were found in vivo by transferring Ia-positive macrophages in semi-syngeneic combinations or by using X irradiation (103). Irradiated mice, which lose their stem-cell function had a loss of Ia-positive macrophages. Thus in tissues, at the population level the macrophage function of antigen presentation is radiosensitive. The syn

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thesis of Ia by the macrophages could be prolonged briefly following the uptake of particulate materials (102).

The response of macrophages to lymphokines has been studied both in vivo and in vitro. In vivo intraperitoneal administration of the lymphokine for 2–3 days, every 12 hr, resulted in the induction of exudates with a high ratio of Ia-positive/Ia-negative macrophages (94, 103). The cells responding in vivo were young, immature macrophages (103). This was surmised from studies using X-irradiated mice. Their peritoneal macrophages were unresponsive even in conditions where X irradiation was given shortly after induction of exudate rich in Ia-negative cells. The X-irradiated mice, on the other hand, developed Ia-positive exudates if reconstituted with bone marrow cells, regardless of whether or not the bone marrow was depleted of Ia-positive cells. Thus, the responding cells derived from Ia-negative precursors found in abundance in the bone-marrow inoculum.

The response of the macrophage to the lymphokine is a complex event when evaluated in cell culture (95–97, 104). Beller & Ho distinguished several stages: an essential initial period of adherence followed by a second period where the cell was receptive to the lymphokine and during which Ia was absent from the cell membrane. During this period, the cell synthesized new messenger RNA for the Ia molecules. In a final period, the cell then expressed Ia on its surface. Uptake of particulate compounds resulted in a fresh burst of Ia expression. Interestingly, the response to the lymphokine depended on the type of macrophage, a finding that confirmed the results found in vivo. That is to say, while monocytes responded well with a brief period of latency (i.e. the period between exposure to the lymphokine and the first appearance of Ia on the membrane), the resident macrophage responded sluggishly following a prolonged period of interaction with the lymphokine. Exudate macrophages, on the other hand, were intermediate in their response.

The major lymphokine responsible for Ia induction is gamma-interferon, as initially indicated by Oppenheim's laboratory (105). Biochemical manipulations that enriched for or depleted of interferon correlated with the enrichment or loss of the Ia-inducing lymphokine. Furthermore, antibodies to gamma-interferon abolished the Ia-inducing activity (105). Recently, interferon derived by recombinant DNA technology has been available and shown to have Ia-inducing properties (104, 106–108).

Studies with bone-marrow-derived macrophages have been important in several aspects. In all instances, bone-marrow cells were induced to grow and differentiate to macrophages by exposure to macrophage growth factors found in a series of cell-conditioned media. The amount of macrophage that developed Ia following exposure to conditioned media has varied from 0 to 60% or more, most likely because of the use of widely diverse conditioned media

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(109–113). Using L cell–conditioned media, the percentage of Ia-positive macrophages developing Ia spontaneously was small, although these respond well to lymphokines. In an assay where individual colonies were examined for the response to lymphokines, all colonies contained cells capable of making Ia—i.e. one could not distinguish sets of bone-marrow-derived macrophages that were either Ia-positive or Ia-negative (112). It is noteworthy that the bone-marrow macrophages showed a clear antagonism between their stimulation of growth by the conditioned media and their differentiation to express Ia by lymphokine (112). In the presence of the growth factor, the response to the lymphokine was markedly reduced. Bone-marrow macrophages that expressed Ia were capable of presenting antigen to T cells (109–111, 107).

In summary, the expression of Ia takes place primarily in the immature macrophages if properly stimulated. The stimulants for Ia induction can be classified into a thymus-independent and thymus-dependent. The latter are represented by gamma-interferon secreted by activated T cells. Each tissue has a characteristic ratio of Ia-positive/Ia-negative macrophages reflecting most likely the balance of positive and negative modulatory influences (see below). Clearly, a number of conditions affect the biology of the macrophage in a particular local environment. Ia expression requires the continuous presence of the stimulant, rapidly decaying in its absence. Ia synthesis is not a marker of stable subsets of macrophages; rather it is an expression of an activated state of the cell. While it is the fate of all Ia-positive macrophages to become Ia-negative, not all Ia-negative macrophages must follow a pathway of activation resulting in the expression of Ia. Clearly, a number of stimulants produce macrophages lacking in the synthesis and expression of these key regulatory proteins. Finally, it is noteworthy that in humans the expression of Ia molecules by monocytes takes place in a large percentage of them [e.g. (114–116)]. As in other species, Ia decays in culture (115) and can be increased by gamma-interferon and inhibited by endotoxin (116).

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Inhibition of Ia Expression

Three sets of molecules inhibit Ia expression—prostaglandins of the E class (117,118), alpha-fetoprotein (119), and glucocorticoids (120). The first two may be responsible for the low number of Ia-positive macrophages during neonatal development.

The mouse is a species where the newborn has a highly immature immune system. Newborns had few Ia-positive macrophages and consequently showed a deficit in antigen presentation to T cells (121, 122). By 7–14 days after birth, the Ia-positive macrophage reached the level found in adults. The thymus gland, in contrast, contained, aside from Ia-positive stromal cells, a high percentage of Ia-positive macrophages (123). This finding indicates that the absence of Ia-positive macrophages in the neonate is not the result of a defec

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tive stem cell. Indeed, macrophages from newborns, when cultured with lymphokines, synthesized Ia.

The paucity of Ia-positive macrophages was due to inhibitory influences found in the neonatal environment. Indeed, spleen cells from newborns transferred to an adult mouse intraperitoneally inhibited the T-cell induction of Ia-positive macrophages (124). This inhibition was, in great part, accounted for by a secretory component, sensitive to indomethacin or aspirin, strongly suggesting the involvement of prostaglandins. Addition of purified E prostaglandins to cultures of murine macrophages markedly inhibited the synthesis and membrane expression of Ia molecules but not of Class I molecules or of Fc or C3 receptors [addition of dibutyryl cyclic AMP to macrophages also inhibited Ia expression (117)]. The amount of E_2 prostaglandin required to inhibit Ia synthesis depended on the amount of lymphokine added to the culture. The 50% inhibitory dose was about 10^{-9}–10^{-10} M, concentrations found physiologically in some tissues.

Prostaglandin E could also play a modulatory role in some tissues in adults, under normal or altered conditions (124). Thus, the low number of Ia-positive macrophages in the peritoneal cavity most likely reflected a high local level of these products. Intraperitoneal injection of indomethacin into normal adult mice markedly increased the percentage of peritoneal Ia-positive macrophages. The effects of endotoxin in inhibiting Ia have been explained on the basis of its stimulation of prostaglandin secretion (118).

In the neonate as well as in the adult peritoneal cavity, the cell responsible for prostaglandin production is a mononuclear phagocyte. The neonatal cell appears to be an immune phagocyte in cell cycle since X irradiation stopped its inhibitory effect. It therefore appears that the macrophage can regulate its own program of Ia induction and that among the autoregulatory molecules are the E-type prostaglandins. We do not yet know what conditions in the neonatal environment affect macrophage PG production.

Not all arachidonate-derived molecules have been evaluated for their effect in Ia induction. Curiously, thromboxane counteracted the inhibitory effect of PGE_2 (117). This finding argues that a careful scrutiny of the metabolites of arachidonate is required and that the effects on Ia might reflect the balance between inhibitory and stimulatory metabolites.

A recent study has shown that alpha-fetoprotein, a major plasma protein of the fetus and the neonate, also inhibited Ia synthesis by the macrophage (119). Alpha-fetoprotein was not cytotoxic to macrophages but selectively reduced Ia expression at concentrations of about 10^{-6} M. The inhibitory effect of alpha-fetoprotein on macrophage Ia is of interest in the light of previous studies showing its effect in a number of immunological reactions in tissue culture. Alpha-fetoprotein reduced mixed-lymphocyte reactions, antibody formation, phytohemagglutinin response of T cells, and induced suppressor T cells [e.g.

Annual Reviews

(125, 126)]. All these effects could well be explained by its inhibition of Ia molecules on the macrophage.

We have considered the significance of low Ia-positive macrophages during development and speculated about its relationship to the hyporesponsiveness to antigens seen during this period of life (127). Our speculation is that the neonate is placed in jeopardy during this critical period because another, more important event—i.e. autoreactivity—must be controlled. A paucity of Ia-macrophages may lean the balance towards suppression rather than induction at a critical time when self-proteins must be recognized as tolerogens.

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Relationship Between Ia Expression and Presentation

Fluctuations in expression of Ia by the macrophages correlated with changes in antigen presentation (97, 108, 128). The loss of Ia that took place upon tissue culture of the macrophage resulted in a marked to complete reduction in antigen presentation (108). In contrast, the acquisition by macrophages of Ia induced by exposure to lymphokine resulted in a proportional increase in its accessory cell function. This relationship between Ia and antigen presentation may require stringent conditions in order to be seen in cultures (108). These populations of macrophages with varying numbers of Ia-positive macrophages showed only slight differences in antigen presentation. However, fixation of the cells (after uptake of the antigen) resulted in excellent correlation between number of Ia-positive macrophages (and total amount of surface Ia detected immunochemically) and the degree of antigen-dependent T-cell stimulation. Most likely during culture in closed systems a number of variables are reflected in the function of the macrophage—i.e. production of interferon, prostaglandins, etc. It should be stressed that antigen-handling parameters (rate of catabolism, exocytosis of antigen, etc) were identical in Ia-positive or Ia-negative macrophages and in normal or stimulated macrophages (59). Thus, antigen handling is a constitutive function insensitive to modulatory events. [The surface expression of Ia in tumor macrophages also correlated with the acquisition and degree of antigen presentation to T cells (107, 128, 129)].

The findings, therefore, that Ia-positive macrophages present antigen and activate T cells, which, in turn, call forth for Ia-positive macrophages, imply that a loop or circuit of cellular interactions promotes the presentation of antigen, a vital function of the immune process.

Two features to be considered in the context of the antigen-presenting function of Ia-bearing macrophages are: (*a*) the relationship between induction of Ia molecules and of cytocidal activity, and (*b*) the release of IL-1. Limited studies have indicated that the induction of cytocidal activity accompanies, at some state, the induction of Ia. Both cytocidal macrophages (i.e. capable of killing tumor cells or microbes) and Ia-bearing macrophages develop as a result of T-cell stimulation. A single lymphokine—gamma-interferon—could

Annual Reviews

induce both activities (130, 131). In culture, the two activities were dissociated. Cytotoxicity developed early, and then decayed preceding the expression of Ia. The relationship between the two in vivo has not been fully explained. Certainly, Ia-positive macrophages in tissues did not exhibit cytotoxicity (132). However, the Ia-positive macrophages in strong or continuous cellular immune reactions, which are strongly Ia-positive, may exercise both functions (132).

IL-1 has been thought to be a molecule vitally involved in T-cell activation (133). Previous studies have stressed the wide range of biological functions of this molecule, which range from promoting thymocyte growth to inducing fever [reviewed in (134, 135)]. The relationship between IL-1 production and the antigen-presenting activity of the macrophage has yet to be critically studied. Uptake of some particles, compounds that activate macrophages, and T cell–macrophage interaction during antigen presentation, enhanced IL-1 secretion (136–139). These observations support the notion that IL-1 becomes operative during presentation. An essential role for IL-1 is made dubious by recent studies showing that either B cell lines that do not secrete IL-1 (see below) or fixed macrophages can present antigen and induce T cells to secrete IL-2 or proliferate. One study has shown, however, that fixed human monocytes required soluble IL-1 to present antigen (63); another reached the same conclusion using spleen B cells (140). Thus the requirements for IL-1 may vary during the life of the T cell. T cells, at some steps of their activation cycle, may not require this molecule. This issue of IL-1 secretion, therefore, needs more research.

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ANTIGEN PRESENTATION AND Ia EXPRESSION IN CELLS OTHER THAN THE MACROPHAGE

Many cells besides the macrophages express Ia antigens. Do all these Ia-positive cells present all, some, or no antigens? A brief review on these questions follows.

B cells constitutively express Ia on their cell surface, although there are differences in the amounts of Ia molecules per cell that affect their interactions with T cells and protein antigens (141–143). To some extent, Ia molecules can be increased following stimulation such as with anti-Ig antibodies (144) or endotoxin (145). B cells can present some antigen to T cells. Studies on antigen presentation have been done with B cells isolated from murine spleens (140, 146, 147) or with B cell tumors (60, 61, 147–150).

Grey, Chesnut, and associates studied proliferation of rabbit IgG-primed T cells upon interaction with B cells incubated with rabbit anti-mouse Ig. B cells were able to handle the anti-Ig and present the immunogen to T cells, inducing their proliferation (146). This induction was restricted by the MHC. The curious finding was that presentation by T cells of normal rabbit IgG was poor.

Annual Reviews

In contrast, macrophages were able to present equally well both preparations, either normal IgG or rabbit IgG with anti-mouse Ig antibody activity. Subsequent studies indicated that B cells isolated from a normal spleen were inefficient in presenting keyhole limpet hemocyanin; in contrast, B-cell lymphomas were highly active. Activation of B cells by treatment with endotoxin resulted in the acquisition of antigen-presenting function. The differences in presentation of normal rabbit Ig or rabbit anti-mouse IgG were explained by the stimulatory effect of the latter compound on B cells. The reason for the lack of presentation of unstimulated B cells is not clear. Small B cells took up less antigen, which may be one critical factor. As far as is known, normal or stimulated cells did not secrete IL-1. We found a marked enhancement of presentation of KLH by unstimulated B cells in the presence of IL-1 (140).

B cells can internalize and degrade proteins (152). The presentation of proteins by B cells had the same characteristics described in the first section of this review—i.e. it required a handling step sensitive to lysosomotropic drugs (60, 61). It is not known, however, whether B cells will be able to present particulate antigens such as those on red cells or microbes. B cells internalize these large antigens to a limited extent.

Langerhans cells of the skin exhibit dendritic morphology; some bear Fc and C3 receptors, and many have a high density of Ia antigens (82, 153–157). Some Langerhans cells have a peculiar cytoplasmic structure, the Birbeck granule, which serves as an identifier (82). Langerhans cells are found in thymus and in the thymus-dependent areas of lymphoid organs (82). It is likely that the spleen dendritic cells isolated by Steinman and associates belong to the Langerhans cell lineage (158). In vivo studies have indicated an important role for Langerhans cells in contact sensitivity: Their elimination from areas of the skin resulted in a loss of delayed sensitivity at the site; moreover, antigen applied in such areas triggered the induction of T suppressor cells (159). Langerhans cells isolated from skin stimulated antigen-primed T cells (156). Dendritic cells of the spleen were potent inducers of autologous and allogeneic mixed-lymphocyte reactions (160–162) and stimulated T-cell proliferation to some antigens (163–165). Ia expression on Langerhans or dendritic cells may be constitutive, but the studies that address this point are few. The role of such cells in handling proteins or particulate antigens is not known.

Ia antigens have now been found to be expressed in hematopoietic (166), epithelial (167–171), connective tissue (172), and endothelial cells (172–174) of various tissues under certain conditions. Studies to this effect have been done using immunocytochemistry in tissue sections, or by isolating the cells from each organ. Examples have been found of Ia expression under normal (i.e. unstimulated) conditions or following activation by a number of stimulants. Most keratinocytes, intestinal epithelium, liver cells, thyroid follicular

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cells, mammary gland epithelium, vascular endothelium, and fibroblasts did not normally express Ia (167, 168, 171, 172). Immunofluorescence revealed Ia in what appear to be intracellular sites in some renal tubules (in the rat) (170). Epithelial cells of the thymus cortex, on the other hand, appeared normally to have Ia molecules (78–81). Human myeloid cells expressed Ia before their final differentiation to granulocytes (166).

Three conditions have been cited as resulting in induction of Ia: (*a*) Mammary gland epithelium expressed Ia during lactation or following administration of estrogen and prolactin; in contrast, administration of androgens counteracted the stimulation of prolactin (169). (*b*) Human thyroid cells were stimulated by lectin in culture (this may have resulted from residual T cells in the culture) (171) or in vivo during a number of thyroid abnormalities (some of which may be immunologically mediated) (175). (*c*) A number of cells expressed Ia following T-cell stimulation in vivo or in culture: Keratinocytes and intestinal epithelium became positive after systemic graft-vs-host reactions (this was done in the rat by injecting parental lymphocytes into an F_1; the Ia was that of the host) or in local sites during contact sensitivity (176–178). Endothelial cells and fibroblasts became positive in culture following administration of gamma-interferon and stimulated the mixed-lymphocyte reaction (174).

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Summary

The functional significance of multiple cells—among lymphoid and nonlymphoid cells—capable of having Ia molecules on their membranes must be critically addressed. Ia is absolutely required before a cell can interact with helper T cells, but it is not clear whether the presence of this protein is all that is needed for antigen presentation. Indeed, at present, except for the macrophage, few cells have been studied for antigen presentation using a wide range of protein antigens, either soluble or particulate.

On the basis of the studies discussed in the first section, it appears that the recruitment of most helper–T cell clones takes place by APC that can internalize and process the protein antigens, be they soluble or part of the structure of microorganisms. The fact that helper T cells are programmed to recognize antigen in the context of Ia, and therefore on an APC such as the macrophage, forces recognition of antigens that are altered or processed. Indeed, proteins in their native state may not remain membrane-bound for long periods; the T cells, therefore, have the opportunity to recognize the altered fragments. To this issue is added the requirement for the T-cell receptor to interact with Ia molecules. The available information, therefore, leads one to conclude that APC deficient in their capacity to internalize and process proteins will not be able to present them. The finding that small peptides from a previous catab-

Annual Reviews

olism of proteins can be presented without further handling implies that APC with limited processing capacity could be involved in presentation of such small peptides.

The different Ia-positive APC of the lymphoid organs may interact to different extents with protein antigens and collaborate with each other to bring about an effective stimulation of the clones of helper T cells. The macrophage, being the most ubiquitous cell and the one capable of interacting with many proteins, is our candidate as the major APC involved in the recruitment and enlargement of clones of T cells. The observations that macrophages can release proteins partially altered implies that there may be cooperativity among the various APC. Data for this have been obtained. Most likely B cells will be found to have a limited capacity to present all antigens because of their inherent difficulties in internalizing large particulate materials. In such instances, B cells may interact with the solubilized proteins released by the macrophages (1, 59). The same may apply to the Langerhans/dendritic cells. We have previously speculated that the antigen-presenting function of the B cell may be a means for acquiring T-cell help and, therefore, autoregulating its own differentiation (1, 127). Antigen presentation by B cells may explain the apparent discrepancy of two observations: (*a*) the different patterns of recognition by B and T cells, and (*b*) the finding that hapten and carrier determinants must be linked in the same molecule for optimal presentation. B cells can recognize globular proteins in their native state via surface Ig and then process them and present the processed (i.e. carrier) determinants to T cells. Thus, the determinants recognized by B and T cells are in the same molecule and are recognized in different time sequences.

A related issue concerns the functional significance of Ia in various cells of nonlymphoid tissues. One speculation is that the Ia in these cells could have a regulatory role not related to its modulation of antigen presentation to T cells. The thymus epithelial cell is one example where Ia is thought to regulate the development of T-cell clones in an antigen-independent way. In general, functions of Class II molecules aside from those involving lymphocyte reactions are not known. We think that expression of Ia molecules must be critically controlled not only at the level of the macrophage but also on all tissue cells (127). For example, a wide representation of Ia-positive macrophages may open the possibility of uncontrolled antigen presentation. To this effect, we have been impressed by a wide number of Ia-positive macrophages in the *lpr* autoimmune strain of mice (179, 180) accounted for, in part, by spontaneous secretion of lymphokine (180). Tissue cells having Ia may develop the potential to present self-antigens, disturbing the delicate homeostasis for tolerance, with resulting autoimmunity. An attractive hypothesis to this effect has just been formulated on the basis of finding Ia in thyroid cells in pathological states (181).

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Annual Reviews

Literature Cited

1. Unanue, E. R. 1981. The regulatory role of macrophages in antigenic stimulation. Part Two: Symbiotic relationship between lymphocytes and macrophages. *Adv. Immunol.* 31:1
2. Moller, G., ed. 1978. Role of macrophages in the immune response. *Immunol. Rev.* 40:3
3. Gell, P. G. H., Benacerraf, B. 1959. Studies of hypersensitivity to denatured proteins in guinea pigs. *Immunology* 2:64
4. Brown, R. K., Delaney, R., Levine, L., van Vunakis, H. 1959. Studies on the antigenic structure of ribonuclease. *J. Biol. Chem.* 234:2043
5. Freedman, M. H., Sela, M. 1966. Recovery of antigenic activity upon reoxidation of completely reduced polyalanylated rabbit immunoglobulin G. *J. Biol. Chem.* 241:2383
6. Crumpton, M. J., Sonall, P. A., Jr. 1967. Conformation of immunologically active fragments of sperm whale myoglobin in aqueous solution. *J. Mol. Biol.* 26:143
7. Gerwing, J., Thompson, K. 1968. Studies on antigenic properties of egg-white lysozyme. I. Isolation and characterization of a tryptic peptide from reduced and alkylated lysozyme exhibiting haptenic activity. *Biochemistry* 7:3888
8. Sela, M. 1969. Antigenicity: some molecular aspects. *Science* 166:1365
9. Schechter, B., Schechter, I., Ramachandran, J., Conway-Jacobs, A., Sela, M., Benjamini, E., Shimizu, M. 1971. Synthetic agents with sequential and conformation-dependent determinants containing the same L-tyrosyl-L-alanyl-L-glutamyl sequence. *Eur. J. Biochem.* 20:309
10. Young, J. D., Leung, C. Y. 1970. Immunochemical studies on lysozyme and carboxymethylated lysozome. *Biochemistry* 9:2755
11. Schirrmacher, V., Wigzell, H. 1972. Immune responses against native and chemically modified albumins in mice. *J. Exp. Med.* 136:1616
12. Goetzl, E. J., Peters, J. H. 1972. Immunologic alterations in bovine serum albumin resulting from partial or complete reduction and alkylation. *J. Immunol.* 108:785
13. Thompson, K., Harris, M., Benjamini, E., Mitchell, G., Noble, M. 1972. Cellular and humoral immunity: a distinction in antigenic recognition. *Nature* 238:20
14. Scibienski, R. J. 1973. Denaturation of lysozyme by Freund's complete adjuvant. *J. Immunol.* 111:114
15. Ishizaka, K., Okudaira, H., King, T. 1975. Immunogenic properties of modified antigen E. II. Ability of urea-denatured antigen and E-polypeptide chain to prime T cells specific for antigen E. *J. Immunol.* 114:110
16. Attasi, M. Z. 1978. Precise determination of the entire antigenic structure of lysozyme. *Immunochemistry* 15:909
17. Chesnut, R., Endres, R., Grey, H. M. 1980. Antigen recognition by T cells and B cells: recognition of cross-reactivity between native and denatured forms of globular antigens. *Clin. Immunol. Immunopathol.* 15:397
18. Maizels, R. A., Clarke, J. A., Harvey, M. A., Miller, A., Sercarz, E. E. 1980. Epitope specificity of the T cell proliferative response to lysozyme: proliferative T cells react predominantly to different determinants from those recognized by B cells. *Eur. J. Immunol.* 10:509
19. Senyk, G., Williams, E. B., Nitecki, D. E., Goodman, J. W. 1971. The functional dissociation of an antigen molecule: specificity of humoral and cellular immune response to glucagon. *J. Exp. Med.* 133:1295
20. Rosenthal, A. S., Barcinski, M. A., Blake, J. T. 1977. Determinant selection is a macrophage-dependent immune response gene function. *Nature* 267:156
21. Ishii, N., Baxevanis, C. B., Nagy, Z. A., Klein, J. 1981. Responder T cells depleted of alloreactive cells react to antigen presented on allogenic macrophages from nonresponder strains. *J. Exp. Med.* 154:978
22. Clark, R. B., Shevach, E. M. 1982. Generation of T cell colonies from responder strain 2 guinea pigs that recognize the copolymer L-glutaminic acid, L-lysine in association with nonresponder strain 13 Ia antigens. *J. Exp. Med.* 155:635
23. Keck, K. 1975. Ir gene control of immunogenicity of insulin and A chain loop as a carrier determinant. *Nature* 254:78
24. Barcinski, M. A., Rosenthal, A. S. 1977. Immune response gene control of determinant selection. I. Intramolecular mapping of the immunogenic sites on insulin recognized by guinea pig T and B cells. *J. Exp. Med.* 145:726
25. Sercarz, E. E., Yowell, R. L., Turkin, D., Miller, A., Araneo, B. A., Adorini, L. 1978. Differential functional speci-

Annu. Rev. Immunol. 1984.2:395-428. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annu. Rev. Immunol. 1984.2:395-428. Downloaded from arjournals.annualreviews.org
by HINARI on 08/27/07. For personal use only.

ficity repertoires for suppressor and helper T cells. *Immunol. Rev.* 39:108
26. Corradin, G., Chiller, J. M. 1979. Lymphocyte specificity to protein antigens. II. Fine specificity of T cell activation with cytochrome C and derived peptides as antigenic probes. *J. Exp. Med.* 149:436
27. Thomas, D. W., Meltz, S. K., Wilner, G. D. 1979. Nature of T lymphocyte recognition of macrophage-associated antigens. I. Response of guinea pig T cells to human fibrinopeptide B. *J. Immunol.* 123:759
28. Thomas, D. W., Meltz, S. K., Wilner, G. D. 1979. Nature of T lymphocyte recognition of macrophage-associated antigens. II. Macrophage determination of guinea pig T cell responses to human fibrinopeptide B. *J. Immunol.* 123:1299
29. Berzofsky, J. A., Richman, L. K., Killon, D. J. 1979. Distinct H-2-linked Ir genes control both antibody and T cell responses to different determinants on the same antigen, myoglobin. *Proc. Natl. Acad. Sci. USA* 76:4046
30. Richman, L. R., Strober, W., Berzofsky, J. A. 1980. Genetic control of the immune response to myoglobin. III. Determinant-specific, two Ir gene phenotype is regulated by the genotype of reconstituting Kuppfer cells. *J. Immunol.* 124:619
31. Ultee, M. E., Margoliash, E., Lipkowski, A., Flouret, G., Solinger, A. M., Lebwohl, D., Matis, L. A., Chen, C., Schwartz, R. H. 1980. The T lymphocyte response to cytochrome c. II. Molecular characterization of a pigeon cytochrome c determinant recognized by proliferating T lymphocytes of the B10.A mouse. *Mol. Immunol.* 17:809
32. Thomas, D. W., Hsieh, K. H., Schauster, J. L., Wilner, G. D. 1981. Fine specificity of genetic regulation of guinea pig T lymphocyte responses to angiotensin II and related peptides. *J. Exp. Med.* 153:583
33. Thomas, D. W., Danho, W., Bullesbach, E., Fohles, J., Rosenthal, A. S. 1981. Immune response gene control of determinant selection. III. Polypeptide fragments of insulin are differentially recognized by T but not by B cells in insulin-immune guinea pigs. *J. Immunol.* 126:1095
34. Thomas, D. W., Hoffman, M. D., Wilner, G. D. 1982. T lymphocyte recognition of peptide antigens: evidence favoring the formation of neoantigenic determinants. *J. Exp. Med.* 156:289
35. Matis, L. A., Longo, D. L., Hedrick, S. M., Hannum, C., Margoliash, E., Schwartz, R. H. 1983. Clonal analysis of the Major Histocompatibility Complex restriction and the fine specificity of antigen recognition in the T cell proliferative response to cytochrome c. *J. Immunol.* 130:1527
36. Heber-Katz, E., Hansburg, D., Schwartz, D. H. 1983. The Ia molecule of the antigen-presenting cell plays a critical role in immune response gene regulation of T cell activation. *J. Mol. Cell. Immunol.* 1:3
37. Manca, F., Clarke, J. A., Sercarz, E. E., Miller, A. 1983. T cells with different specificity exist for a single determinant on lysozyme. In *Ir Genes,* ed. C. W. Pierce, S. E. Cullen, J. A. Kapp, B. D. Schwartz, D. D. Shreffler, p. 305. Clifton, New Jersey: Humana Press
38. Sela, M. 1966. Immunologic studies with synthetic polypeptides. *Adv. Immunol.* 5:30
39. Jones, V. E., Leskowitz, S. 1965. Role of the carrier in the development of delayed sensitivity to the azophenylarsonate group. *Nature* 207:596
40. Colotti, C., Leskowitz, S. 1970. The role of immunogenicity in the induction of tolerance with conjugates of arsanilic acid. *J. Exp. Med.* 131:571
41. Komatsu, T., Lawn, C. Y., Amsden, A., Leskowitz, S. 1983. Hapten-specific T cell response to azobenzenearsonate-n-acetyl-1-tyrosine in the Lewis rat. III. Effects of peptide-spacer structure on eliciting ABA-specific helper activity with TNP-haptenated ABA peptide Ficoll. *J. Immunol.* 130:586
42. Janeway, C. A., Jr. 1976. The specificity of T lymphocyte responses to chemically defined antigens. *Transplant. Rev.* 29:164
43. Unanue, E. R. 1978. The regulation of lymphocyte functions by the macrophage. *Immunol. Rev.* 40:15
44. Ellner, J. J., Rosenthal, A. S. 1975. Quantitative and immunologic aspects of the handling of 2,4 dinitrophenyl guinea pig albumin by macrophages. *J. Immunol.* 114:1563
45. Ellner, J. J., Lipsky, P. E., Rosenthal, A. S. 1977. Antigen handling by guinea pig macrophages: further evidence for the sequestration of antigen relevant for activation of primed T lymphocytes. *J. Immunol.* 118:2053
46. Thomas, D. W., Shevach, E. M. 1978. Nature of the antigenic complex recognized by T lymphocytes. VI. The effect of anti-TNP antibody on T cell responses to TNP-conjugated macrophages. *J. Immunol.* 121:1145
47. Erb, P., Feldmann, M. 1975. The role

Annual Reviews

of macrophages in the generation of T helper cells. III. Influence of macrophage-derived factors in helper cell induction. *Eur. J. Immunol.* 5:759

48. Puri, J., Lonai, P. 1980. Mechanism of antigen binding by T cells. H-2 (I-A)-restricted binding of antigen plus Ia by helper cell. *Eur. J. Immunol.* 10:273
49. Freedman, A., Zerubarel, R., Gitler, C., Cohen, I. R. 1983. Molecular events in the processing of avidin by antigen-presenting cells (APC). III. Activation of T lymphocyte lines and H-2 restriction are mediated by processed avidin associated with I region gene products. *Immunogenetics* 18:291
50. Ziegler, H. K., Unanue, E. R. 1981. Identification of a macrophage antigen-processing event required for I-region-restricted antigen presentation to T lymphocytes. *J. Immunol.* 127:1869
51. Ziegler, H. K., Unanue, E.R. 1982. Decrease in macrophage antigen catabolism by ammonia and chloroquine is associated with inhibition of antigen presentation to T cells. *Proc. Natl. Acad. Sci. USA* 79:175
52. Lipsky, P. E., Rosenthal, A. S. 1975. Macrophage-lymphocyte interaction. II. Antigen-mediated physical interactions between immune guinea pig lymph node lymphocytes and syngeneic macrophages. *J. Exp. Med.* 141:138
53. Ziegler, H. K., Unanue, E. R. 1979. The specific binding of *Listeria*-immune T lymphocytes to macrophages. I. Quantitation, role of H-2 gene products. *J. Exp. Med.* 150:1143
54. Werdelin, O., Braendstrup, O., Shevach, E. M. 1979. Specific absorption of T lymphocytes committed to soluble protein antigens by incubation on antigen-pulsed macrophage monolayers. *J. Immunol.* 123:1755
55. Werdelin, O., Shevach, E. M. 1979. Role of nominal antigen and Ia antigen in the binding of antigen-specific T lymphocytes to macrophages. *J. Immunol.* 123:2779
56. Ohkuma, S., Poole, B. 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agent. *Proc. Natl. Acad. Sci. USA* 75:3327
57. Tietze, C., Schlesinger, P., Stahl, P. 1980. Chloroquine and ammonia ion inhibit receptor-mediated endocytosis of mannose-glyconjugates by macrophages: apparent inhibition of receptor recycling. *Biochem. Biophys. Res. Commun.* 93:1
58. Gordon, A. H., D'Arcy Hart, P., Young, M. R. 1980. Ammonia inhibits phagosome-lysosome fusion in macrophages. *Nature* 286:79
59. Allen, P. M., Beller, D. I., Braun, J., Unanue, E. R. 1984. The handling of *Listeria monocytogenes* by macrophages: the search for an immunogenic molecule in antigen presentation. *J. Immunol.* 132:323
60. Chesnut, R. S., Colon, S., Grey, G. M. 1982. Requirements for the processing of antigen by antigen-presenting B cells. I. Functional comparison of B cell tumors and macrophages. *J. Immunol.* 129:2382
61. Grey, H. M., Colon, S., Chesnut, R. S. 1982. Requirements for the processing of antigen by antigen-presenting B cells. II. Biochemical comparison of the fate of antigen in B cell tumors and macrophages. *J. Immunol.* 129:2389
62. Lee, K. C., Wong, M., Spitzer, D. 1982. Chloroquine as a probe of antigen processing by accessory cells. *Transplantation* 34:150
63. Scala, G., Oppenheim, J. J. 1983. Antigen presentation by human monocytes: evidence for stimulant processing and requirement for interleukin-1. *J. Immunol.* 131:1160
64. Friedman, A., Zerubarel, R., Gitler, C., Cohen, I. R. 1983. Molecular events in the processing of avidin by antigen-presenting cells (APC). II. Identical processing by APC of H-2 high and low responder mouse strains. *Immunogenetics* 18:277
65. McKean, D. J., Nilson, A., Infante, A. J., Kazim, L. 1983. Biochemical characterization of B lymphoma cell antigen processing and presentation to antigen-reactive T cells. *J. Immunol.* 131:2726
66. Shimonkevitz, R., Kappler, J., Marrack, P., Grey, H. M. 1983. Antigen recognition by H-2-restricted T cells. I. Cell-free antigen processing. *J. Exp. Med.* 158:303
67. Allen, P. M., Unanue, E. R. 1984. Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. *J. Immunol.* 132:1077
68. Allen, P. M., Strydom, D. J., Unanue, E. R. 1984. Processing of lysozyme by macrophages; identification of the determinant recognized by two T cell hybridomas. *Proc. Natl. Acad. Sci. USA*. In press.
69. Geuze, H. J., Slot, J. W., Strous, G. J. A. M., Lodish, H. F., Schwartz, A. L. 1983. Intracellular site of asialoglycoprotein receptor-ligand uncoupling: double-label immunoelectron microscopy during receptor-mediated endocytosis. *Cell* 32:277

Annu. Rev. Immunol. 1984.2:395-428. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

70. Benacerraf, B. 1978. A hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages to B lymphocytes. *J. Immunol.* 120:1809
71. Cowing, C., Schwartz, B. D., Dickler, H. B. 1978. Macrophage Ia antigens. I. Macrophage populations differ in their expression of Ia antigens. *J. Immunol.* 120:378
72. Beller, D. I., Unanue, E. R. 1980. Ia antigens and antigen-presenting function of thymic macrophages. *J. Immunol.* 124:1433
73. Raviola, E., Karnovsky, M. J. 1972. Evidence for a blood-thymus barrier using electron-opaque tracers. *J. Exp. Med.* 136:466
74. Longo, D. L., Schwartz, D. H. 1980. T cell specificity for H-2 and Ir gene phenotype correlates with the phenotype of thymic antigen-presenting cells. *Nature* 287:44
75. Barclay, A. N., Mayrhofer, G. 1981. Bone marrow origin of Ia-positive cells in the medulla of the rat thymus. *J. Exp. Med.* 153:1667
76. Beller, D. I., Unanue, E. R. 1977. Thymocyte maturation *in vitro* by a secretory product from macrophages. *J. Immunol.* 118:1780
77. Beller, D. I., Unanue, E. R. 1978. Thymic macrophages modulate one stage of T cell differentiation *in vitro*. *J. Immunol.* 121:1861
78. Rouse, R. V., van Ewijk, W., Jones, P. P., Weissman, I. L. 1979. Expression of MHC antigens by mouse thymic dendritic cells. *J. Immunol.* 122:2508
79. Wekerle, H., Ketelsen, U. P., Ernst, M. 1980. Thymic mouse cells. Lymphoepithelial cell complexes in murine thymus: morphological and serological characterization. *J. Exp. Med.* 151:925
80. Jenkinson, E. J., Owen, J. J. T., Aspinwall, R. 1980. Lymphocyte differentiation and major histocompatibility complex antigen expression in the embryonic thymus. *Nature* 284:177
81. Janossy, G., Thomas, J. A., Bollum, J. J., Granger, S., Pizzdo, G., Brandstock, K. F., Wong, K., McMichael, A., Ganeshaguru, K., Hoffbrand, A. V. 1980. The human thymic microenvironment: an immunohistologic study. *J. Immunol.* 125:202
82. Silberg-Sinakin, S., Baer, R. L., Thorbecke, G. J. 1978. Langerhans cells: a review of their nature with emphasis on their immunologic functions. *Progr. Allergy* 24:268
83. Humphrey, J. H., Grennan, D. 1981. Different macrophage populations distinguished by means of fluorescence polysaccharides. Recognition and properties of marginal-zone macrophages. *Eur. J. Immunol.* 11:221
84. Alblas, A. B., van Furth, R. 1979. Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. *J. Exp. Med.*. 149:1504
85. Weinberg, D. S., Unanue, E. R. 1981. Antigen-presenting function of alveolar macrophages: uptake and presentation of *Listeria monocytogenes*. *J. Immunol.* 126:794
86. Lipscomb, M. F., Toews, G. B., Lyons, C. R., Uhr, J. W. 1981. Antigen presentation by guinea pig alveolar macrophages. *J. Immunol.* 126:286
87. Schreiner, G. F., Kiely, J.-M., Cotran, R. S., Unanue, E. R. 1981. Characterization of resident glomerular cells expressing Ia determinants and manifesting genetically restricted interaction with lymphocytes. *J. Clin. Invest.* 68:920
88. Schreiner, G. F., Cotran, R. S. 1982. Localization of an Ia-bearing glomerular cell in the mesangium. *J. Cell Biol.* 94:483
89. Richman, L. D., Klingenstein, R. J., Richman, J. A., Strober, W., Berzofsky, J. A. 1979. The murine Kuppfer cell. I. Characteristics of the cell serving accessory function in antigen-specific T cell proliferation. *J. Immunol.* 123:2602
90. Rogoff, T. M., Lipsky, P. E. 1980. Antigen presentation by isolated guinea pig Kuppfer cells. *J. Immunol.* 124:1740
91. Lu, C. Y., Peters, E., Unanue, E. R. 1981. Ia-bearing macrophages in athymic mice: antigen presentation and regulation. *J. Immunol.* 126:2496
92. Tzehoval, E., Segal, S., Feldman, M. 1979. Thymus-derived lymphocytes control the expression of immunogenic properties of peritoneal macrophages. *Proc. Natl. Acad. Sci. USA* 76:4056
93. Beller, D. I., Kiely, J.-M., Unanue, E. R. 1980. Regulation of macrophage populations. I. Preferential induction of Ia-rich peritoneal exudates by immunological stimuli. *J. Immunol.* 124:1426
94. Scher, M. G., Beller, D. I., Unanue, E. R. 1980. Demonstration of a soluble mediator that induces exudates rich in Ia-positive macrophages. *J. Exp. Med.* 152:1684
95. Steinman, R. M., Nogueira, M., Witmer, M. D., Tydings, J. D., Mellman, I. S. 1982. Lymphokines enhance the expression and synthesis of Ia antigens on cultures of mouse peritoneal macrophages. *J. Exp. Med.* 155:629
96. Steeg, P., Moore, R. N., Oppenheim,

Annu. Rev. Immunol. 1984.2:395-428. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

J. J. 1981. Regulation of macrophage Ia antigen expression by products of activated spleen cells. *J. Exp. Med.* 152:1734

97. Beller, D. I., Ho, K. 1982. Regulation of macrophage populations. V. Evaluation of the control of macrophage Ia expression *in vitro*. *J. Immunol.* 129:971
98. Ezekowitz, R. A. B., Austyn, J., Stahl, P. D., Gordon, S. 1981. Surface properties of bacillus Calmette-Guerin-activated mouse macrophages. Reduced expression of mannose-specific endocytosis, Fc receptors, and antigen F4/80 accompanies induction of Ia. *J. Exp. Med.* 154:60
99. Behbehani, K., Pan, S., Unanue, E. R. 1981. Marked increase in Ia-bearing macrophages during *Trypanosoma cruzi* infection. *Clin. Immunol. Immunopathol.* 19:190
100. Stadecker, M., Wyler, D., Wright, J. 1982. Ia antigen expression and antigen presentation function by macrophages isolated from hypersensitivity granulomas. *J. Immunol.* 128:2739
101. Welhausen, S. R., Boros, D. L. 1982. Comparison of Fc, C3 receptors, and Ia antigens on the inflammatory macrophages isolated from vigorous or immunomodulated liver granulomas of schistosome-infected mice. *J. Reticuloendothel. Soc.* 30:191
102. Beller, D. I., Unanue, E. R. 1981. Regulation of macrophage populations. II. Synthesis and expression of Ia antigens by peritoneal exudate macrophages is a transient event. *J. Immunol.* 126:263
103. Scher, M. G., Unanue, E. R., Beller, D. I. 1982. Regulation of macrophage populations. III. The immunologic induction of exudates rich in Ia-positive macrophages is a radiosensitive process. *J. Immunol.* 138:447
104. King, D. P., Jones, P. P. 1983. Induction of Ia and H-2 antigens on a macrophage cell line by immune interferon. *J. Immunol.* 131:135
105. Steeg, P. S., Moore, R. N., Johnson, H. M., Oppenheim, J. J. 1982. Regulation of murine Ia antigen expression by a lymphokine with immune interferon activity. *J. Exp. Med.* 156:1780
106. Basham, T. Y., Merigan, T. C. 1983. Recombinant interferon-gamma increases HLA-DR synthesis and expression. *J. Immunol.* 130:1492
107. Zlotnick, A., Shimonkevitz, R. P., Geffer, M. L., Kappler, J., Marrack, P. 1983. Characterization of the gamma-interferon–mediated induction of antigen-presenting ability in P388D1 cells. *J. Immunol.* 131:2814
108. Beller, D. I. 1984. Functional significance of the regulation of macrophage Ia expression. *Eur. J. Immunol.* In press
109. Stern, A. C., Erb, P., Gislar, R. H. 1979. Ia-bearing bone marrow cultured macrophages induce antigen-specific helper T cells for antibody synthesis. *J. Immunol.* 123:612
110. Erb, P., Stern, A. C., Alkan, S. S., Studer, S., Zoumbov, E., Gisler, R. H. 1980. Characterization of accessory cells required for helper T cell induction *in vitro:* evidence for a phagocytic, Fc receptor, and Ia-bearing cell type. *J. Immunol.* 125:2504
111. Lee, K.-C., Wong, M. 1980. Functional heterogeneity of culture-grown bone marrow–derived macrophages. *J. Immunol.* 125:86
112. Calamai, E. G., Beller, D. I., Unanue, E. R. 1982. Regulation of macrophage populations. IV. Modulation of Ia expression in bone marrow–derived macrophages. *J. Immunol.* 128:1692
113. Lee, K.-C., Wong, M. 1982. Functional heterogeneity of culture-grown bone marrow–derived macrophages. II. Lymphokine stimulation of antigen-presenting function. *J. Immunol.* 128:2487
114. Albrechtsen, D. 1977. HLA-D-associated "Ia-like" antigens on human macrophages. *Scand. J. Immunol.* 6:907
115. Smith, B. R., Ault, K. A. 1981. Increase of surface Ia-like antigen expression on human monocytes independent of antigen stimuli. *J. Immunol.* 127:2020
116. Yen, A. W., Parmely, J. J. 1981. Modulation of Ia-like antigen expression and antigen-presenting activity of human monocytes by endotoxin and zymosan. *J. Immunol.* 127:2245
117. Snyder, D. S., Beller, D. I., Unanue, E. R. 1982. Prostaglandins modulate macrophage Ia expression. *Nature* 299:163
118. Steeg, P. S., Johnson, H. M., Oppenheim, J. J. 1982. Regulation of murine macrophage Ia antigen expression by an immune interferon-like lymphokine: inhibitory effects of endotoxin. *J. Immunol.* 129:2402
119. Lu, C. Y., Changelian, P. S., Unanue, E. R. 1984. Alpha-fetoprotein inhibits macrophage expression of Ia antigen. *J. Immunol.* In press
120. Snyder, D. D., Unanue, E. R. 1982. Corticosteroids inhibit murine macrophage Ia expression and interleukin-1 production. *J. Immunol.* 129:1803
121. Lu, C. Y., Calamai, E. G., Unanue, E. R. 1979. A defect in the antigen-presenting function of macrophages from neonatal mice. *Nature* 282:327

Annu. Rev. Immunol. 1984.2:395-428. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

122. Nadler, P. I., Klingenstein, R. J., Hodes, R. J. 1980. Ontogeny of murine accessory cells: Ia antigen expression and accessory cell function in *in vitro* primary antibody responses. *J. Immunol.* 125:914
123. Lu, C. Y., Beller, D. I., Unanue, E. R. 1980. During ontogeny, Ia-bearing accessory cells are found early in the thymus but late in the spleen. *Proc. Natl. Acad. Sci. USA* 77:1597
124. Snyder, D. S., Lu, C. Y., Unanue, E. R. 1982. Control of macrophage Ia expression in neonatal mice—role of a splenic suppressor cell. *J. Immunol.* 128:1458
125. Murgita, R. A., Tomasi, T. B., Jr. 1975. Suppression of the immune response by alpha-fetoprotein. I. The effects of mouse alpha-fetoprotein on the primary and secondary antibody response. *J. Exp. Med.* 141:269
126. Peck, A. B., Murgita, R. A., Wigzell, H. 1982. Cellular and genetic restrictions in the immunoregulatory activity of alpha-fetoprotein. III. Role of the MLC-stimulating cell population in alpha-fetoprotein-induced suppression of T cell–mediated cytotoxicity. *J. Immunol.* 128:1134
127. Unanue, E. R., Beller, D. I., Lu, C. Y., Allen, P. M. 1984. Antigen presentation: comments on its regulation and mechanisms. *J. Immunol.* 132:1
128. Walker, E. B., Lanier, L. L., Warner, N. L. 1982. Concomitant induction of the self-surface expression of Ia determinants and accessory cell function by a murine macrophage tumor cell line. *J. Exp. Med.* 155:629
129. Birmingham, J. R., Chesnut, R. W., Kappler, J. W., Marrack, P., Kubo, R., Grey, H. M. 1982. Antigen presentation to two cell hybridomas by a macrophage cell line: an inducible function. *J. Immunol.* 128:1491
130. Meltzer, M. S., Benjamin, W. R., Farrar, J. J. 1982. Macrophage activation for tumor cytotoxicity: induction of macrophage tumoricidal activity by lymphokines from EL-4, a continuous T cell line. *J. Immunol.* 129:2802
131. Schreiber, R. D., Pace, J. L., Russell, S. W., Altman, A., Katz, D. H. 1983. Macrophage-activating factor produced by a T cell hybridoma: physiocochemical and biosynthetic resemblance to gamma-interferon. *J. Immunol.* 131:826
132. Blumenthal, E. J., Roberts, A. K., Vasil, A., Talmage, D. W. 1983. Macrophage activation: dissociation of cytotoxic activity from Ia-A antigen expression. *Proc. Natl. Acad. Sci. USA* 80:2031
133. Gery, I., Gershon, R. K., Waksman, B. H. 1972. Potentiation of T lymphocyte responses to mitogens. I. The responding cell. *J. Exp. Med.* 136:128
134. Beller, D. I., Unanue, E. R. 1982. Reciprocal regulation of macrophage and T cell function by way of soluble mediators. *Lymphokines* 6:25
135. Mizel, S. B. 1982. Interleukin-1 and T cell activation. *Immunol. Rev.* 63:51
136. Unanue, E. R., Kiely, J.-M., Calderon, J. 1976. The modulation of lymphocyte functions by molecules secreted by macrophages. II. Conditions leading to increased secretion. *J. Exp. Med.* 144:155
137. Farr, A. G., Dorf, M. E., Unanue, E. R. 1977. Secretion of mediators following T lymphocyte-macrophage interaction is regulated by the major histocompatibility complex. *Proc. Natl. Acad. Sci. USA* 74:3452
138. Meltzer, M. S., Oppenheim, J. J. 1977. Bidirectional amplification of macrophage-lymphocyte interactions: enhanced lymphocyte activation factor production by activated adherent mouse peritoneal cells. *J. Immunol.* 118:77
139. Mizel, S. B., Oppenheim, J. J., Rosenstreich, D. L. 1978. Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line, P388D1. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* 120:1497
140. Kammer, G. M., Unanue, E. R. 1980. Accessory cell requirement in the proliferative response of T lymphocytes to hemocyanin. *Clin. Immunol. Immunopathol.* 15:434
141. Press, J. L., Klinman, N. R., McDevitt, H. O. 1976. Expression of Ia antigens on hapten-specific B cells. I. Delineation of B cell subpopulations. *J. Exp. Med.* 144:414
142. Greenstein, J. L., Lord, E. L., Horan, P., Kappler, J. W., Marrack, P. 1981. Functional subsets of B cells defined by quantitative differences in surface I-A. *J. Immunol.* 126:2419
143. Mond, J. J., Kessler, S., Findelman, F. D., Paul, W. E., Scher, I. 1980. Heterogeneity of Ia expression on normal B cells, neonatal B cells, and on cells from B cell-defective CBA/N mice. *J. Immunol.* 124:1675
144. Mond, J. J., Seghal, E., Kung, H., Finkelman, F. D. 1981. Increased expression of I-region-associated antigen (Ia) on B cells after cross-linking of surface immunoglobulin. *J. Immunol.* 127:881
145. Monroe, J., Cambier, J. 1983. Level of mIa expression on mitogen-stimulated

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murine B lymphocytes is dependent on protein in cell cycle. *J. Immunol.* 130:626

146. Chesnut, R. W., Grey, H. M. 1981. Studies on the capacity of B cells to serve as antigen-presenting cells. *J. Immunol.* 126:1075
147. Chesnut, R. W., Colon, S., Grey, H. M. 1982. Antigen presentation by normal B cells, B cell tumors, and macrophages: functional and biochemical comparison. *J. Immunol.* 128:1764
148. McKean, D. J., Infante, A. J., Nilson, A., Kimoto, M., Fathman, C. G., Walker, E., Warner, N. L. 1981. Major histocompatibility complex-restricted antigen presentation to antigen-reactive T cells by B lymphocyte tumor cells. *J. Exp. Med.* 154:1419
149. Glimcher, L. H., Kim, K. J., Green, I., Paul, W. E. 1982. Ia antigen-bearing B cell tumor lines can present protein antigen and alloantigen in a major histocompatibility complex-restricted fashion to antigen-reactive T cells. *J. Exp. Med.* 155:445
150. Kappler, J., White, J., Wegmann, D., Mustain, E., Marrack, P. 1982. Antigen presentation by Ia-positive B cell hybridomas to H-2-restricted T cell hybridomas. *Proc. Natl. Acad. Sci. USA* 79:3604
151. Walker, E., Warner, N. L., Chesnut, R. W., Kappler, J., Marrack, P. 1982. Antigen-specific I-region interactions *in vitro* between tumor cell lines and T cell hybridomas. *J. Immunol.* 128:2164
152. Engers, H. D., Unanue, E. R. 1973. The fate of anti-Ig-surface Ig complexes on B lymphocytes. *J. Immunol.* 110:465
153. Rowden, G., Lewis, M. G., Sullivan, A. K. 1977. Ia antigen expression on human epidermal Langerhans cells. *Nature* 268:247
154. Klarskog, L., Malmnas-Tjernlund, U., Forsum, U., Peterson, P. A. 1977. Epidermal Langerhans cells express Ia antigens. *Nature* 268:248
155. Stingl, G., Katz, S. I., Shevach, E. M., Wolff-Schreiner, E. C., Green, I. 1978. Detection of Ia antigens on Langerhans cells in guinea pig skin. *J. Immunol.* 120:570
156. Stingl, G., Katz, S. I., Clement, L., Green, I., Shevach, E. M. 1978. Immunological functions of Ia-bearing epidermal Langerhans cells. *J. Immunol.* 121:2005
157. Tamaki, K., Stingl, G., Gullino, M., Sachs, D. H., Katz, S. I. 1979. Ia antigens in mouse skin are predominantly expressed as Langerhans cells. *J. Immunol.* 123:784
158. Steinman, R. M., Kaplan, G., Witmer, M. D., Cohn, Z. A. 1979. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance *in vitro*. *J. Exp. Med.* 149:1
159. Toews, G. B., Bergstresser, P. D., Streilein, J. W. 1980. Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *J. Immunol.* 124:445
160. Steinman, R. M., Witmer, M. G. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed lymphocyte reaction in mice. *Proc. Natl. Acad. Sci. USA* 75:5132
161. Nussenzweig, M. C., Steinman, R. M. 1980. Contribution of dendritic cells to stimulation of the murine syngeneic mixed leukocyte reaction. *J. Exp. Med.* 151:1196
162. Van Voorhis, W. E., Valinsky, J., Hoffman, E., Luban, J., Hair, L. S., Steinman, R. M. 1983. The relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. *J. Exp. Med.* 158:174
163. Sunshine, G. H., Katz, D. R., Feldmann, M. 1980. Dendritic cells induce T cell proliferation to synthetic antigens under Ir gene control. *J. Exp. Med.* 152:1817
164. Klingert, W. E. F., LaBaglie, J. H., Bowers, W. E. 1982. Accessory and stimulating properties of dendritic cells and macrophages isolated from various rat tissues. *J. Exp. Med.* 156:1
165. Lee, K.-C., Guidos, C., Wong, M., Spitzer, D. 1983. Differential activities of dendritic cells and macrophages in immune stimulation. In *Intracellular Communication in Leukocyte Function*, ed. J. W. Parker, R. L. O'Brien, p. 295. Chichester: J. Wiley
166. Winchester, R. J., Ross, C. D., Jarowski, C. I., Wang, C. Y., Halper, J., Broxmeyer, H. E. 1977. Expression of Ia-like antigen molecules on human granulocytes during early phases of differentiation. *Proc. Natl. Acad. Sci. USA* 74:4012
167. Wiman, K., Curman, B., Forsum, U., Klareskog, L., Malmnas-Tjernlund, U., Rask, L., Tragardh, L., Peterson, P. A. 1978. Occurrence of Ia antigens on tissues of non-lymphoid origin. *Nature* 276:711
168. Scott, H., Solheim, B. G., Brandtzaeg, R., Thorsby, E. 1980. HLA-DR-like antigens in the epithelium of the human small intestine. *Scand. J. Immunol.* 12:77
169. Klareskog, L., Forsum, U., Peterson, P. A. 1980. Hormonal regulation of the

Annual Reviews

expression of Ia antigens on mammary gland epithelium. *Eur. J. Immunol.* 10:958

170. Hart, D. N. J., Fabre, J. W. 1981. Endogenously produced Ia antigens within cells of convoluted tubules of rat kidney. *J. Immunol.* 126:2109
171. Pujol-Borrell, R., Hanafusa, T., Chiorato, L., Bottazzo, G. F. 1983. Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. *Nature* 304:71
172. Pober, J. S., Collins, T., Gimbrone, M. A., Jr., Cotran, R. S., Gitlin, J. D., Fiers, W., Clayberger, C., Krensky, A. M., Burakoff, J. S., Reiss, C. S. 1983. Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature* 305:726
173. Hirschberg, H., Braathen, L. R., Thorsby, E. 1982. Antigen presentation by vascular endothelial cells and epidermal Langerhans cells: the role of HLA-DR. *Immunol. Rev.* 66:57
174. Pober, J. S., Gimbrone, M. A. Jr., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W., Ault, K. A. 1983. Ia expression by vascular endothelium is inducible by activated T cells and by human gamma-interferon. *J. Exp. Med.* 157:1339
175. Hanafusa, T., Pujol-Borrell, R., Chiorato, L., Russell, R. C. G., Coniach, D., Bottazzo, G. F. 1983. Aberrant expression of HLA-DR antigen on thymocytes in Grave's disease: relevance for autoimmunity. *Lancet* 2:1111
176. Lampert, I. A., Suitters, A. J., Chisholm, P. M. 1981. Expression of Ia antigen on epidermal keratinocytes in graft-versus-host disease. *Nature* 293:149
177. Mason, D. W., Dullman, M., Barclay, A. N. 1981. Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. *Nature* 293:150
178. Barclay, A. N., Mason, D. W. 1982. Induction of Ia antigen in rat epidermal cells and gut epithelium by immunological stimuli. *J. Exp. Med.* 156:1665
179. Kelley, V. E., Roths, J. B. 1982. Increase in macrophage Ia expression on autoimmune mice: role of the *lpr* gene. *J. Immunol.* 129:923
180. Lu, C. Y., Unanue, E. R. 1982. Spontaneous T cell lymphokine production and enhanced macrophage Ia expression and tumoricidal activity in MRL-*lpr* mice. *Clin. Immunol. Immunopathol.* 25:213
181. Bottazzo, G. F., Pujol-Borrell, R., Hanafusa, T., Feldmann, M. 1983. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* 2:1115

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1

THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23

THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51

THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67

TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103

SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127

REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka* 159

TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183

COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199

NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219

TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239

CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257

THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283

INTERLEUKIN 2, *Kendall A. Smith* 319

THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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IMMUNOBIOLOGY OF EOSINOPHILS

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INTRODUCTION

The eosinophilic leukocyte was discovered by Paul Ehrlich in 1879 when he stained fixed blood smears with aniline dyes (1). By the early part of this century a voluminous literature on eosinophils had accumulated and the association of blood eosinophilia with helminth infection and bronchial asthma was established. Later, tissue eosinophilia was shown following cutaneous allergic reactions (2). However, only in the past decade has the striking ability of the eosinophil to kill helminths been shown. This observation has raised the possibility that the same toxic properties of the eosinophil, beneficial when focused on a helminth invader, might be turned against the host during hypersensitivity diseases. Finally, new information regarding eosinophil granule proteins has been obtained and the role of these proteins in eosinophil function has been investigated.

In this review we summarize present knowledge of the eosinophil with emphasis on its granule proteins and on its likely role in helminth infection and hypersensitivity diseases.

EOSINOPHIL-ASSOCIATED PROTEINS

The granules of the eosinophil are distinctive, first in that they stain avidly with acid dyes and second in their characteristic appearance in the electron microscope. Three types of eosinophil granules are recognized. Primary granules are round, uniformly electron-dense, and characteristic of eosinophilic promyelocytes (3). During differentiation, these granules develop cores and are called secondary or specific granules. The secondary or specific granules

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Annual Reviews

(Figure 1) consist of an electron-dense core and an electron-radiolucent matrix (4). Peroxidase activity is localized to the granule matrix, whereas the granule core shows a periodicity in both longitudinal and cross-sectional dimensions and, therefore, has been termed a crystalloid. A third type of granule, the small granule, is reported to contain acid phosphatase and arylsulfatase (5).

Because of the distinctive nature of the secondary or crystalloid-containing granule, it has been assumed that investigation of the properties of its contents would aid in understanding the functions of the eosinophil. Therefore, we discuss in detail the properties of the presently recognized eosinophil granule–associated proteins.

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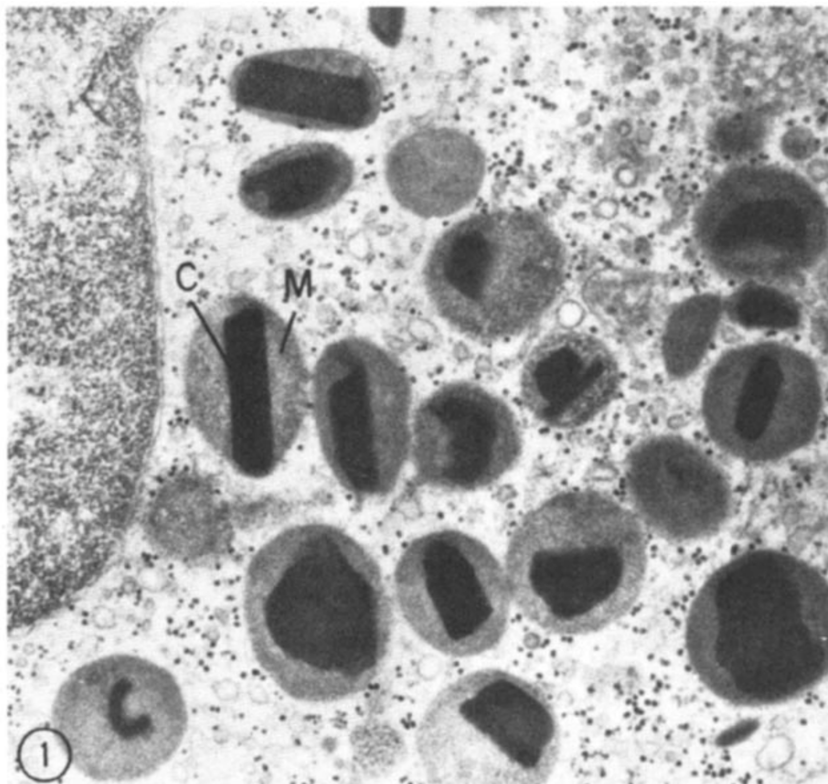

Figure 1 Electron photomicrograph of specific granules in a human eosinophil leukocyte showing dense cores (C) of various shapes embedded in a less dense matrix (M). (× 28,000). [From (4) with permission]

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Major Basic Protein

Studies of isolated eosinophil granules revealed the presence of a major basic protein (MBP) identified by polyacrylamide gel electrophoresis (PAGE) of solubilized guinea-pig eosinophil granules (6). Guinea-pig MBP had a molecular weight of 11,000 in sodium dodecyl sulfate (SDS) PAGE, accounted for 53.2 ± 3.9 ($\bar{X} \pm$ SGM) of the granule protein, and lacked peroxidase activity. Its pI was too high to measure because most of the protein applied to the focusing gel migrated into the alkaline wick. MBP was purified from acid-solubilized guinea-pig eosinophil granules by gel filtration on Sephadex G-50 (7). Biochemical analyses of MBP were hampered by a propensity of the molecule to aggregate. Indeed, simply concentrating dilute solutions or exposing solutions to air resulted in aggregation as judged by the appearance of extra bands in SDS-PAGE. These extra bands were multiples of the 11,000-dalton monomer, and they were depolymerized by exposure to disulfide bond–reducing agents. When freshly isolated MBP was alkylated with iodoacetamide, polymerization did not occur. The physicochemical properties of MBP are shown in Table 1. The presence of tryptophan indicates that MBP is not a histone-like protein.

Subsequently, an MBP-like molecule was found in human and rat eosinophils (8,9). Amino acid compositions showed a high content of arginine in both molecules. Human MBP had a molecular weight of 9,300 and rat MBP, a molecular weight of 10,000–12,000. Analyses of the immunochemical relationship of MBP molecules showed little cross-reactivity (10). Measurement of guinea-pig MBP by double antibody radioimmunoassay was achieved by using a buffer containing protamine to reduce nonspecific binding of ^{131}I-MBP (11). MBP antigenic determinants survived reduction and alkylation in the absence of denaturing solvents; in the presence of 6M guanidinium hydrochloride, reduction and alkylation destroyed MBP immunoreactivity. Exposure to 6M guanidinium hydrochloride in the absence of reducing agents caused a 50% reduction in immunoreactivity. Polymerized MBP was only one-tenth as

Table 1 Physicochemical properties of guinea-pig MBP

Isoelectric point, pH 10 or greater
Aggregates to disulfide-linked polymers
Molecular weight 11,000
Extinction coefficient at 277 nm, $E_{1cm}^{1\%} = 26.7$
Contains six half-cystine residues and two reactive sulfhydryl groups
Contains 13% arginine and 4% tryptophan

Annual Reviews

reactive as monomer MBP, illustrating the need for reduction and alkylation of the molecule before measurement by radioimmunoassay.

LOCALIZATION OF MBP IN THE EOSINOPHIL GRANULE CORE Localization of MBP was pursued by immunoelectron microscopy and by isolation of granule crystalloids (12). Using immunoperoxidase electron microscopy, we found antibodies to MBP bound to the crystalloid core of the granule. However, this result was limited in that one does not know whether MBP is the only protein present in the core. SDS-PAGE analyses of protein derived from solubilized crystalloids showed a band in the expected position for MBP, and quantitative scanning of these gels showed that 79–91% of the dye staining was associated with the MBP band. Comparison of the immunochemical reactivity of core protein and authentic MBP showed that the radioimmunoassay inhibition curves were essentially identical. Thus, core protein could not be immunochemically distinguished from MBP, and essentially all of the protein in the core could be accounted for as MBP. Finally, radiolabeled core protein reacted with antibody to MBP; and the extinction coefficients ($E^{1\%}_{1cm}$) of core protein, 26.7, and MBP, 26.8 ± 1 ($\bar{X} \pm$ SEM), were very similar. These results support the conclusion that the core of the eosinophil granule is a crystal of MBP.

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LOCALIZATION OF IMMUNOREACTIVE MBP IN OTHER CELLS Studies of the Charcot-Leyden crystal (CLC) protein (see below) indicated that it is present in human basophils (13), which raised the possibility that MBP might also be present in basophils. To test this hypothesis, basophil-containing mononuclear cell preparations were stained with fluorescein-conjugated antihuman IgE antibody, and basophils were purified using fluorescence-activated cell sorter (FACS) to sort brightly fluorescent cells (14). With this approach it was found that (*a*) enrichment for surface IgE-positive cells (of which greater than 95% were basophils) also enriched for cells staining for MBP by immunofluorescence, (*b*) MBP appeared to be localized in the granules of the basophils as judged by the punctate staining of the cytoplasm, (*c*) MBP was measurable in extracts of basophils by radioimmunoassay, and (*d*) slopes of the dose-response curves for the basophil extracts were not statistically different from those of eosinophil MBP. The MBP content of normal basophils averaged 140 ng per 10^6 cells whereas normal eosinophils contained 5000 ng per 10^6 cells. MBP was also detected by immunofluorescence and radioimmunoassay in cells from a patient with basophil leukemia. This result along with the finding that CLC protein is present in basophils (13) indicates certain biochemical similarities between eosinophils and basophils. Furthermore, studies of granulocyte colonies from human peripheral blood indicate that "eosinophil-type" colonies may contain both MBP and histamine (15, 16). Taken together, these results suggest the existence of a common precursor for eosinophils and basophils.

Annual Reviews

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The HL-60 cell line, derived from a patient with acute promyelocytic leukemia (17), can be induced to mature to cells with many functional, morphological, and biochemical features of neutrophils or macrophages (18, 19). Two reports suggest that HL-60 cells can also differentiate to eosinophils (20, 21). Fischkoff et al found that culture of HL-60 cells at alkaline pH for one week resulted in differentiation to eosinophils (S. A. Fischkoff, A. Pollack, G. J. Gleich, J. R. Testa, S. Misawa, T. Reber, unpublished observations). Immunofluorescent staining for MBP of unstimulated HL-60 cells showed that 80–85% were positive, many with bright cytoplasmic fluorescence. Culture of HL-60 cells with dimethylsulfoxide caused differentiation to neutrophils (18); this was associated with a loss of staining for MBP. In contrast, cells stimulated by culture at pH 7.8 retained MBP staining and up to 30% resembled eosinophils when stained with Wright-Giemsa stain. These results suggest that HL-60 cells may be well-suited for study of eosinophilopoiesis and that they constitutively produce MBP.

Finally, as will be discussed below in more detail, MBP is elevated in sera of pregnant women (22), and recent studies indicate that MBP is localized to placental X-cells and placental giant cells (D. E. Maddox, G. M. Kephart, C. B. Coulam, J. H. Butterfield, K. Benirschke, G. J. Gleich, submitted for publication).

FUNCTIONS OF MBP Initial studies of MBP function (7) did not provide many clues. MBP did not increase vascular permeability and had only weak antibacterial activity. It precipitated deoxyribonucleic acid, neutralized the activity of heparin, and activated papain, the latter presumably through the activity of the two reactive sulfhydryl groups; however, all of these activities were predictable based on the physicochemical properties of MBP.

Insights into the potential functions of MBP came about as a consequence of studies of its effect on schistosomules of *Schistosoma mansoni* (23). These studies showed that MBP as well as certain other basic proteins, including protamine, damaged schistosomules. MBP bound to the membrane of the schistosomules and caused its disruption. In these experiments MBP stabilized by alkylation was tested; more recent experiments indicate that the toxicity of native and alkylated MBP for schistosomules is similar (24). When eosinophils in the presence of antibody attacked the schistosomule, they deposited MBP on the surface of the parasite, and increased concentrations of MBP could be detected in culture supernatants. Subsequently, eosinophils but not neutrophils were shown to adhere irreversibly to schistosomules in the presence of antibody (25). MBP and protamine enhanced the adherence of both eosinophils and neutrophils to the schistosomules (26). Concanavalin A also caused adherence of eosinophils to schistosomules, and this adherence could be made irreversible and converted into an attack on the parasite by addition of the

Annual Reviews

calcium ionophore, A23187. Under these conditions, MBP was released into the culture supernatants. These studies indicate that the irreversible adherence of eosinophils to schistosomules is associated with degranulation and release of MBP. Additional studies on the effect of MBP on newborn larvae of *Trichinella spiralis* showed that all larvae cultured with MBP at concentrations of 5×10^{-5}M or higher were rendered immobile (27). The effect of MBP was detected as early as two hours after treatment when the larvae became progressively stiffened and sluggish. Controls, consisting of other basic proteins, were negative save for polyarginine, which killed 20% of larvae in 24 hr. MBP also killed the bloodstream trypomastigote stage of *Trypanosoma cruzi*; this effect was inhibited by heparin, by antibody to MBP, but not by normal rabbit serum; and the toxic effect of MBP was destroyed by heating at 56°C for 30 min (28). The antigenic activity of MBP is also abolished by heating at 56°C for 30 min (11, 29).

Because MBP was toxic to schistosomules, its capacity to damage mammalian cells was tested. Initial experiments showed that MBP damaged two varieties of murine ascites tumor cells (23). This observation raised the possibility that MBP might damage other cells, and, as discussed below, further work has suggested that the eosinophil causes damage during hypersensitivity reactions.

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Eosinophil Peroxidase (EPO)

As noted above, EPO is localized in the granule matrix of numerous species, including laboratory animals (30-32) and humans (33). The intensity of peroxidase staining is so great that it can be used to enumerate eosinophils, as in the automated continuous-flow cytochemistry devices used to perform differential counts. Interestingly, the eosinophils of many members of the cat family including the lion, the tiger, and the domestic cat do not contain peroxidase (34).

PHYSICOCHEMICAL PROPERTIES OF EPO Partially purified EPO showed spectral differences from neutrophil myeloperoxidase and a Soret maximum of 40? nm for oxidized EPO and 437 nm for reduced EPO (35). Guinea-pig EPO, purified from marrow cells by detergent extraction, ion exchange chromatography, and gel filtration (36), was homogeneous by immunodiffusion and existed as a monomer of 75,000 daltons and a dimer of 150,000 daltons. Both monomer and dimer had the same specific activity, the same absorption spectrum, and a Soret maximum at 425 nm, and the same ratio of absorbance at 415 nm and 280 nm. EPO evidently consists of a single polypeptide chain as indicated by a single band in SDS-PAGE.

Annual Reviews

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FUNCTIONS OF EPO EPO catalyzes the oxidation of many substances by hydrogen peroxide. Klebanoff showed that myeloperoxidase (MPO) plus hydrogen peroxide and free iodide can iodinate and kill bacteria and that the activity of this system is increased in phagocytizing neutrophils (37). The combination of MPO, H_2O_2 and halide also will kill viruses (38), mycoplasma (39), and fungi (40). Because eosinophils generate considerable H_2O_2 (41-43), the activities of the EPO + H_2O_2 + halide system in the killing of microorganisms have been explored. Migler et al found that eosinophil extracts killed *Staphylococcus aureus* and *Escherichia coli*, but only in the presence of iodide, not chloride (44). Using purified EPO, Jong et al showed that EPO could kill *E. coli* in the presence of H_2O_2 and iodide as well as chloride and bromide (45). They attributed the difference between their results and those of Migler et al to the presence of gelatin in the reaction mixture; Jong et al showed that gelatin and albumin inhibit killing of *E. coli* by the EPO system. In additional studies EPO in the presence of H_2O_2 and halide enhanced the killing of schistosomula (46), toxoplasma (47), trypanosoma (48), mast cells (49), and tumor cells (50). It is clear from these studies that this system can mediate toxicity to numerous targets.

Subsequently, Henderson et al showed that EPO binds to mast cells and that the EPO–mast cell complex retains the ability to catalyze iodination of proteins and killing of microorganisms (51). They suggested that such complexes could form extracellularly and affect the inflammatory response. In an interesting extension of this line of work, Henderson et al (49) showed that EPO supplemented by H_2O_2 and halide induced mast cell degranulation and histamine release. At low EPO concentrations this reaction was noncytotoxic, but at higher concentrations ultrastructural evidence of mast-cell demage was seen. The EPO–mast cell granule complex was more effective than free EPO in stimulating mast-cell secretion. Henderson and co-workers proposed that eosinophils, either by secretion or by cell lysis, release EPO; EPO in the presence of H_2O_2 (generated by eosinophils or other phagocytes in the area), chloride, and iodide initiates mast-cell secretion. At pH 7.4, EPO was active in the presence of physiological concentrations of chloride plus iodide at 10^{-6}M; the iodide concentration is above that present in extracellular fluid. Thus, these findings suggest a role for the EPO + H_2O_2 + halide system in the inflammatory response. Whether the proposed reactions occur in vivo will require further study. The possibility that EPO plays a role in hypersensitivity reactions is strengthened by observations showing leakage of EPO from granules into cytoplasm and apparent extracellular release following allergen provocation of human nasal mucous membranes (52).

Subsequent studies of the EPO + H_2O_2 + halide system indicate that EPO binds to microbes such as *S. aureus* (52), *Toxoplasma gondii* (47), and *T. cruzi*

Annual Reviews

(48) and markedly potentiates their killing by mononuclear phagocytes. Tumor cells also adsorb EPO, and this potentiates their lysis by H_2O_2 (54). Moreover, EPO-coated tumor cells are spontaneously lysed by macrophages; this damage is inhibited by catalase (indicating a need for H_2O_2) and by azide (indicating a need for enzymically active EPO). These results point to a synergistic action between the cytophilic cationic EPO and H_2O_2, spontaneously released from macrophages, causing tumor-cell destruction (54).

Eosinophil Cationic Proteins (ECP)

Analyses by Olsson & Venge of cationic proteins of human leukemic myeloid cells showed seven cationic protein components (55). In their experiments leukocytes obtained from patients with myeloid leukemia were disrupted, the granule fraction was obtained by centrifugation, and granules were extracted at pH 4. Granule proteins were separated by gel filtration, preparative electrophoresis, and ion-exchange chromatography, and their physicochemical and immunological properties were compared. Four of the components formed a group with molecular weights of 25,500–28,500 and with similar amino acid compositions. The remaining three components had molecular weights from 21,000–29,000 and showed immunologic identity; the amino acid composition of component 5 differed greatly from those of components 1, 2, and 3. Subsequent investigations showed that components 5, 6, and 7 were derived from eosinophils (56), and they have been termed eosinophil cationic proteins (ECP) (57).

PHYSICOCHEMICAL PROPERTIES OF ECP The isoelectric points of ECP are greater than pH 11 and they contain 11% arginine and 10 half-cystines. Component 5 has a molecular weight of 21,000 and is composed of a single polypeptide chain as determined by SDS-PAGE (57). Of interest is the presence of 2.5 moles of zinc per mole of protein (56). Although ECP forms aggregates, these are depolymerized by detergents, indicating that they are not formed by covalent bonds as is the case with MBP. Finally, ECP accounts for approximately 30% of the weight of the granule's proteins.

The physicochemical properties described above seem to differentiate MBP from ECP, and recently, in an exchange of reagents with Venge & Olsson we have found that the proteins are immunologically distinct (58). Moreover MBP and ECP elute from Sephadex G-50 at different volumes, as shown in Figure 2; ECP emerges in the valley between the second and third peaks. ECP has not been localized within the eosinophil granule; the finding that MBP constitutes the crystalline core points to the granule matrix as the likely source of ECP. Although the term ECP was initially used to refer to a family of proteins, components 5–7, evidently component 5 is the most abundant of these; and ECP, as used hereafter, refers to that component.

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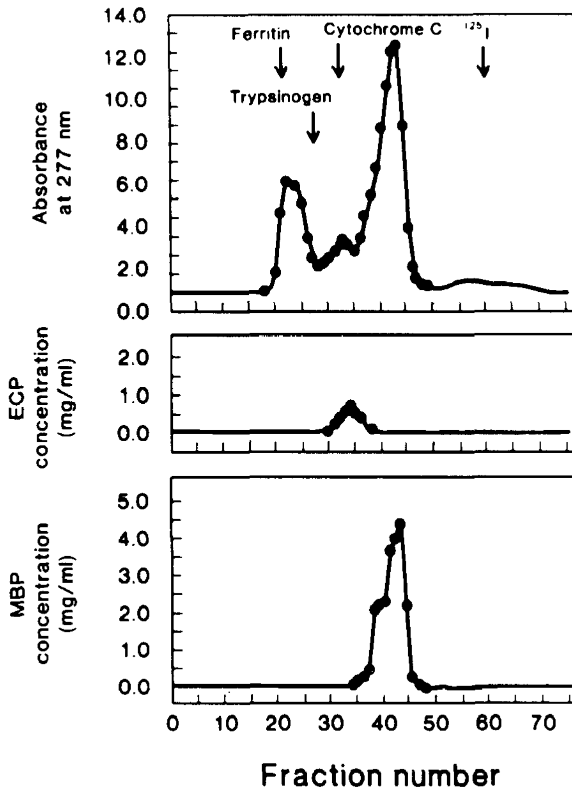

Figure 2 MBP and ECP levels by radioimmunoassay of Sephadex G-50 column fractions of acid-solubilized eosinophil granules. Molecular weight markers include ferritin (750,000), trypsinogen (24,000), cytochrome C (12,400) and ^{125}I. The upper panel shows the absorbance profile at 277 nm, indicating the three peaks characteristically seen when eosinophil granules are fractionated on Sephadex G-50. The first peak contains the enzymes including eosinophil peroxidase. The second peak contains EDN and ECP, and the third peak contains MBP. The middle panel shows the quantity of ECP in individual column fractions as determined by radioimmunoassay, and the lower panel shows the quantity of MBP in individual columns as determined by radioimmunoassay. [From (58) with permission]

FUNCTIONS OF ECP ECP did not possess bactericidal or esterolytic activity; it did not contract the guinea-pig ileum, nor did it have any inhibitory effect on histamine-induced or guinea-pig anaphylotoxin-induced contractions of the ileum (56). ECP bound to heparin and neutralized its anticoagulant activity (57). ECP shortened the coagulation time of normal plasma in a dose-dependent manner when incubated for 30 seconds; when the incubation with normal plasma was prolonged beyond 10 minutes, there was a marked lengthening of the clotting time (59). ECP shortened the recalcification time of plasmas defi-

Annual Reviews

cient in Factors V, VII, VIII, IX, X, and XI, but prolonged the recalcification time of Factor XII-deficient plasma. This suggests that Factor XII was the target of ECP interaction. Kallikrein activation, which is dependent on Factor XII, was also enhanced by ECP (59).

An effect of ECP on fibrinolysis is suggested by experiments showing that ECP enhanced hydrolysis of a plasmin-specific substrate by plasmin in a dose-related fashion (60). In contrast, plasminogen activation by streptokinase was abolished by ECP; this effect was presumably due to the formation of a precipitate between streptokinase and ECP. The enhancement of plasminogen activation by ECP was not associated with the formation of a complex between ECP and fibrinogen. Finally, ECP is reported to produce the Gordon phenomenon (see below) at very low dosages (0.1–0.3 μg) (61), amounts about 10–100-fold less than those needed for the eosinophil-derived neurotoxin (62).

Finally, as discussed more fully below, ECP is a potent toxin for schistosomules of *S. mansoni*.

The Eosinophil-Derived Neurotoxin (EDN)

Eosinophils contain a powerful neurotoxin that can severely damage myelinated neurons in experimental animals (63-65). M. H. Gordon first described this neurotoxic reaction in 1933 (63); it is now known as the "Gordon phenomenon" in his honor. Because patients with the idiopathic hypereosinophilic syndrome and cerebrospinal fluid eosinophilia exhibit varied neurologic abnormalities (66-71), EDN may play an important role in central nervous system disease in humans.

PHYSICOCHEMICAL PROPERTIES OF EDN Using the production of the Gordon phenomenon as an assay, certain of the properties of the EDN were determined (72). EDN was partially purified by ultracentrifugation of sonicated human eosinophils followed by fractionation of the supernatant on Sephadex G-50 columns at neutral pH. Fractions with neurotoxic activity eluted at an apparent molecular weight of approximately 15,000. The partially purified material withstood lyophilization and dialysis, but its neurotoxic activity was destroyed by heating at 90°C. In a subsequent study, extracts of whole human eosinophils and purified eosinophil granules were sequentially fractionated by gel filtration at acid and alkaline pH. Fractions were analyzed by SDS-PAGE and for their ability to produce the Gordon phenomenon by intrathecal injection into rabbits (62). In confirmation of prior studies, extracts of whole eosinophils possessed potent neurotoxic activity. Similarly, extracts of highly purified eosinophil granules were also active. Fractionation of eosinophil-granule extracts on Sephadex G-50 at pH 4.3 yielded three major peaks comparable to those shown in Figure 2. Eosinophil enzymes including peroxidase are included in peak 1 while MBP constitutes the protein in peak 3. Neither peak 1 nor peak 3 protein

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produced the Gordon phenomenon. Fractions comprising peak 2 were concentrated and fractionated on Sephadex G-50 at pH 7.4. A single peak resulted, and injection of 50 μg (assuming $E_{1cm}^{1\%}$ = 10.0 at 277 nm) caused the Gordon phenomenon. By SDS-PAGE the active material gave a single band with a molecular weight of 18,000. This component was purified from whole eosinophil extracts of two other patients and showed a single band by SDS-PAGE, and both preparations produced the Gordon phenomenon; EDN from these patients had molecular weights of 19,200 and 17,700, respectively. In these same studies purified CLC protein (lysophospholipase) and MBP did not produce the Gordon phenomenon. Recently, two granule-derived proteins, ECP and eosinophil protein X (EPX), were reported to cause the Gordon phenomenon (61); ECP was an exceedingly potent neurotoxin. This report speculated that EPX (73) may be identical to EDN (61); it raises the possibility that ECP accounts for the only neurotoxic activity, the activity reported for EPX and for EDN being due to contamination with ECP. Clearly, further studies are needed to determine whether there are two granule molecules with neurotoxic activity or only one.

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LOCALIZATION OF EDN Extracts of highly purified eosinophil granules produced the Gordon phenomenon (62); this result points to the eosinophil granule as the source of EDN. Because MBP forms the crystalloid, it seems likely that EDN is present in the granule matrix.

FUNCTIONS OF EDN The only function presently associated with EDN is its ability to provoke the Gordon phenomenon. When injected intrathecally into experimental animals, usually rabbits and guinea pigs, EDN produces a predictable syndrome that begins with stiffness, most pronounced in the forelimbs, and mild ataxia, followed by incoordination and severe ataxia such that the animals have difficulty remaining upright. The final phase of the Gordon phenomenon is characterized by severe weakness and muscle wasting (72). Some animals develop nystagmus and jerky, repetitive head movements. No evidence of neurologic abnormalities of higher level functions are observed; the animals remain alert and eat and drink in a normal fashion, provided food and water are placed within reach. The latent period between injection of purified EDN and the onset of neurologic manifestations ranged from 3 to 11 days (62).

The histopathologic changes produced in rabbits have been thoroughly described (72). On light microscopy, abnormalities are concentrated in the cerebellum, pons, and spinal cord. A hallmark of the Gordon phenomenon is the disappearance of Purkinje cells from the cerebellum. In addition, the white matter of the cerebellum, pons, and spinal cord shows a gross spongiform change; the grey matter remains essentially normal (72).

Annual Reviews

Charcot-Leyden Crystal Protein (CLC) (Lysophospholipase)

The Charcot-Leyden crystal was initially described in 1853 in a patient witl leukemia and later in 1872 in the sputa of patients with asthma. Since thei the appearance in tissues and body fluids of these hexagonal bipyramida crystals has been a hallmark of the eosinophil.

PHYSICOCHEMICAL PROPERTIES OF CLC PROTEIN Initial studies of CLC indi cated the presence of a low-molecular-weight protein (74,75) and suggeste that CLC formed from cytoplasm (76). In subsequent studies, purified CL(dissolved in acid or detergent and analyzed by SDS-PAGE gave a single ban that stained for protein (8). Similarly, CLC gave a single symmetrical peal when analyzed by gel filtration in 6M guanidinium hydrochloride after reduc tion and carboxymethylation. The molecular weight of CLC was 12,980 ± 23 ($\overline{X}$ ± SD; n = 5). The presence of a single band by SDS-PAGE and a singl peak by gel filtration in the presence of denaturing solvents indicated a singl polypeptide chain. CLC protein contained only 1.2% carbohydrate and had 280 nm $E^{1\%}_{1cm}$ = 12.0. Amino acid analyses showed 117–119 amino acids, i keeping with the molecular weight, with six residues of arginine, 18–19 o glutamic acid, and 12 of valine (8).

A subsequent study of the immunochemical properties of CLC was aide by the finding that slow freezing of eosinophil extracts markedly increased th yield of crystals (77). CLC were solubilized by exposure of the lyophilize crystals to slightly basic solutions; at pH 9 in borate-HCl such solutions con tained up to 868 μg/ml. SDS-PAGE analyses of these solutions showed single band with a molecular weight of 13,000 when CLC protein was reduce prior to electrophoresis, whereas nonreduced samples showed a minor ban at 26,000 daltons, presumably a dimer. This model is strengthened by th finding that CLC protein has one sulfhydryl group. Electrophoresis of CL(protein in PAGE (in the absence of SDS) at pH 8.9 yielded multiple bands reduction prior to analysis failed to eliminate this heterogeneity. Similarl electrofocusing of CLC protein showed two major and four minor bands witl isoelectric points between 5.7 and 5.1. Immunodiffusion analyses of CL(protein showed a single precipitin band. The materials in the major bands wer immunochemically identical (77) when analyzed by agar gel diffusion and b radioimmunoassay. This result indicated that the presence of multiple band by PAGE analysis was due to aggregation, carbohydrate heterogeneity, o possibly small variations in amino acid sequence rather than contaminatin proteins or markedly different types of CLC protein.

FUNCTIONS OF CLC PROTEIN CLC protein did not increase vascular permea bility as assessed in guinea pigs pre-injected with a blue dye, did not contrac

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the isolated guinea-pig ileum, and did not antagonize the effect of bradykinin or histamine (8). Earlier histochemical analyses had shown the prominent lysophospholipase activity in rat eosinophils (78); lysophospholipase activity was also shown by biochemical titration of liberated fatty acid from lysolecithin (79). Later, the finding of increased concentrations of lysophospholipase activity in eosinophils was confirmed (80); a mean activity of 79 units per 5×10^6 cells was present in eosinophils compared to 10 units per 5×10^6 neutrophils, 28 units per 5×10^6 mononuclear cells, and 0.4 units per 5×10^6 platelets. Subsequent work showed that lysophospholipase purified by gel filtration of sonicated eosinophils on Sephadex G-100, by organomercurial agarose chromatography, and by heparin-Sepharose chromatography formed crystals typical of CLC (81). The fall-through fractions from the heparin-Sepharose column were homogeneous by SDS-PAGE and by PAGE at pH 8.9. The molecular weight of lysophospholipase activity was $17{,}200 \pm 260$ ($\bar{X} \pm$ SGM; $n = 9$). When purified lysophospholipase activity was concentrated, crystals with a morphology identical to CLC formed (81). The cause of the difference between the molecular weight of CLC protein found earlier, 13,000 (8, 77), and that found for lysophospholipase, 17,200 (81), is not clear at this point. The value of 13,000 for the CLC protein (77) is consistent with the molecular weight determined by agarose column chromatography under highly denaturing conditions using 6M guanidinium hydrochloride (8).

The experiments discussed above indicate that lysophospholipase activity is associated with a protein that crystallizes in vitro to give CLC. Naturally occurring CLC derived from feces reacted with antisera to purified lysophospholipase by indirect immunofluorescence, and after purification fecal CLC yielded a single protein band that migrated identically to eosinophil lysophospholipase on SDS-PAGE (82). These data strongly support the proposal that CLC formed in vitro is the same as that occurring naturally. Finally, lysophospholipase (EC 3.1.1.5) catalyzes the inactivation of lysophospholipids by removal of a single fatty acid (81). Lysophospholipids are formed by the action of phospholipase A_2, which catalyzes the hydrolysis of fatty acid from the 2-position of phospholipids. Because arachidonic acid can be esterified to phospholipids at the 2-position, phospholipase A_2 can liberate arachidonic acid and thus provide substrate for the formation of cyclooxygenase and lysooxygenase products. Lysophospholipids formed as a consequence of phospholipase A_2 activity are potentially cytotoxic and can be neutralized by lysophospholipase, which deacylates at the 1-position.

LOCALIZATION OF CLC PROTEIN Several lines of evidence suggest that lysophospholipase (CLC protein) is localized in the plasma membrane of the eosinophil (80). For example, granule fractions showed no lysophospholipase activity, whereas activity was found in fractions containing magnesium-depen-

Annual Reviews

dent adenosine triphosphatase activity, a membrane marker. That lysophos pholipase activity was not free was shown by the observation that it could b sedimented at 100,000 *g*. Also, the lysophospholipase activity of intact eo sinophils was inhibited by cell-impermeant probes such as *p*-chloromercuri benzene sulfonate. As already noted, CLC protein is present not only in eo sinophils but also in basophils (13).

Other Eosinophil Proteins

Numerous enzymes have been associated with the eosinophil, including aci glycerophosphatase, adenosine triphosphatase, alpha-mannosidase, arylsul fatase, collagenase, beta-glucouronidase, ribonuclease, cathepsin, acid an alkaline phosphatase, histaminase, and phospholipase D (83-90). In addition a phospholipid exchange protein is reported as preferentially associated wit the eosinophil (80). The subunit composition of the arylsulfatase from eosi nophils has recently been reported (91).

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THE FUNCTIONS OF THE EOSINOPHIL

Helminth Infections

By the beginning of this century the striking association between helmint infection and peripheral blood eosinophilia had been established [for a concis summary see (92)]. However, it was not until the 1970s that evidence sup porting a role for the eosinophil in parasite damage was obtained. This evi dence consists of (*a*) experiments testing the effect of anti-eosinophil serur (AES) on immunity to helminths, (*b*) experiments testing the direct effect o eosinophils on helminths, and (*c*) demonstration of eosinophil infiltration abou and degranulation onto the parasite.

The effect of AES has been tested on animals infected with three hel minths—*S. mansoni* (93, 94), *T. spiralis* (95), *Trichostrongylus colubriformi* (96)—and a tick, *Amblyoma americanum* (97). In the case of *S. mansoni* mice were infected once by exposure to schistosomules, and a monospecifi antimouse eosinophil serum was administered before a subsequent challeng of schistosomula (93). The AES-treated animals had a marked reduction i the number of peripheral blood eosinophils and a concomitant loss of immunit as judged by the number of schistosomula in the lungs. A subsequent test c the effect of AES on the destruction of *S. mansoni* eggs suggested that ec sinophil depletion delayed egg destruction (94). The suppression of eg destruction resulted in a significant increase in the severity of liver disease a judged by an increased portal pressure and splenic weight. A test of the effec of AES on naive mice primarily infected with *T. spiralis* showed no reductio in the number of intestinal worms, whereas a marked reduction in the numbe of encysted muscle larvae was observed (95). This result suggested that th

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eosinophil acts on newborn larvae or muscle larvae but not on intestinal worms. In the case of *T. colubriformis*, guinea pigs were treated with AES to determine whether susceptibility and immunity to this intestinal parasite were altered (96). The results showed a significant increase in susceptibility and a reduction in immunity. Finally, analyses of the effect of AES and anti-basophil serum (ABS) on infection with the tick *A. americanum* indicated that ABS completely abolished and AES significantly reduced immunity to the tick (97). Both antisera had the expected effect on their respective targets in peripheral blood. However, in tissue from tick feeding sites, whereas AES reduced only the number of eosinophils, ABS reduced both basophils and eosinophils. These results point to a role for the basophil in tick immunity and to a cooperation between these cells in that immunity. In this cooperation basophils presumably attract eosinophils to the tissue sites and both participate in the attack on the tick.

Concerning the ability of eosinophils to damage parasites, numerous studies have comfirmed the first reports (98,99) on this phenomenon showing that schistosomules coated with IgG antibody bind eosinophils that attack the parasite (100). In this system the damage to the schistosomule is correlated with the number of eosinophils; neutrophils are not active (101). Moreover, the attack on the parasite is associated with degranulation as shown by the concomitant release of MBP (23). Eosinophil-mediated damage to the schistosomule can also be produced by the use of Concanavalin A to bind eosinophils and calcium ionophore to cause their degranulation (26). The ability of the eosinophil to damage schistosomules has been confirmed by others using rat (102, 103) and mouse (104) eosinophils. Certain of these experiments have pointed to a synergy between eosinophils and mast cell–derived mediators in the damage to the schistosomules (103, 105). In rats, C3 bound to schistosomules mediates eosinophil adherence to the schistosomule (102,106), and activation of complement by the classical pathway enhances the antibody-dependent action of human eosinophils (107). Eosinophils also damage a variety of other antibody-coated targets, including newborn larvae of *T. spiralis*, *T. cruzi* epimastigotes, nucleated mammalian target cells, and chicken erythrocytes [reviewed in (108)]. Controversy exists about the ability of the neutrophil to damage schistosomules, one study showing activity (107), another not (101). The reason for this discrepancy is not clear; a thorough discussion of the factors likely to be important has been presented (108).

Eosinophils accumulate about parasites in vivo and degranulate there. Hsu et al showed eosinophils in close contact with the surface of schistosomules in the skin of monkeys immune to *S. japonicum* (109), and they noticed that many of the challenge larvae were destroyed in the skin at sites where eosinophils were numerous. Similar observations have been made in other species (110-112). Recent observations indicate that eosinophils degranulate and deposit

Annual Reviews

toxic granule proteins on their targets in vivo (113). In humans, microfilariae of *Onchocerca volvulus* and eosinophils are present in skin of patients with onchocerciasis. During the Mazzotti reaction (an acute exacerbation of chronic onchocercal dermatitis characterized by intense pruritus, edema, erythema and urticaria, and induced by treatment with diethylcarbamazine), immunofluorescent staining for MBP showed a marked extracellular deposition of MBP surrounding degenerating microfilariae. Taken together with the findings noted in the preceding portions of this section, this observation strongly supports the hypothesis that the eosinophil functions as a killer cell for parasites both in vitro and in vivo and in experimental animals and humans.

MECHANISMS OF EOSINOPHIL DAMAGE TO PARASITES As noted above, the eosinophil granule is a rich source of cationic proteins. Three of these, MBP, ECP, and EPO, damage helminths directly. Comparison of the toxicity of ECP and MBP indicates that ECP is about 10-fold more active on a molar basis (24, 114). MBP (23) and EPO (115) are released during the attack on the schistosomula membrane. EPO may kill schistosomules either in conjunction with H_2O_2 + halide or directly (46). Eosinophils produce H_2O_2 on stimulation with a parasite (116), and therefore this should be available for the reaction with EPO and halide. Of interest is the finding that eosinophils can kill schistosomules under anaerobic conditions (117).

ENHANCEMENT OF EOSINOPHIL DAMAGE TO PARASITES Several factors enhance the eosinophil's capacity to damage parasites. First, tissue culture supernatants rich in T cell–derived eosinophil stimulation promoter (118) also enhanced the ability of eosinophils from normal mice to kill *S. mansoni* eggs in vitro (118). Second, eosinophils incubated with the eosinophil chemotactic factor of anaphylaxis (ECF-A) show an increased number of complement receptors (119, 120), and such eosinophils show an enhanced ability to kill schistosomules. Because mast cells contain ECF-A, this may mediate the enhancement of eosinophil killing by mast cell products noted earlier (103). Third, the finding that patients with eosinophilia also had an enhanced ability to kill schistosomules in vitro (121) suggested that eosinophil colony-stimulating factors (CSF) might act not only to stimulate eosinophil production by the bone marrow, but also to activate the eosinophil. A test of this hypothesis indicated that purified CSF-alpha both enhanced eosinophil cytotoxicity and promoted eosinophil colony growth (122). CSF-alpha enhanced the adherence step of the killing reaction, and schistosomules were coated with several layers of eosinophils. The enhancement of eosinophil adherence to schistosomules by CSF-alpha was temperature dependent and was not blocked by puromycin.

Factors derived from monocytes also enhance eosinophil killing of schistosomules (123, 124). One of these factors, derived from an adherent mono-

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nuclear cell positive for nonspecific esterase, resists boiling, has a molecular weight of 35,000–45,000 as judged from Sephadex G-200 fractionation, and does not increase the number of Fc receptors on eosinophils (123). Because this factor appears to be of monocyte origin, it is not the eosinophil stimulation promoter referred to above. The other monocyte-derived factor was produced by plastic-adherent cells, 91% peroxidase-positive, from human peripheral blood (124). The macrophage-like histiocytic cell line U937 and a promyelocytic leukemia cell line, HL-60, also produce factors enhancing eosinophil cytotoxicity (124).

Lastly, T lymphocytes stimulated with Concanavalin A in the presence of 2-mercaptoethanol produce a factor enhancing eosinophil cytotoxicity (124); the relationship of this factor to the eosinophil stimulation promoter requires further investigation.

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Hypersensitivity Reactions

The results above indicate that the eosinophil is well endowed with machinery to destroy multicellular parasites. Furthermore, it can be activated by several factors that enhance its destructive capability. These findings would be in keeping with an inimical role for the eosinophil in hypersensitivity reactions. However, because earlier findings showed that the eosinophil possesses enzymes able to degrade mediators of anaphylaxis, it has been regarded as a regulatory cell for control of immediate hypersensitivity reactions. As detailed below, this view must now be enlarged to include a role for the cell as an effector of damage in hypersensitivity reactions.

EOSINOPHIL MODULATION OF IMMEDIATE HYPERSENSITIVITY REACTION The eosinophil possesses numerous enzymes, and certain of these enzymes can neutralize mediators of anaphylaxis, including slow-reacting substance (125), histamine (89) and platelet-activating factor (90). Therefore, it was postulated that one function of the eosinophil was containment of the inflammation following immediate-type hypersensitivity reactions (126). Subsequently it was demonstrated that stimulation of human eosinophils by allergens or anti-IgE causes liberation of an inhibitor of leukocyte histamine release, and this inhibitor was identified as prostaglandin E_1 and E_2 (127, 128). MBP binds heparin, neutralizing its anticoagulant activity (7). In addition, EPO + H_2O_2 + halide are able to degrade leukotrienes (129). Finally, eosinophils are attracted to sites of anaphylactic reactions and interactions of IgE with many antigens, including those derived from parasites and pollen (109, 130-134). These eosinophils should therefore be available for the reactions described above.

The hypothesis that the eosinophil modulates immediate hypersensitivity reactions, although attractive, must be viewed with caution. Recent data have not verified the hypothesis, and certain of the original bases of the hypothesis

Annual Reviews

have not been confirmed. For example, it is now recognized that leukotrienes (LT) C, D, and E account for the activity of the slow-reacting substance of anaphylaxis (135, 136). LT-C is an acidic lipid substituted at the 6-position with the tripeptide glutathione through a thiol-ether linkage. The absence of a sulfate ester suggests that LT-C is not susceptible to the hydrolytic action of arylsulfatase. Commercial arylsulfatase contains protease activity; this could cleave the glutathione moiety, thus inactivating SRS-A (137). When purified eosinophil arylsulfatase (91) was tested for its ability to inactivate the contraction of the guinea-pig ileum by synthetic LT-C4 and LT-D4, the purified enzyme did not degrade the activity of the leukotrienes when tested on them at pH 5.7 or neutral pH (138). Similarly, recent data suggest that eosinophil phospholipase D degrades a factor showing platelet lytic activity but not the platelet-activating factor (139). Finally, a test of the hypothesis that eosinophils inactivate mediators was conducted using AES and glucocorticoids to ablate eosinophils from passively sensitized guinea pigs (140). These experiments showed that neither administration of AES nor methylprednisolone altered passive or systemic anaphylactic reactions when compared to controls. Tests of the effect of ablation of eosinophils on histamine release in the passively sensitized peritoneal cavity of the guinea pig showed a reduction in the levels of histamine. In contrast, histamine release following intraperitoneal injection of compound 48/80 was not affected by either AES or glucocorticoid administration, suggesting that stores of histamine were not depleted by these agents. This study concluded that the presence of eosinophils might actually contribute to histamine release.

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EFFECTOR FUNCTION IN HYPERSENSITIVITY REACTIONS During the attack by eosinophils on schistosomules, the external membrane is disrupted, indicating the existence of powerful membrane-active effector systems that are brought into action by degranulation. Presumably, MBP, ECP, and the EPO + H_2O_2 + halide system all play a role in this damage. Because certain hypersensitivity diseases are associated with eosinophilia (141) and because there is a positive association between eosinophilia and organ dysfunction in bronchial asthma (142), the possibility that the eosinophil produces organ damage in hypersensitivity reactions was tested.

Initial studies showing that MBP was toxic to murine tumor cells (23) prompted a test of guinea-pig MBP on cells from organs that are infiltrated with eosinophils in disease (143). Dermal, intestinal, splenic, and peripheral blood mononuclear cells were all damaged by MBP at concentrations from [illegible] $\times 10^{-6}$M to 8×10^{-5}M in a dose-related manner. Cilia in tracheal explants ceased beating at about 10^{-5}M of MBP. Measurement of MBP in body fluids showed concentrations as high as 14 μg/ml, 1.5×10^{-6}M, in serum; 3[illegible]

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μg/ml, 3.2×10^{-6}M, in pleural fluid; and up to 93 μg/ml, 1×10^{-5}M, in sputa from patients with bronchial asthma. These results indicated that concentrations of MBP in the toxic range were present in human body fluids.

A further study of the effect of MBP on respiratory epithelium using guinea-pig tracheal rings showed that MBP in low doses produced exfoliation of epithelial cells and impairment of ciliary beating (144). In contrast, another basic protein, protamine, 1×10^{-4}M, produced neither exfoliation nor alteration of ciliary beating when incubated with tracheal rings for 72 hr. Microscopic examination of fixed tissue showed varying degrees of epithelial damage. With the lowest concentration of MBP tested, 9×10^{-7}M, the epithelium was disrupted and damaged cells were free in the lumen. With MBP levels of 4.5×10^{-6}M and 9×10^{-6}M, the epithelium showed detachment of ciliated and brush cells and destruction of individual cells, leaving only basal cells. Exfoliated cells showed disruption of the cellular membrane and liberation of cell contents. Cilia were stripped from cells and exhibited a distinctive beaded appearance. The lamina propria appeared edematous, with separation of collagen fibrils. These effects of MBP on bronchial epithelium were remarkably similar to the pathologic changes in human bronchial asthma. Specifically, excessive shedding and desquamation of the bronchial epithelium down to the level of the lamina propria are reported as constant findings in bronchial asthma (145-148). The superficial columnar cells undergo detachment, leaving behind them a layer of basal cells, and from these regeneration of the mucosa takes place.

The findings that MBP damaged guinea-pig respiratory tissue and that the damage mimicked in part the pathology of asthma were consistent with the hypothesis that eosinophils, known to be a hallmark of bronchial asthma, damage bronchial epithelium in asthma. Both human and guinea-pig MBP damaged respiratory epithelium of both species, indicating that the MBP was not preferentially active on one or the other tissue (144, 149). To determine its prevalence MBP was measured in the sera and sputa of patients with respiratory disease in general and with asthma in particular (149). First, sputa of 100 patients with various respiratory diseases were examined for their content of MBP by radioimmunoassay. Sputa of 13 patients contained MBP levels greater than 0.1 μg/ml, and 11 of these patients had asthma. Second, sputa of 15 patients hospitalized for asthma were analyzed sequentially for MBP content. MBP levels were elevated in all patients and rose from admission to day 3 before falling by day 8. The peak levels of sputum MBP in the 15 patients ranged from 0.3 μg/ml to 92.9 μg/ml, with a geometric mean of 7.1 μg/ml. These results indicated that an elevated sputum MBP was a good marker for bronchial asthma, and that high concentrations of MBP were present in sputa of some patients with asthma. Certain sputa containing high MBP

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levels had been processed within a few minutes after expectoration, suggesting that the elevated MBP was not merely a consequence of in vitro cell death and liberation of granule contents.

A second set of experiments tested the hypothesis that eosinophils mediate tissue damage. Lung tissues from 9 autopsies of patients dead of asthma, between 1967 and 1979, were examined for intracellular and extracellular MBP. An immunofluorescence procedure (developed for tissue localization of MBP in the guinea pig) could detect MBP in formalin-fixed, paraffin-embedded tissues (150, 151). Examination of sections stained with hematoxylin and eosin (H&E) showed that all the patients had a thickened basement membrane zone, goblet-cell hyperplasia, and peribronchial inflammatory infiltrates with eosinophils in the lamina propria. Smooth muscle hypertrophy was seen in all but one of the patients, and mucous gland hyperplasia was seen in seven. Squamous metaplasia, mucus plugs, Creola bodies, epithelial damage, and frank denudation of respiratory epithelium occurred more variably. Examination of sections by immunofluorescence showed MBP staining that was intracellular as well as extracellular. The sites at which MBP was localized and the frequency of staining at these sites differed among the patients. Striking abnormalities were seen in patients dead of status asthmaticus, while patients dead of other causes with associated severe asthma showed these to a lesser degree. Patients whose deaths were not due to asthma showed neither epithelial damage nor extracellular MBP immunofluorescence. One pattern of MBP immunofluorescence (Figure 3) was diffuse staining of a mucus plug with brilliant streaks and whorls. In addition, the epithelial surface of this bronchiole was stained in a patchy, scalloped manner. Other patterns included diffuse extracellular MBP immunofluorescence in the lamina propria corresponding to the presence of necrotic, amorphous, eosinophilic material on the H&E-stained section. Another striking abnormality was intense extracellular MBP staining of damaged epithelium associated with eosinophilic infiltration in the lamina propria and frank destruction of the basement membrane zone.

These results indicate that eosinophils degranulate in tissues and that MBP, a granule constituent, is present at sites of tissue damage in asthma. Recently, both human and horse eosinophils were shown to produce leukotrienes (152-155), including both LT-C4 and -D4. Comparison of the quantity of LT-C produced by purified normal leukocytes indicated that neutrophils produced 7.5 ± 4 ng/10^6 cells whereas eosinophils produced 38 ± 3 ng/10^6 cells (154). These results indicate that in asthma, local eosinophils might secrete these potent smooth muscle contractants, thus inducing bronchospasm.

As already noted, eosinophils may also induce inflammation by stimulating mast cells via the EPO + H_2O_2 + halide system (49). MBP also activates purified human basophils and rat mast cells to release histamine in an energy-, temperature-, and calcium-dependent manner (156). MBP also stimulates wheal-

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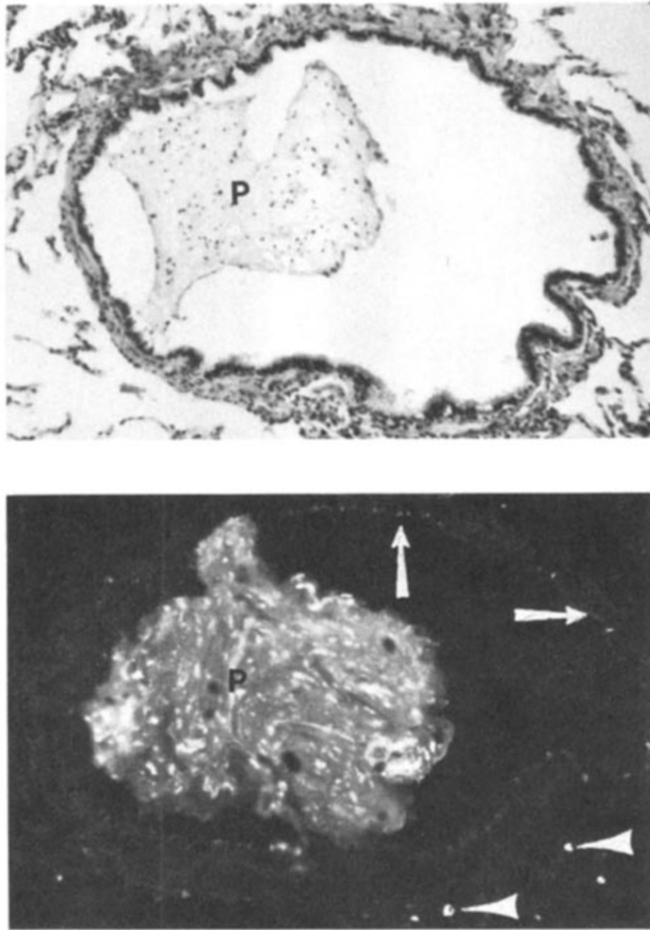

Figure 3 Serial sections from a bronchiole with a mucus plug (P) from a patient with asthma. (*Top*) Sections stained with H&E; (*bottom*) sections stained with anti-MBP. Note the diffuse staining of the plug (P) with brilliant swirls as well as the patchy, less intense fluorescence of the luminal surface of the respiratory epithelium (arrows). There are discrete eosinophils in the peribronchial area (arrowheads). (× 100) [Reproduced from (151) with permission]

and-flare skin reactions in a dose-related manner (G. J. Gleich, D. A. Loegering, unpublished observations). Thus, eosinophils can cause inflammation and tissue damage in bronchial asthma through a variety of mechanisms (summarized in Figure 4).

The eosinophil may also participate in inflammatory skin diseases. For example, a study of serum MBP in chronic urticaria showed an elevated level

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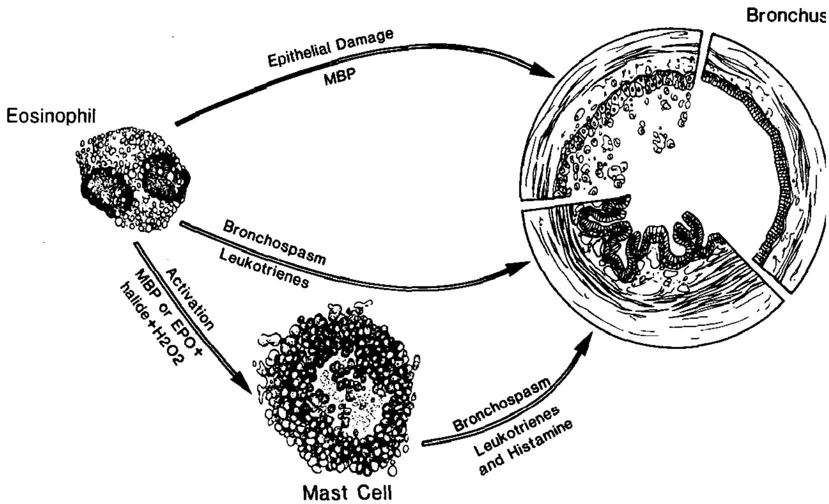

Figure 4 The role of eosinophils in airway obstruction in bronchial asthma. The effects on th bronchus are shown by comparing a section of normal bronchus (upper right) with a sectio damaged by MBP (upper left) that shows epithelial desquamation and with a section (bottom that shows smooth muscle hypertrophy, constriction, and edema of the lamina propria resultin in a reduction in the caliber of the airway (MBP, eosinophil granule major basic protein; EPO eosinophil peroxidase; H_2O_2, hydrogen peroxide). [From (157) by permission]

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in the absence of peripheral-blood eosinophilia in 38% of patients (29). Sub sequently, immunofluorescence localization of MBP in skin from patients witl chronic urticaria showed that extracellular MBP was present in 12 of 28 biop sies (43%); in contrast, skin from patients with dermatitis herpetiformis, pem phigus vulgaris, ichthyosis vulgaris, actinic keratosis, basal-cell carcinoma and familial benign pemphigus did not show MBP outside of eosinophil (158). Because eosinophils can stimulate mediator release from mast cells, i is possible that they act upon the mast cell in certain cases of chronic urticaria In eosinophilic cellulitis (Wells syndrome) patients have recurrent edematou and infiltrative plaques and show a characteristic histological picture includin diffuse infiltration with eosinophils, histiocytes, and foci of amorphous eosin ophilic material associated with connective tissue fibers of the dermis, th so-called "flame figures." Immunofluorescence localization of MBP showe striking staining of "flame figures," suggesting that MBP and/or other eosin ophil-granule constituents play a role in the pathogenesis of these lesions b altering collagen (159).

Another syndrome in which eosinophils may play a pathogenic role is epi sodic angioedema associated with eosinophilia. Four patients with this syn drome presented with recurrent attacks of angioedema, urticaria, and feve

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(G. J. Gleich, A. L. Schroeter, J. P. Marcoux, M. I. Sachs, E. J. O'Connell, P. F. Kohler, submitted for publication). During attacks, leukocyte counts were as high as 108,000 per mm^3, with 92% eosinophils and body weights increased up to 18%. The disease clearly waxed and waned in concert with the number of eosinophils in the peripheral blood. By electron microscopy, peripheral-blood eosinophils showed alteration of granules, and dermal eosinophils showed a spectrum of abnormalities including frank destruction and loss of their contents into the spaces among the collagen bundles. By immunofluorescence, MBP was present outside of the eosinophil around blood vessels and collagen bundles. Mast cells showed evidence of degranulation by electron microscopy. These observations suggest that activated eosinophils localize in the skin, release their granule constituents, and cause mast-cell degranulation with resultant edema.

Peripheral-blood eosinophilia is also associated with the development of cardiac disease, especially in the hypereosinophilic syndrome (160-163). The pathogenesis of the cardiac involvement is obscure. Cationic eosinophil-derived products damage myocardial cells in vitro (164) so that eosinophil degranulation onto myocardium could be important in the pathogenesis of the disease. Immunofluorescent localization of MBP in cardiac tissue from patients with the hypereosinophilic syndrome shows diffuse extracellular staining of subendomyocardium and, in some early cases, of the basement membrane. In contrast, during the fibrotic stage of the disease, no extracellular MBP was present and eosinophils themselves were scanty (S. J. Ackerman, P.-C. Tai, C. J. F. Spry, G. J. Gleich, unpublished observations).

Thus, there is considerable evidence that eosinophils are associated with disease, especially bronchial asthma, and that they have the potential for producing the symptoms and signs of these diseases.

EOSINOPHILS AND REPRODUCTION

A considerable literature exists showing that eosinophils cyclically infiltrate rodent reproductive tissues. Infiltration of the rat uterus by eosinophils, coincident with estrous cycle (165), was observed as early as 1956, and this observation was confirmed and extended (166, 167). Since then, various investigations have shown (*a*) that injection of estrogen into castrate or immature rodents leads to immediate uterine eosinophilia (168), (*b*) that the uterine eosinophil is marrow-derived (169), (*c*) that the number of uterine eosinophils varies more than 100-fold during the normal estrous cycle (170), (*d*) that uterine content of eosinophil peroxidase varies directly with the estrous cycle (171), (*e*) that eosinophils possess a cell-surface estrogen receptor (172), and (*f*) that estrogen-induced uterine eosinophilia apparently has no dependence upon uterine mast-cell activity (173).

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In contrast, few observations relate eosinophils to human reproductive physiology. Cyclic eosinopenia correlating with ovulation has been reported (174), and cyclic variations in endometrial eosinophils and in their uptake of tritiated estradiol have also been observed (175). During studies of hypersensitivity diseases in pregnancy, we found that serum levels of immunoreactive MBP were elevated in all pregnant women, increasing during pregnancy and decreasing to normal levels following parturition. MBP levels increased during early gestation and plateaued around 7,500 ng/ml by the 20th week (more than 10-fold normal). Levels returned to normal following delivery, with a $t_{1/2}$ of 13.7 days. The MBP in pregnancy serum was remarkably similar to the eosinophil granule MBP by a variety of criteria. However, no correlation between serum MBP level and peripheral-blood eosinophil count existed in pregnant women, in contrast to previous studies of patients with eosinophilia (29). Also, levels of three other eosinophil-associated proteins, including CLC, EDN, and ECP, were normal or low in sera from pregnant women, whereas the serum levels of these proteins were elevated in patients with eosinophilia. Surprisingly, the immunoreactive MBP molecule in pregnancy serum emerges from Sephadex G-50 columns with an elution volume indicative of higher molecular weight than granule MBP. These findings suggest that the MBP in sera from pregnant women is derived from a source other than the eosinophil. Localization of immunoreactive MBP in placentae by immunofluorescence showed staining of anchoring villae, placental X cells, and placental giant cells (176). Placental septal cysts stain brightly for MBP. Aspiration of septal cysts yielded fluid containing 100 μg/ml immunoreactive MBP, a 6-fold greater concentration than the highest levels measured in maternal blood. This immunoreactive MBP also has a higher molecular weight than granule MBP. Moreover, eosinophils could not be demonstrated in the placenta. These results support the hypothesis that an immunoreactive MBP is produced by the placenta. The function of this MBP of pregnancy is presently obscure. Because it is present in placental giant cells and because these cells are thought to play a key role in the invasion of the endometrium at implantation, the cytotoxic MBP may play a role in the invasive processes of early pregnancy. However, this hypothesis fails to account for the continued production of MBP throughout gestation. Further work is needed to isolate the MBP of pregnancy and investigate its properties and functions.

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Literature Cited

1. Hirsch, J. G., Hirsch, B. I. 1980. Paul Ehrlich and the discovery of the eosinophil. In *The Eosinophil in Health and Disease*, ed. A. A. F. Mahmoud, K. F. Austen, p. 3. NY: Grune & Stratton
2. Samter, M. 1980. Eosinophils: the first 90 years. See Ref. 1, p. 25
3. Zucker-Franklin, D. 1980. Eosinophil structure and maturation. See Ref. 1, p. 43
4. Miller, F., DeHarven, E., Palade, G. E. 1962. The structure of the eosinophil leukocyte granules in rodents and man. *J. Cell. Biol.* 31:349
5. Parmley, R. T., Spicer, S. S. 1974. Cytochemical and ultrastructural identification of a small type granule in human late eosinophils. *Lab. Invest.* 30:557
6. Gleich, G. J., Loegering, D. A., Maldonado, J. E. 1973. Identification of a major basic protein in guinea pig eosinophil granules. *J. Exp. Med.* 137:1459
7. Gleich, G. J., Loegering, D. A., Kueppers, F., Bajaj, S. P., Mann, K. G. 1974. Physiochemical and biological properties of the major basic protein from guinea pig eosinophil granules. *J. Exp. Med.* 140:313
8. Gleich, G. J., Loegering, D. A., Mann, K. G., Maldonado, J. E. 1976. Comparative properties of the Charcot-Leyden crystal protein and the major basic protein from human eosinophils. *J. Clin. Invest.* 57:633
9. Lewis, D. M., Loegering, D. A., Gleich, G. J. 1976. Isolation and partial characterization of a major basic protein from rat eosinophil granules. *Proc. Soc. Exp. Biol. Med.* 152:512
10. Lewis, D. M., Loegering, D. A., Gleich, G. J. 1976. Antiserum to the major basic protein of guinea pig eosinophil granules. *Immunochemistry* 13:743
11. Wassom, D. L., Loegering, D. A., Gleich, G. J. 1979. Measurement of guinea pig eosinophil major basic protein by radioimmunoassay. *Mol. Immunol.* 16:711
12. Lewis, D. M., Lewis, J. C., Loegering, D. A., Gleich, G. J. 1978. Localization of the guinea pig eosinophil major basic protein to the core of the granule. *J. Cell. Biol.* 77:702
13. Ackerman, S. J., Weil, G. J., Gleich, G. J. 1982. Formation of Charcot-Leyden crystals by human basophils. *J. Exp. Med.* 155:1597
14. Ackerman, S. J., Kephart, G. M., Habermann, T. M., Greipp, P. R., Gleich, G. J. 1983. Localization of eosinophil granule major basic protein in human basophils. *J. Exp. Med.* 158:946
15. Denburg, J. A., Richardson, M., Telizyn, S., Bienenstock, J. 1983. Basophils/mast cell precursors in human peripheral blood. *Blood* 61:775
16. Denburg, J. A., Richardson, M., Telizyn, S., Gleich, G., Dor, P., Bienenstock, J. 1983. Basophils and eosinophils share a common committed hemopoietic progenitor. *Clin. Res.* 31:163A (Abstr.)
17. Gallagher, R., Collins, S., Trujillo, J., McCredie, K., Ahearn, M., Tsai, S., Metzgar, R., Aulakh, G., Ting, R., Ruscetti, F., Gallo, R. 1979. Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia. *Blood* 54:713
18. Collins, S. J., Ruscetti, F. W., Gallagher, R. E., Gallo, R. C. 1978. Terminal differentiation of human promyelocytic leukemia cells induced by dimethyl sulfoxide and other polar compounds. *Proc. Natl. Acad. Sci. USA* 75:2458
19. Rovera, G., Santoli, D., Damsky, C. 1979. Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc. Natl. Acad. Sci. USA* 76:2779
20. Lu, L., Broxmeyer, H. E., Pelus, L. M., Andreeff, M., Moore, M. A. S. 1981. Detection of Luxol-fast-blue positive cells in human promyelocytic leukemia cell line HL-60. *Exp. Hematol.* 9:887
21. Metcalf, D. 1983. Clonal analysis of the response of HL60 human myeloid leukemia cells to biological regulators. *Leukemia Res.* 2:117
22. Maddox, D. E., Butterfield, J. H., Ackerman, S. J., Coulam, C. B., Gleich, G. J. 1983. Elevated serum levels in human pregnancy of a molecule immunochemically similar to eosinophil granule major basic protein. *J. Exp. Med.* 158:1211
23. Butterworth, A. E., Wassom, D. L., Gleich, G. J., Loegering, D. A., David, J. R. 1979. Damage to schistosomula of *Schistosoma mansoni* induced directly by eosinophil major basic protein. *J. Immunol.* 122:221
24. Ackerman, S. J., Gleich, G. J., Loegering, D. A., Butterworth, A. E. 1983. Comparative toxicity of purified cationic proteins of the human eosinophil granule for schistosomula of *S. mansoni*. *Fed. Proc.* 42:1247 (Abstr.)
25. Vadas, M. A., Butterworth, A. E., Sherry, B., Dessein, A., Hogan, M., Bout, D., David, J. R. 1980. Interactions between human eosinophils and schistosomules of *Schistosoma mansoni*. II. Stable and irre-

Annual Reviews

versible antibody-dependent eosinophil adherence. *J. Immunol.* 124:1441
26. Butterworth, A. E., Vadas, M. A., Wassom, D. L., Dessein, A., Hogan, M., Sherry, B., Gleich, G. J., David, J. R. 1979. Interactions between human eosinophils and schistosomules of *Schistosoma mansoni*. II. The mechanisms of irreversible eosinophil adherence. *J. Exp. Med.* 150:1456
27. Wassom, D. L., Gleich, G. J. 1979. Damage to *Trichinella spiralis* newborn larvae by eosinophil major basic protein. *Am. J. Trop. Med. Hyg.* 28:860
28. Kierszenbaum, F., Ackerman, S. J., Gleich, G. J. 1981. Destruction of bloodstream forms of *Trypanosoma cruzi* by eosinophil granule major basic protein. *Am. J. Trop. Med. Hyg.* 30:775
29. Wassom, D. L., Loegering, D. A., Solley, G. O., Moore, S. B., Schooley, R. T., Fauci, A. S., Gleich, G. J. 1981. Elevated serum levels of the eosinophil granule major basic protein in patients with eosinophilia. *J. Clin. Invest.* 67:651
30. Bainton, D. F., Farquhar, G. M. 1970. Segregation and packaging of granule enzymes in eosinophilic leukocytes. *J. Cell Biol.* 45:54
31. Kelenyi, G., Zombai, E., Nemeth, A. 1965. Histochemische und elektronenmikroskopische Beobachtungen an der spezifischen Granulation der eosinophilen Granulozyten. *Acta Histochem.* 22:77
32. Yamada, E., Yamauchi, R. 1966. Some observations on the cytochemistry and morphogenesis of the granulocytes in the rat bone marrow as revealed by electron microscopy. *Acta Haematol. Jpn.* 29:530
33. Enomoto, I., Kitani, T. 1966. Electron microscopic studies on peroxidase and acid phosphatase reaction in human leukocytes in normal and leukemic cells and on phagocytosis. *Acta Haematol. Jpn.* 29:554
34. Unditz, E., Lang, M., Van Oyi, E. 1956. La reaction proxydasique des eosinophiles: comme moyen de toxonomie. *Le Sang* 27:513
35. Archer, G. T., Air, G., Jackas, M., Morell, D. B. 1965. Studies on rat eosinophil peroxidase. *Biochem. Biophys. Acta* 99:96
36. Dresser, R. K., Himmelhock, S. R., Evans, W. H., Januska, M., Mage, M., Shelton, E. 1972. Guinea pig heterophil and eosinophil peroxidase. *Arch Biochem. Biophys.* 148:452
37. Klebanoff, S. J. 1967. Iodination of bacteria: a bactericidal mechanism. *J. Exp. Med.* 126:1063
38. Bilding, M. E., Klebanoff, S. J. 1970. Peroxidase-mediated virucidal systems. *Science* 167:195
39. Jacobs, A. A., Loew, I. E., Paul, B. B., Strauss, R. R., Sbarra, A. T. 1972. Mycoplasmacidal activity of peroxidase-H_2O_2-halide system. *Immunology* 51:127
40. Lehrer, R. I. 1969. Antifungal effects of peroxidase systems. *J. Bacteriol.* 99:361
41. Baehmer, R. L., Johnston, R. B., Jr. 1971. Metabolic and bactericidal activities of human eosinophils. *Br. J. Haematol.* 20:277
42. Klebanoff, S. J., Durack, D. T., Rosen, H., Clark, R. A. 1977. Functional studies on human peritoneal eosinophils. *Infect. Immunol.* 17:167
43. Klebanoff, S. J., Jong, E. C., Henderson, W. R., Jr. 1980. The eosinophil peroxidase: purification and biological properties. See Ref. 1, p. 99
44. Migler, R., DeChatelet, L. R., Bass, D. A. 1978. Human eosinophilic peroxidase: role in bactericidal activity. *Blood* 51:445
45. Jong, E. C., Henderson, W. R., Klebanoff, S. J. 1980. Bactericidal activity of eosinophil peroxidase. *J. Immunol.* 124:1378
46. Jong, E. C., Mahmoud, A. A. F., Klebanoff, S. J. 1981. Peroxidase-mediated toxicity to schistosomula of *Schistosoma mansoni*. *J. Exp. Med.* 126:468
47. Locksley, R. M., Wilson, C. B., Klebanoff, S. J. 1982. Role of endogenous and acquired peroxidase in the toxoplasmacidal activity of murine and human mononuclear phagocytes. *J. Clin. Invest.* 69:1099
48. Nogueira, N. M., Klebanoff, S. J., Cohn, Z. A. 1982. *Trypanosoma cruzi*: sensitization to macrophage killing by eosinophil peroxidase. *J. Immunol.* 128:1705
49. Henderson, W. R., Chi, E. Y., Klebanoff, S. J. 1980. Eosinophil peroxidase-induced mast cell secretion. *J. Exp. Med.* 152:265
50. Jong, E. C., Klebanoff, S. J. 1980. Eosinophil-mediated mammalian tumor cell cytotoxicity: role of the peroxidase system. *J. Immunol.* 124:1949
51. Henderson, W. R., Jong, E. C., Klebanoff, S. J. 1980. Binding of eosinophil peroxidase to mast cell granules with retention of peroxidatic activity. *J. Immunol.* 124:1383
52. Watanabe, K., Hasegawa, M., Saito, Y., Takayama, S. 1977. Eosinophilic leukocytes in nasal allergy-movement of enzymes. *Clin. Allergy* 7:263
53. Ramsey, P. G., Martin, T., Chi, E., Klebanoff, S. J. 1982. Arming of mononuclear phagocytes by eosinophil peroxidase bound to *Staphylococcus aureus*. *J. Immunol.* 128:415
54. Nathan, C. F., Klebanoff, S. J. 1982.

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by HINARI on 08/27/07. For personal use only.

Augmentation of spontaneous macrophage-mediated cytolysis by eosinophil peroxidase. *J. Exp. Med.* 155:1291

55. Olsson, I., Venge, P. 1974. Cationic proteins of human granulocytes. II. Separation of the cationic proteins of the granules of leukemic myeloid cells. *Blood* 44:235
56. Olsson, I., Venge, P., Spitznagel, J. K., Lehrer, R. I. 1977. Arginine-rich cationic proteins of human eosinophil granules. *Lab. Invest.* 36:493
57. Venge, P., Dahl, R., Hallgren, R., Olsson, I. 1980. Cationic proteins of human eosinophils and their role in the inflammatory reaction. See Ref. 1, p. 131
58. Ackerman, S. J., Loegering, D. A., Venge, P., Olsson, I., Harley, J. B., Fauci, A. S., Gleich, G. J. 1983. Distinctive cationic proteins of the human eosinophil granule: major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin. *J. Immunol.* 131:2977
59. Venge, P., Dahl, R., Hallgren, R. 1979. Enhancement of Factor XII dependent reactions by eosinophil cationic protein. *Thromb. Res.* 14:641
60. Dahl, R., Venge, P. 1979: Enhancement of wokinase-induced plasminogen activation by the cationic protein of human eosinophil granulocytes. *Thromb. Res.* 14:599
61. Fredens, K., Dahl, R., Venge, P. 1982. The Gordon phenomenon induced by eosinophil cationic protein and eosinophil protein X. *J. Allergy Clin. Immunol.* 70:361
62. Durack, D. T., Ackerman, S. J., Loegering, D. A., Gleich, G. J. 1981. Purification of human eosinophil-derived neurotoxin. *Proc. Natl. Acad. Sci. USA* 78:5165
63. Gordon, M. H. 1933. Remarks on Hodgkin's disease. A pathogenic agent in the glands and its application in diagnosis. *Br. Med. J.* 1:641
64. King, L. S. 1939. Encephalopathy following injections of bone marrow extract. *J. Exp. Med.* 70:303
65. Turner, J. C., Jackson, H., Jr., Parker, F., Jr. 1938. The etiologic relation of the eosinophil to the Gordon phenomenon in Hodgkin's disease. *Am. J. Med. Sci.* 195:27
66. Schwartz, A. M., Lapham, L. W., Van den Noort, S. 1966. Cytologic and cytochemical studies of neuroglia. IV. Experimentally induced protoplasmic astrocytosis in Bergman glia of cerebellum. *Neurology* 16:1118
67. Terplan, K., Kraus, R., Barnes, S. 1957. Eosinophilic meningo-encephalitis, with predominantly cerebellar changes caused by Trichinella infection. *J. Mt. Sinai Hosp.* 24:1293
68. Benvenisti, D. S., Ultmann, J. E. 1969. Eosinophilic leukemia. Report of five cases and review of the literature. *Ann. Intern. Med.* 71:731
69. Chusid, M. J., Dale, D. D., West, B. C., Wolff, S. M. 1975. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. *Medicine* 54:1
70. Snead, O. C., Kalavsky, S. M. 1976. Cerebrospinal fluid eosinophilia. A manifestation of a disorder resembling multiple sclerosis in childhood. *J. Pediatr.* 89:83
71. Yii, C.-Y. 1976. Clinical observations on eosinophilic meningitis and meningoencephalitis caused by *Angiostrongylus cantonensis* in Taiwan. *Am. J. Trop. Med. Hyg.* 25:233
72. Durack, D. T., Sumi, S. M., Klebanoff, S. J. 1979. Neurotoxicity of human eosinophils. *Proc. Natl. Acad. Sci. USA* 76:1443
73. Peterson, C. G. B., Venge, P. 1983. Purification and characterization of a new cationic protein-eosinophil protein-X (EPX) from granules of human eosinophils. *Immunology* 50:19
74. Buddecke, E., Essellier, A. F., Morti, H. R. 1956. Über die chemische Natur der Charcot-Leydenschen Kristalle. *Hoppe-Seyler's Z. Physiol. Chem.* 305:203
75. Hornung, M. 1962. Preservation, recrystallization and preliminary biochemical characterization of Charcot-Leyden crystals. *Proc. Soc. Exp. Biol. Med.* 110:119
76. Archer, G. T., Blackwood, A. 1965. Formation of Charcot-Leyden crystals in human eosinophils and basophils and study of the composition of the isolated crystal. *J. Exp. Med.* 122:173
77. Ackerman, S. J., Loegering, D. A., Gleich, G. J. 1980. The human eosinophil Charcot-Leyden crystal protein: biochemical characteristics and measurement by radioimmunoassay. *J. Immunol.* 125:2118
78. Ottolenghi, A., Pickett, J. P., Greene, W. B. 1967. Histochemical demonstration of phospholipase B (lysolecithinase) activity in rat tissues. *J. Histochem. Cytochem.* 14:907
79. Ottolenghi, A. 1969. The relationship between eosinophilic leukocytes and phospholipase B activity in some rat tissues. *Lipids* 5:531
80. Weller, P. F., Wasserman, S. I., Austen, K. F. 1981. Selected enzymes preferentially present in the eosinophil. See Ref. 1, pp. 115
81. Weller, P. F., Goetzl, E. J., Austen, K.

Annual Reviews

Annu. Rev. Immunol. 1984.2:429-459. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

F. 1980. Identification of human eosinophil lysophospholipase as the constituent of Charcot-Leyden crystals. *Proc. Natl. Acad. Sci. USA* 77:7440

82. Weller, P. F., Bach, D., Austen, K. F. 1982. Human eosinophil lysophospholipase: the sole protein component of Charcot-Leyden crystals. *J. Immunol.* 128:1346
83. Archer, G. T., Hirsch, J. G. 1963. Isolation of granules from eosinophil leukocytes and study of their enzyme content. *J. Exp. Med.* 118:277
84. Heyneman, R. A., Monbaliu-Bauwens, D., Vercauteren, R. E. 1975. Hydrolytic enzymes in neutrophil and eosinophil leukocytes. *Comp. Biochem. Physiol.* 50B:463
85. Bassett, E. G., Baker, J. R., Baker, P. A., Meyers, D. B. 1976. Comparison of collagenase activity in eosinophil and neutrophil fractions from rat peritoneal exudates. *Aust. J. Exp. Biol. Med. Sci.* 54:459
86. DePierre, J. W., Karnovsky, M. L. 1974. Ecto-enzymes of the guinea pig polymorphonuclear leukocyte. II. Properties and suitability as markers for the plasma membrane. *J. Biol. Chem.* 249:7121
87. West, B. C., Gelb, N. A., Rosenthal, A. S. 1975. Isolation and partial characterization of human eosinophil granules. *Am. J. Pathol.* 81:575
88. Williams, D. M., Linder, J. E., Hill, M. W., Gillett, R. 1978. Ultrastructural localization of alkaline phosphatase in rat eosinophil leukocytes. *J. Histochem. Cytochem.* 26:862
89. Ziegler, R. S., Yurdin, D. L., Colten, H. R. 1976. Histamine metabolism. II. Cellular and subcellular localization of the catabolic enzymes, histaminase and histamine methyl transferase in human leukocytes. *J. Allergy. Clin. Immunol.* 58:172
90. Kater, L. A., Goetzl, E. J., Austen, K. F. 1980. Isolation of human eosinophil phospholipase D. *J. Clin. Invest.* 58:1173
91. Weller, P. F., Austen, K. F. 1983. Human eosinophil arylsulfatase B. Structure and activity of the purified tetrameric lysosomal hydrolase. *J. Clin. Invest.* 71:114
92. Dessein, A. J., David, J. R. 1982. The eosinophil in parasitic diseases. In *Advances in Host Defense Mechanisms,* ed. J. I. Gallin, A. S. Fauci, p. 243. NY: Raven
93. Mahmoud, A. A. F., Warren, K. S., Peters, P. A. 1975. A role for the eosinophil in acquired resistance to *Schistosoma mansoni* infection as determined by antieosinophil serum. *J. Exp. Med.* 142:805
94. Olds, G. R., Mahmoud, A. A. F. 1980. Role of host granulomatous response in murine schistosomiasis mansoni. *J. Clin. Invest.* 66:1191
95. Grove, D. I., Mahmoud, A. A. F., Warren, K. S. 1977. Eosinophils in resistance to *Trichinella spiralis*. *J. Exp. Med.* 145:755
96. Gleich, G. J., Olson, G. M., Herlich, H. 1979. The effect of antiserum to eosinophils on susceptibility and acquired immunity of the guinea pig to *Trichostrongylus colubriformis*. *Immunology* 37:873
97. Brown, S. J., Galli, S. J., Gleich, G. J., Askenase, P. W. 1982. Ablation of immunity to *Amblyoma americanum* by antibasophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J. Immunol.* 129:790
98. Butterworth, A. E., Sturrock, R. F., Houba, V., Mahmoud, A. A. F., Sher, A., Rees, P. H. 1975. Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* 256:727
99. Butterworth, A. E., David, J. R., Franks, D., Mahmoud, A. A. F., David, P. H., Sturrock, R. F., Houba, V. 1977. Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labeled schistosomula of *Schistosoma mansoni:* damage by purified eosinophils. *J. Exp. Med.* 145:136
100. Butterworth, A. E., Remold, H. G., Houba, V., David, J. R., Franks, D., David, P. H., Sturrock, R. F. 1977. Antibody-dependent eosinophil-mediated damage to ^{51}Cr-labeled schistosomula of *Schistosoma mansoni*: Mediation by IgG, and inhibition by antigen-antibody complexes. *J. Immunol.* 118:2230
101. Vadas, M. A., David, J. R., Butterworth, A. E., Pisani, N. T., Siongok, T. A. 1979. A new method for the purification of human eosinophils and neutrophils, and a comparison of the abilities of these cells to damage schistosomula of *Schistosoma mansoni*. *J. Immunol.* 122:1228
102. McLaren, D. J., Ramalho-Pinto, F. J., Smithers, S. R. 1978. Ultrastructural evidence for complement and antibody-dependent damage to schistosomula of *Schistosoma mansoni* by rat eosinophils in vitro. *Parasitology* 77:313
103. Capron, M., Capron, A., Torpier, G., Bazin, H., Bout, D., Joseph, M. 1978. Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG_{2a} antibody and role of mast cells. *Eur. J. Immunol.* 8:127
104. Kassis, A. I., Aikawa, M., Mahmoud,

Annual Reviews

A. A. F. 1979. Mouse antibody-dependent eosinophil and macrophage adherence and damage to schistosomula of *Schistosoma mansoni*. *J. Immunol.* 122:398

105. Capron, M., Rousseaux, J., Mazingue, C., Bazin, H., Capron, A. 1978. Rat mast cell-eosinophil interaction in antibody-dependent cytotoxicity to *Schistosoma mansoni* schistosomula. *J. Immunol.* 121:2518
106. Ramalho-Pinto, F. J., McLaren, D. J., Smithers, S. R. 1978. Complement-mediated killing of schistosomula of *Schistosoma mansoni* by rat eosinophils in vitro. *J. Exp. Med.* 147:147
107. Anwar, A. R. E., Smithers, S. R., Kay, A. B. 1979. Killing of schistosomula of *Schistosoma mansoni* coated with antibody and/or complement by human leukocytes in vitro: Requirement for complement in preferential killing by eosinophils. *J. Immunol.* 122:628
108. Butterworth, A. E., Vadas, M. A., David, J. R. 1980. Mechanisms of eosinophil mediated helminthotoxicity. See Ref. 1, p. 253
109. Hsu, S. Y. L., Hsu, H. F., Penick, G. E., Lust, G. L., Osborne, J. W. 1974. Dermal hypersensitivity to schistosome cercariae in rhesus monkeys during immunization and challenge. I. Complex hypersensitivity reactions of a well-immunized monkey during the challenge. *J. Allergy Clin. Immunol.* 54:339
110. von Lichtenberg, F., Sher, A., Gibbons, N., Doughty, B. L. 1976. Eosinophil-enriched inflammatory response to schistosomula in the skin of mice immune to *Schistosoma mansoni*. *Am. J. Pathol.* 84:479
111. Moqbel, R. 1980. Histopathological changes following primary, secondary and repeated infection of rats with *Strongyloides ratti* with special reference to tissue eosinophils. *Parasite Immunol.* 2:11
112. Knopf, P. M. 1979. *Schistosoma mansoni*: Peripheral and tissue eosinophilia in infected rats. *Exp. Parasitol.* 47:232
113. Kephart, G. M., Gleich, G. J., Connor, D. H., Gibson, D. W., Ackerman, S. J. 1984. Deposition of eosinophil granule major basic protein onto microfilariae of *Onchocera volvulus* in the skin of patients treated with diethylcarbamazine. *Lab. Invest.* 50:51
114. McLaren, D. J., McKean, J. R., Olsson, I., Venge, P., Kay, A. B. 1981. Morphological studies on the killing of schistosomula of *Schistosoma mansoni* by human eosinophil and neutrophil cationic proteins in vitro. *Parasite Immunol.* 3:359
115. McLaren, D. J., Mackenzie, C. D., Ramalho-Pinto, F. J. 1977. Ultrastructural observations on the in vitro interaction between rat eosinophils and some parasitic helminths (*Schistosoma mansoni*, *Trichinella spiralis* and *Nippostrongylus brasiliensis*). *Clin. Exp. Immunol.* 30:105
116. Kazura, J. W., Blumer, J., Mahmoud, A. A. F. 1979. Parasite-stimulated production of H_2O_2 from human eosinophils and neutrophils. *Clin. Res.* 27:515A (Abstr.)
117. Pincus, S. H., Butterworth, A. E., David, J. R., Robbins, M., Vadas, M. A. 1981. Antibody-dependent eosinophil-mediated damage to schistosomula of *Schistosoma mansoni*: lack of requirement for oxidative metabolism. *J. Immunol.* 126:1794
118. James, S. L., Colley, D. G. 1978. Eosinophil-mediated destruction of *Schistosoma mansoni* eggs. III. Lymphokine involvement in the induction of eosinophil functional abilities. *Cell Immunol.* 38:48
119. Anwar, A. R. E., Kay, A. B. 1977. The ECF-A tetrapeptides and histamine selectively enhanced human eosinophil complement receptors. *Nature* 269:522
120. Anwar, A. R. E., Kay, A. B. 1978. Enhancement of human eosinophil complement receptors by pharmacologic mediators. *J. Immunol.* 121:1245
121. David, J. R., Vadas, M. A., Butterworth, A. E., Azevedo de Brito, P., Carvalho, E. M., David, R. A., Bina, J. C., Andrade, Z. A. 1980. Enhanced helminthotoxic capacity of eosinophils from patients with eosinophilia. *N. Engl. J. Med.* 303:1147
122. Dessein, A. J., Vadas, M. A., Nicola, N. A., Metcalf, D., David, J. R. 1982. Enhancement of human blood eosinophils cytotoxicity by semi-purified eosinophil colony-stimulating factor(s). *J. Exp. Med.* 156:90
123. Veith, M. C., Butterworth, A. E. 1983. Enhancement of human eosinophil-mediated killing of *Schistosoma mansoni* larvae by mononuclear cell products in vitro. *J. Exp. Med.* 157:1828
124. Dessein, A. J., Lenzi, H. L., David, J. R. 1983. Modulation of the cytotoxicity of human blood eosinophils by factors secreted by monocytes and T lymphocytes. *Monogr. Allergy* 18:45
125. Wasserman, S. I., Goetzl, E. J., Austen, K. F. 1975. Inactivation of slow-reacting substance of anaphylaxis by human eosinophil arylsulfatase. *J. Immunol.* 114:645
126. Goetzl, E. J., Wasserman, S. I., Austen, K. F. 1975. Eosinophil polymorphonu-

Annu. Rev. Immunol. 1984.2:429-459. Downloaded from arjournals.annualreviews.org
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Annual Reviews

clear leukocyte function in immediate hypersensitivity. *Arch. Pathol.* 99:1
127. Hubscher, T. 1975. Role of the eosinophil in the allergic reactions. I. EEI-an eosinophil-derived inhibitor of histamine release. *J. Immunol.* 114:1379
128. Hubscher, T. 1975. Role of the eosinophil in the allergic reactions. II. Release of prostaglandins from human eosinophilic leukocytes. *J. Immunol.* 114:1389
129. Henderson, W. R., Jong, A., Klebanoff, S. J. 1982. Eosinophil peroxidase-mediated inactivation of leukotrienes B_4, C_4 and D_4. *J. Immunol.* 128:2609
130. Samter, M. 1949. The response of eosinophils in the guinea pig to sensitization, anaphylaxis and various drugs. *Blood* 4:217
131. Eidinger, D., Wilkinson, R., Rose, B. 1964. A study of cellular responses in immune reaction utilizing the skin window technique. I. Immediate hypersensitivity reaction. *J. Allergy* 35:77.
132. Lowell, F. C. 1967. Clinical aspects of eosinophilia in atopic disease. *J. Am. Med. Assoc.* 202:875
133. Solley, G. O., Gleich, G. J., Jordon, R. E., Schroeter, A. L. 1976. The late phase of the immediate wheal and flare skin reaction. *J. Clin. Invest.* 58:408
134. Dessein, A. J., Parker, W., James, S. L., David, J. R. 1981. IgE antibody in resistance to infections. I. Selective suppression of the IgE antibody response in rats diminishes the resistance and the eosinophil response to *Trichinella spiralis* infection. *J. Exp. Med.* 153:423
135. Murphy, R. C., Hammarstrom, S, Samuelsson, B. 1979. Leukotriene C: a slow-reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. USA* 76:4275
136. Lewis, R. A., Austen, K. F., Drazen, J. M., Clark, D. A., Marfat, A., Corey, E. J. 1980. Slow reacting substances of anaphylaxis: identification of leukotriene C-1 and D from human and rat sources. *Proc. Natl. Acad. Sci. USA* 77:3710
137. Parker, C. W., Koch, D. A., Huber, M. M., Falkenhein, S. F. 1980. Arylsulfatase inactivation of slow reacting substance. Evidence for proteolysis as a major mechanism when ordinary commercial preparations of the enzyme are used. *Prostaglandins* 20:887
138. Weller, P. S. 1983. Functional and biochemical properties of selected protein constituents of human eosinophils. In *The Immunobiology of the Eosinophil*, T. Yoshida, M. Torisu, p. 145. NY: Elsevier
139. Valone, F. H., Whitmer, D. I., Pickett, W. C., Austen, K. F., Goetzl, E. J. 1979. The immunological generation of a platelet-activating factor and a platelet-lytic factor in the rat. *Immunology* 37:841
140. Gleich, G. J., Olson, G. M., Loegering, D. A. 1979. The effect of ablation of eosinophils on immediate-type hypersensitivity reactions. *Immunology* 38:343
141. Cohen, S. G., Ottesen, E. A. 1983. The eosinophil, eosinophilia and eosinophil-related disorders. *Allergy: Principles and Practice*, E. Middleton, Jr., C. E. Reed, E. F. Ellis, p. 701. St. Louis, MO: C. V. Mosby
142. Horn, B. R., Robin, E. D., Theodore, J., Van Kessel, A. 1975. Total eosinophil counts in the management of bronchial asthma. *N. Engl. J. Med.* 292:1152
143. Gleich, G. J., Frigas, E., Loegering, D. A., Wassom, D. L., Steinmuller, D. 1979. Cytotoxic properties of the eosinophil major basic protein. *J. Immunol.* 123:2925
144. Frigas, E., Loegering, D. A., Gleich, G. J. 1980. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab. Invest.* 42:35
145. Naylor, B. 1962. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 17:69
146. Dunnill, M. S. 1971. The pathology of asthma. In *The Identification of Asthma*, R. Porter, J. Birch, p. 35. London: Churchill-Livingstone
147. Cutz, E., Levison, H., Coopers, D. M. 1978. Ultrastructure of the airways in children with asthma. *Histopathology* 2:407
148. Cardell, B. S., Pearson, B. R. S. 1979. Death in asthmatics. *Thorax* 14:341
149. Frigas, E., Loegering, D. A., Solley, G. O., Farrow, G. M., Gleich, G. J. 1981. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. *Mayo Clin. Proc.* 56:345
150. Filley, W. V., Ackerman, S. J., Gleich, G. J. 1981. An immunofluorescent method for specific staining of eosinophil granule major basic protein. *J. Immunol. Meth.* 47:227
151. Filley, W. V., Holley, K. E., Kephart, G. M., Gleich, G. J. 1982. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. *Lancet* 2:11
152. Jorg, A., Henderson, W. R., Murphy, R. C., Klebanoff, S. J. 1982. Leukotriene generation by eosinophils. *J. Exp. Med.* 155:390
153. Henderson, W. R., Harley, J. B., Fauci,

Annu. Rev. Immunol. 1984.2:429-459. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

A. S., Klebanoff, S. J. 1983. Leukotriene B_4, C_4, and D_4 generation by human eosinophils. *J. Allergy Clin. Immunol.* 71:138A

154. Weller, P. F., Lee, C. W., Foster, D. W., Corey, E. J., Austen, K. F., Lewis, R. A. 1983. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: Predominant production of leukotriene C4. *Proc. Natl. Acad. Sci. USA* 80:762b
155. Ziltener, H. J., Chavaillaz, P.-A., Jorg, A. 1983. Leukotriene formation by eosinophil leukocytes: analysis with ion-pair high pressure liquid chromatography and effect of the respiratory burst. *Hoppe-Seyler's Z. Physiol. Chem.* 364:1029
156. O'Donnell M. C., Ackerman S. J., Gleich G. J., Thomas L. L. 1983. Activation of basophil and mast cell histamine release by eosinophil granule major basic protein. *J. Exp. Med.* 157:1981.
157. Gleich, G. J., Frigas, E., Filley, W. V., Loegering, D. A. 1984. Eosinophils and Bronchial Inflammation. In: *Asthma: Physiology, Immunopharmacology and Treatment*, ed. K. F. Austen, L. M. Lichtenstein. NY: Academic. In press, with permission)
158. Peters, M. S., Schroeter, A. L., Kephart, G. M., Gleich, G. J. 1983. Localization of eosinophil granule major basic protein in chronic urticaria. *J. Invest. Dermatol.* 81:39.
159. Peters, M. S., Schroeter, A. L., Gleich, G. J. 1983. Immunofluorescence identification of eosinophil granule major basic protein in the flame figures of Wells' syndrome. *Br. J. Dermatol.* 109:141
160. Hardy, W. R., Anderson, R. E. 1968. The hypereosinophilic syndromes. *Ann. Int. Med.* 68:1220
161. Chusid, M. J., Dale, D. C., West, B. C., Wolff, S. M. 1975. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. *Medicine* 54:1
162. Parrillo, J. E., Borer, J. S., Henry, W. L., Wolff, S. M., Fauci, A. S. 1979. The cardiovascular manifestations of the hypereosinophilic syndrome: prospective study of 26 patients, with review of the literature. *Am. J. Med.* 67:572
163. Spry, C. J. F. 1982. The hypereosinophilic syndrome: clinical features, laboratory findings and treatment. *Allergy* 37:539
164. Tai, P.-C., Hayes, D. J., Clark, J. B., Spry, C. J. F. 1982. Toxic effects of human eosinophil secretion products on isolated rat heart cells in vitro. *Biochem. J.* 204:75
165. Gansler, H. 1956. Electronenmikroskopische Untersuchungen am Uterusmuskel der Ratte unter Follikelhormonwirkung. *Virchows Arch.* 329:235.
166. Bassett, E. G. 1962. Infiltration of eosinophils into the modified connective tissue of oestrous and pregnant animals. *Nature* 194:1259
167. Ross, R., Klebanoff, S. J. 1966. The eosinophilic leukocyte: fine structure studies of changes in the uterus during the estrous cycle. *J. Exp. Med.* 124:653
168. Tchernitchin, A., Roorijck, J., Tchernitchin, X., Vandenhende, J., Galand, P. 1974. Dramatic early increase in uterine eosinophils after estrogen administration. *Nature* 248:142
169. Lobel, B. L., Levy, E., Kisch, E. S., Shelesnyak, M. C. 1967. Studies on the mechanism of nidation. XXVIII. Experimental investigation on the origin of eosinophilic granulocytes in the uterus of the rat. *Acta Endocrinol.* 55:451
170. Rytomaa, T. 1960. Organ distribution and histochemical properties of eosinophil granulocytes in rat. *Acta Pathol. Microbiol. Scand.* 50 (Suppl. 140):1
171. Lucas, F. V., Neufeld, H. A., Utterback, J. G., Martin, A. P., Stotz, E. 1955. The effect of estrogen on the production of a peroxidase in the rat uterus. *J. Biol. Chem.* 214:775
172. Tchernitchin, A. 1967. Autoradiographic study of (6,7-^{3}H)oestradiol-17B incorporation into rat uterus. *Steroids* 10:661
173. Clark, K. E., Farley, D. B., Van Orden, D. E., Brody, M. J. 1977. Estrogen-induced uterine hyperemia and edema persist during histamine receptor blockade. *Proc. Soc. Exp. Biol. Med.* 156:411
174. Pepper, H., Lindsay, S. 1960. Levels of eosinophils, platelets, leukocytes, and 17-hydroxycorticosteroids during normal menstrual cycle. *Proc. Soc. Exp. Biol. Med.* 104:145
175. Tchernitchin, A., Hasbun, J., Pena, G., Vega, S. 1971. Autoradiographic study of the in vitro uptake of estradiol by eosinophils in human endometrium. *Proc. Soc. Exp. Biol. Med.* 137:108
176. Maddox, D. E., Kephart, G. M., Coulam, C. B., Butterfield, J. H., Benischke, K., Gleich, G. J. 1984. Localization of immunoreactive eosinophil major basic protein in human placenta. *J. Exp. Med.* In press

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

Annu. Rev. Immunol. 1984.2:429-459. Downloaded from arjournals.annualreviews.org
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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

Annu. Rev. Immunol. 1984.2:429-459. Downloaded from arjournals.annualreviews.org
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COMPLEMENT AND BACTERIA:

Chemistry and Biology in Host Defense

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INTRODUCTION

The last several years have seen an enormous expansion in our understanding of the details of complement biochemistry and in our appreciation of the role of complement proteins in biologic phenomena. In this review we explore one important aspect of complement function—the role of complement in host defense against bacterial infection. We examine the mechanisms that govern the interaction of complement with bacteria, the role of complement as one of the principal heat-labile opsonin systems in serum, and the events that govern the ability of complement proteins to attack the bacterial cell wall and to lyse the microorganism. We attempt to acquaint the reader with the major recent developments in complement action and biology, assuming a fairly detailed knowledge of complement biochemistry and protein structure.

THE COMPLEMENT SYSTEM

The complement system is comprised of a series of glycoproteins that circulate in the extracellular fluid compartment (1,2,3). These molecules interact in a precise sequence of reactions leading to the production of biologically active cleavage fragments capable of interacting with microorganisms and cells, promoting opsonization on the one hand and direct cell damage on the other. Two major pathways of complement activation are recognized. The classical pathway (CP) is generally activated by the interaction of antibody of the appropriate class and subclass with an antigenic surface. In humans, IgG 1,2, and 3 and

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IgM are capable of activating the CP. Activation involves binding of the macromolecule C1 to the Fc region of the antigen-bound antibody with subsequent activation of the C1 molecule. This activation leads to the generation of an enzyme, C1 esterase, capable of interacting with and cleaving the next two components in the complement cascade, C4 and C2. C1 esterase binds and cleaves C4 with the release of the small cleavage fragment C4a from the C4 molecule. The C4b molecule is capable of interacting with the antigenic particle surface where it can bind covalently, thus continuing the complement cascade. The binding of C4b is similar in many respects to the binding of C3b. We discuss the binding of C3b in the detail below and therefore simply add here that C4b bound to a particle surface is capable of binding the next component in the cascade, C2. C2 bound to the C4b can be cleaved by C1 esterase to form a complex of C4b and C2a, which in turn is capable of cleaving the next component in the cascade, C3. C3 is cleaved into the small and large fragments, C3a and C3b. Following cleavage, the small fragment, C3a, with various inflammation-promoting properties, is released from the C3b molecule. C3b can bind covalently to the complement-activating surface, and can, in turn, bind C5. The C4b2a3b enzyme complex is capable of cleaving bound C5, leading to release of another biologically important fragment, C5a. The larger fragment, C5b, can interact with the next component in the complement cascade, C6, to form a protein complex, C5b6, capable of interacting noncovalently with biological membranes. In the presence of C7, the next component in the complement cascade, insertion of the C5b67 complex into lipid bilayers is initiated. Where on the more complex surface of a bacterium C5b67 may reside is not yet clear; the available data are discussed in detail below. In the presence of C8 and C9, a "membrane attack complex" comprised of C5b, C6, C7, C8, and C9 is formed (4). This complex appears cylindrical on electron microscopy, apparently possessing a hydrophilic central channel and a hydrophobic outer surface. Insertion of this cylinder into a typical cell membrane leads to free communication of small molecules between the inside and outside of the membrane leaflet. The cell cannot maintain its osmotic stability, swells, and dies or lyses.

The second major pathway of complement activation has been termed the alternative pathway (AP) (2). The AP is activated by binding of C3 or C3b to a surface. In the presence of two additional proteins of the alternative pathway, factors B and D, the enzyme complex C3bBb is formed. This complex acts as a C3 convertase, just as the complex composed of C4b and C2a acts as a CP C3 convertase, Like the CP convertase. C3bBb is unstable and decays. Increased stability is provided by the binding of an additional protein of the alternative pathway, properdin. It is clear that immunoglobulin molecules of many classes can increase the rate of assembly of the AP convertase via mechanisms that are not totally clear. Nevertheless, the AP can be activated on the surface of

Annual Reviews

certain bacteria and particles in the absence of specific immunoglobulin. Presumably this involves the binding and attachment of C3 or C3b via an amide or ester linkage through the carboxyl of a thiolester group. The covalently bound C3 or C3b can interact with proteins of the AP to yield a C3-converting enzyme capable of amplifying C3 binding. Once C3b is bound to an activating surface, the later components can bind and activate, in precisely the same way as occurs via CP activation.

The interaction of C3 or C3b with factors B, D, and P leading to amplification of complement action on a particulate surface is regulated by proteins of the C3 inactivator system. This subject is discussed more fully in the section below on opsonization, since the process of complement activation at a particulate surface with binding of complement fragments to that surface is a key to the process of opsonization. Opsonization is of great importance in the host defense mechanism (discussed below). This process involves the interaction of complement fragments with specific receptors on the phagocytic cell surface. Complement may also mediate direct bacterial killing. The presence of C3b on a particle allows for assembly of the membrane attack complex. The precise point of attachment of this cylindrical complex to the cell wall will determine whether it has lytic potential or simply binds in a relatively harmless fashion (see below).

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COMPLEMENT AND OPSONIZATION

Since the turn of the century, it has been known that serum factors are required for the effective binding and ingestion of microorganisms by phagocytic cells. These serum factors have been termed opsonins, from the Greek *opsono,* "to prepare food for" phagocytes. A classic description of opsonin was given in 1906 by George Bernard Shaw in, "The Doctor's Dilemma". The hero, Sir Colenso Ridgeon, was closely patterned after the British scientist, Almroth Wright. "The phagocytes", Ridgeon says, "won't eat the microbes unless the microbes are nicely buttered for them. Well, the patient manufactures the butter for himself alright; that butter . . . I call opsonin." We know that the two principal classes of opsonin are specific serum antibody and complement component fragments. In most situations that have been examined carefully, antibody must bind via its antigen-combining site to the particle to be opsonic. It may then bridge the microorganism and specific surface receptors for the Fc portion of the antibody on phagocytic cells. Both IgG and IgA may mediate phagocytosis via this mechanism. IgG and IgM antibodies may also activate complement, with the deposition of opsonic complement proteins. Complement component fragments may also bind directly to bacterial surfaces without the intervention of antibody. In either case complement, too, may act as a ligand, binding covalently to the microorganism surface and at the same time

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noncovalently to specific receptors on the phagocytic cell. Such bridging increases the contact between microorganism and phagocytic cell, promoting phagocytosis. This interaction of complement fragments with specific phagocytic cell receptors may also induce a physiologic change in the cell, triggering phagocytosis, chemotaxis, secretion of lysosomal contents, etc (45).

A large body of information on the function of complement as an opsonin has accumulated within the last several years. It has been shown that a number of different complement proteins and protein fragments are capable of interacting with specific receptors on the membranes of phagocytic cells. These include the complement fragments C1q, C4b, C3b, C3bi, C3dg, C3d, and possibly C5 or C5b. The specific interactions and their consequences are discussed in detail below.

The experimental system that has been studied most fully examines the interaction of C3 and its various fragments bound to sheep erythrocytes with receptors for C3 on monocyte/macrophages and neutrophils. C3 can be activated by the C3 convertase generated by either the CP or AP. Intact C3 has an internal thiolester linkage between a glutamine and a cysteine within the alpha chain of this two-chain molecule forming a tight ring (5,6). On loss of the C3a portion of the molecule leaving C3b, the thiolester is exposed. Since it is highly reactive, it will either be hydrolyzed or bind to an appropriate nucleophilic substrate, forming either an ester or amide bond. Appropriate substrates for the formation of the covalent bond with C3b are provided by a number of elements on bacterial surfaces including antibody, components of the capsule, and components of the cell wall. A small proportion of C3b may be noncovalently bound but associated with the bacterial surface through hydrophobic or ionic interactions, as well. The biological distinction between covalently and noncovalently bound C3b has not been studied in detail. C3b bound to a particulate surface may participate in a number of biochemical interactions. It may interact with factors B and D of the AP to form a C3 convertase capable of further C3 deposition, allowing activation of the later complement components and triggering of the lytic mechanism (see below). C3b, residing on a particulate surface, can also interact with components of the C3-inactivator system, leading to the formation of a series of cytolytically inactive cleavage fragments as diagrammed in Figure 1. The pathway of interaction of C3b is determined by a number of factors, including the availability of proteins to interact with it in the local environment and the nature of the surface on which the C3b is deposited. C3b on some surfaces is selectively protected from the activity of the C3-inactivator proteins. On such surfaces (so called "protected sites"), C3b is protected from inactivation but can still interact with AP components to assemble an AP convertase. On the other hand, the C3 in sites that are not protected is rapidly cleaved and destroyed. The serum proteins responsible for cleavage and destruction of C3b include

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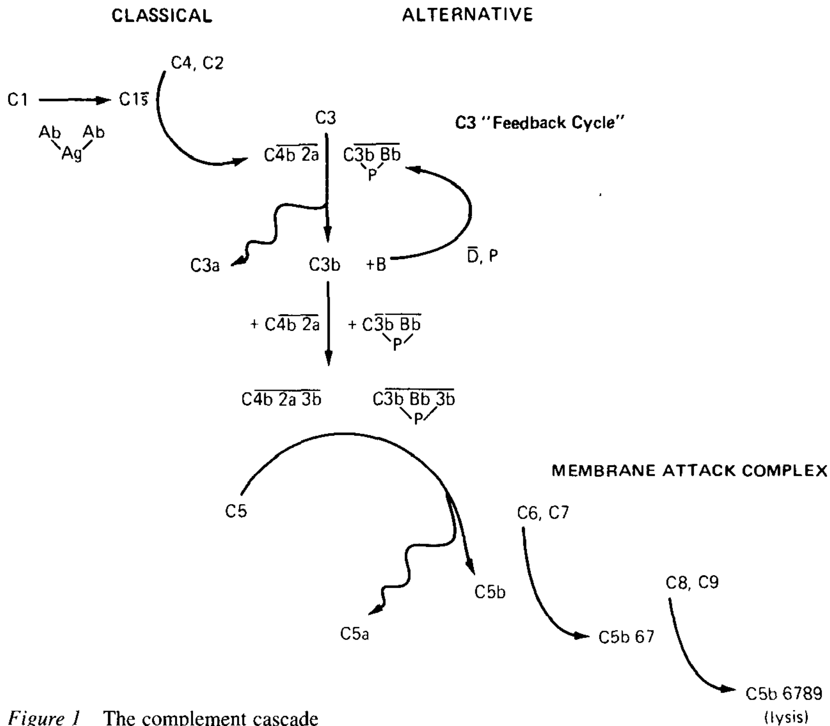

Figure 1 The complement cascade

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factors H and I. Factor H interacts with C3b, preventing activation of the AP by inhibiting interaction of C3b with factor B. In the presence of H, factor I, a serine protease, rapidly cleaves the C3b alpha chain into 68-kd and 43-kd fragments. A 3-kd fragment is thereby released. In serum, this reaction is rapid, but further cleavage of the C3 molecule (now termed C3bi) is slow. Because the covalent binding site of C3b to the microorganism resides in the 68-kd fragment of the alpha chain, following cleavage to C3bi the molecule remains bound to the surface of the microorganism. The intact beta chain remains linked by disulphide bonds to the 68-kd and the 43-kd alpha-chain fragments. Nonspecific proteases, such as those resident in the granules or on the surface of neutrophils can further cleave the 68-kd alpha-chain peptide C3bi into 41- and 27-kd fragments. The major portion of the molecule, termed C3c, is released from the bacterial surface, while the 41-kd peptide, termed C3dg, remains covalently bound. Further proteolytic digestion can release an 8-kd fragment, leaving a 33-kd fragment, C3d, still covalently bound.

Many of the intermediates in this degradation pathway of C3b bind specifically to phagocytic cell receptors. A number of these receptors have been isolated and characterized, and their biochemical specificity has been determined (7,8,9). In some cases the receptors not only bind C3 fragments but

Annual Reviews

also facilitate cleavage or further processing of the fragments. The precise biological role of each of these receptors is, however, still uncertain. Many studies of receptor binding have made use of techniques in which specific C3 fragments are deposited on test particles, such as sheep erythrocytes. The binding of these particles to the phagocytic cell surface ("rosetting") is detected by microscopy. Because none of these receptors shows total specificity for a single C3 fragment, the rosetting of various C3 fragments with more than one receptor has caused confusion. Progress has been made recently with the use of monoclonal antibodies that recognize specific phagocytic cell-membrane receptor proteins.

To date, three distinct receptors for portions of the C3 molecule have been identified (7,9,10). The receptor with the greatest affinity for C3b, termed CR1, is a 250-kd molecule that resides on the surface of B lymphocytes, all phagocytic cells, primate erythrocytes, and several other cell types. The molecule is poorly expressed on resting circulating phagocytic cells; but it appears that rapid "up-regulation" is seen with a variety of stimuli, such as lymphokines and C5a. The molecule binds particles coated with C3b to the phagocyte surface. C3b bound to the C3b receptor can be cleaved by factor I to C3dg even in the absence of factor H. Thus, cleavage of C3b bound to phagocytic cell-surface CR1 proceeds further than C3b cleavage in plasma, which essentially stops at the stage of C3bi. The biological consequences of this interesting series of reactions have not been fully explored.

A second C3 receptor has a higher affinity for C3bi than for C3b. The activity of CR3 is thought to reside in a membrane molecule with chains of 170 kd and 95 kd (10,11). This molecule appears to be one member of a family of receptors that share the same 95-kd beta chain but differ in expression of the alpha chain or 170-kd chain. Recent studies showing that children deficient in the expression of this protein are highly susceptible to bacterial injections (12) represent the best evidence that this receptor is important in phagocytosis. The C3d receptor (CR2) is not of immediate importance to the question of opsonization because it is not thought to be present on phagocytic cells.

In humans, the various complement receptors appear to be mobile in the plane of the phagocytic cell membrane (13,14). When phagocytic cells are plated on a surface to which IgG has been coupled, Fc receptors migrate to the attached side of the cell, leaving C3b receptors available on the exposed upper surface. Similarly, C3 receptors can be modulated to the underside of a cell on a surface coated with C3b, leaving other receptors, such as those for the Fc fragment of IgG, on the upper surface (13,15). Studies of particle phagocytosis have suggested that, under the right circumstances, both C3b and C3bi may mediate phagocytosis of test particles if present in sufficient quantity on the particle surface [reviewed in (7,9)]. However, the state of activation of the phagocytic cell, as well as the milieu in which the phagocytic

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cell exists, seems to play a major role in this process. Resting neutrophils and monocytes bind but do not phagocytize C3b-coated sheep erythrocytes. The particles form rosettes on the surface of the phagocytic cell. If the cell is triggered by a second signal, phagocytosis will proceed; the second signal may be provided by a few molecules of IgG antibody on the particle to be phagocytized (16). Similarly, if the cell has undergone "activation," C3b or C3bi may be a sufficient ligand alone to mediate phagocytosis (17). The biochemical correlates of the activation process are unknown, but clearly marked stimulation of the cell appears to be required for such activation-induced phagocytosis to proceed. It has recently been shown that interaction of fibronectin with its receptor on monocytes can lead to direct phagocytosis of C3b-coated particles in the absence of IgG (18). This has provided a first clue to the potential physiologic initiators of "activation". In any case it is clear that C3 receptors on phagocytic cells may exist in two states, one that leads to attachment without phagocytosis and a second that, like the IgG Fc receptor, mediates ingestion as well as attachment.

It is thought that all opsonins act by immobilizing the particle on the phagocytic cell, thereby promoting phagocytosis. Pseudopodia of the phagocytic cell flow about the bound particle, allowing ingestion to proceed. It has been suggested that the engulfment of the particle by pseudopodia is dependent upon a sequential interaction between opsonin on the particle surface and receptors for that opsonin on the surface of the pseudopodia of the phagocytic cell, drawing the cell membrane about the phagocytosed particle (the so-called "zipper hypothesis") (19,20). It has also been shown that C3b receptors upon cross-linking become associated with the cytoskeletal machinery of the cell (21). This association is not present before the receptors are cross-linked, again suggesting that such association may play a role in the phagocytic process. When these receptors are cross-linked under appropriate circumstances, processes such as enzyme secretion and the formation of active oxygen radicals are stimulated, suggesting that the interaction of the opsonin with these receptors does more than simply facilitate immobilization of the particle to be phagocytosed.

All of these concepts about complement-receptor function are derived from data obtained with defined test particles. The data on the opsonization of bacteria or other microorganisms are much less complete. Ironically, the first demonstration of a cell-surface receptor for a C3 fragment was made in studies of the phagocytosis of *S. pneumoniae* and *Treponema pallidum* (22). Both of these organisms were more efficiently phagocytosed by a mixture of phagocytic cells, serum, and human erythrocytes than by phagocytic cells and serum alone. This was shown to result from a specific interaction of complement bound to the microorganisms with erythrocyte receptors. It was suggested that erythrocytes, which bear on their membranes a molecule since identified as

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CR1, serve as a surface for attachment and immobilization of the microorgan isms, thereby facilitating phagocytosis by the blood neutrophils. As is dis cussed in detail in a later section, the pathway of activation, the presence o antibody and its specificity, and the structure of the microorganisms influence the binding, localization, and effectiveness of complement components a: opsonins. A detailed understanding of opsonization will require the carefu analysis of the mechanisms underlying C3 deposition on the surface of a wide variety of microorganisms, a study of the biochemical structures on the micro organism surface to which the C3 attaches, and a detailed analysis of the availability of C3 to interact with proteins of the alternative pathway, late components of the CP, and the proteins comprising the inactivator system. I will also require a far more advanced understanding of complement receptor and the process of phagocytosis itself. Needless to say, much work remain to be done.

MECHANISM OF COMPLEMENT ACTIVATION BY BACTERIA

Bacteria activate serum complement by a number of different mechanisms Specific antibody to bacterial cell-surface constituents can bind and activate serum C1, leading to activation of the entire CP. Direct demonstration o activation of late-acting complement components in serum by gram negative bacterial lipopolysaccharide (LPS) in the absence of specific antibody was one of the strongest arguments for the existence of an AP. It is now clear tha antibody may facilitate this activation process. In addition, constituents o bacteria other than LPS are capable of direct AP activation as well as antibody independent direct activation of the CP.

Complement activation leads to deposition of the opsonic complement frag ments as well as the bactericidal complex (C5b-9). The location and nature o binding of these complement constituents vary depending on the compositio and organization of the activating surface, but they play a critical role i determining the biological effectiveness of the opsonic and bactericidal mol ecules. Specific antibody, acting through either the CP or AP, may direc complement deposition to biologically effective sites.

Complement and Gram Positive Bacteria

The interactions of gram positive cell walls with the AP have been dissected extensively. The major components of the gram positive cell wall are peptid oglycan, which consists of large polymers of the disaccharide of n-acety glucosamine-n-acetyl muramic acid, and teichoic acid, which is covalently linked to the peptidoglycan backbone (23). The thickness of the peptidoglycan layer of gram positive bacteria (20 nm) is much greater than that of gran

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negative bacteria (2.5 nm) and contributes to the retention of gentian violet in the gram stain procedure. Although the disaccharide backbone of peptidoglycan is the same for all prokaryotes, the cell-wall teichoic acid is variable, even among gram positive bacteria. For example, streptococcal teichoic acid contains ribose and is termed riboteichoic acid, while staphylococcal teichoic acid is a lipoteichoic acid. Despite these differences, all gram positive cell walls appear to activate the AP efficiently in non-immune serum, leading to the deposition of C3b onto the bacterial cell wall (24,25). Further characterization of the cell-wall component that activates complement has been less definitive. Dissociation of the teichoic acid from peptidoglycan requires harsh temperatures and pHs, with denaturant effects on the component structures themselves. Given this caveat, Winkelstein & Tomasz examined the interactions of isolated pneumococcal peptidoglycan and teichoic acid with the AP of guinea-pig serum and concluded that the teichoic acid moiety was responsible for activation (26). On the other hand, similar experiments with *Staphylococcus aureus* suggested that purified peptidoglycan and teichoic acid both could activate the AP in human serum, but that on the intact organism the peptidoglycan was primarily responsible for activation (27). Whether the differences between the two studies result from the use of different organisms, different complement sources, differences in peptidoglycan purification, or some other factor is unknown. Moreover, since these experiments were done before the details of AP activation and control were understood, no molecular explanation for the ability of the various cell-wall components to activate complement was offered. More recent work on complement interactions with *S. pneumoniae* has shown that C3b on intact pneumococci has a 10-fold greater affinity for factor B than for factor H (28). Thus pneumococcal cell walls most likely activate the AP because formation of the AP C3 convertase, C3bBb, is favored over the inactivation reaction. However, these more recent studies did not dissect the molecular interactions of the AP with the isolated teichoic acid or peptidoglycan.

Little is known about the potential contributions of other constituents of gram positive cell walls to complement activation. Studies of two constituents indicate that protein components of cell wall may exist that are important in the interaction of an intact bacterium with complement. The M protein of group A *Streptococcus pyogenes* has been known for some time to be antiphagocytic (29). Probably, this antiphagocytic effect occurs because M protein inhibits interaction of intact streptococcal cell walls with complement, since M protein–deficient streptococcal strains activate the AP far more efficiently than AP-sufficient strains (30).

Protein A of *S. aureus* is a cell-wall protein known to bind to the Fc region of IgG from various species. It has been suggested that this might confer protection from host phagocytic cells on the *S. aureus*, and, indeed, most

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clinical isolates of *S. aureus* contain protein A. However, isolated IgG–proteir A complexes activate complement via the CP in vitro (31), and studies o: phagocytosis suggest that protein A in the cell wall actually increases com- plement-mediated phagocytosis of staphylococci (32). Thus, the net effect o: protein A on host defense against *S. aureus* remains unknown.

Complement and Gram Negative Bacteria

The interaction of complement with gram negative organisms is significantl different from that just described for gram positive bacteria. The difference i: ascribable to specific constituents within the gram negative cell wall. Th gram negative bacterial cell wall is a trilamellar structure, composed of ai outer membrane, a thin peptidoglycan layer, and an inner or cytoplasmi membrane. The outer leaflet of the outer membrane contains lipopolysac charide (LPS) and many proteins serving passive and active transport function: for ions and nutrients. LPS contains three covalently-linked domains: lipid A core polysaccharide, and O-polysaccharide. Lipid A consists of a diglucosa mine subunit with attached fatty acids. The core polysaccharide contains res idues unique to prokaryotes [2-keto 3-deoxyoctonate (KDO) and heptoses] an is relatively invariant among a wide variety of gram negative bacteria. Th O-polysaccharide, composed of repeating subunits of oligosaccharides, impart serologic specificity to gram negative organisms.

Gram negative bacteria, unlike gram positive organisms, can activate com- plement by both the AP and the CP in the absence of antibody. Cooper & Morrison (33) showed that both purified lipid A and so-called deep rough LP (lipid A-KDO) were capable of direct CP activation. This finding with purifie LPS has been confirmed and expanded in a number of laboratories, with th following observations: *(a)* Most types of rough LPS directly bind C1q or $C\bar{1}$ (activated C1). *(b)* The capacity of LPS to bind C1q is modulated by th addition of polysaccharide, since smooth LPS cannot activate the CP in serun (34). *(c)* Studies of bacteria possessing smooth LPS or rough LPS with dif fering extent of core polysaccharide deficiency suggest that stable binding o $C\bar{1}$ to full core or smooth LPS occurs predominantly at low ionic strengths *(d)* Both rough (J5) and smooth (0111B4) strains of *E. Coli* activate precurso C1, but the rough isolate activates about ten times more rapidly and bind: three times more C1. *E. coli* J5 but not 0111B4 binds C1s (35). It is clear tha intact bacteria possessing rough LPS can activate the CP in an antibody independent fashion (36,37), although noncomplement factors may be require for completion of complement activation (38).

Lipopolysaccharide in the absence of antibody is the prototypic activator o the AP pathway. LPS was used by Pillimer (39) in his initial studies of the AI and has been studied extensively more recently. Current understanding of AI activation suggests that LPS provides a "protected site" for C3b depositio

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and C3 convertase formation, a site at which inactivation of bound C3b by factors H and I is relatively inefficient. This idea was confirmed for LPS from *E. coli* O5 by the demonstration that the affinity of factor H for C3b bound to the LPS molecules was far lower than for factor B binding to C3b (40). It appears that the polysaccharide portion of the LPS molecule can activate the AP (34). LPS from gram negative bacteria are heterogeneous in size, since the length of the O-polysaccharide side chain attached to the lipid-A core molecule can vary from a single O-antigen repeating unit to greater than 55–60 O-antigen repeating units. The recent studies of Grossman & Leive (41), using LPS-coated erythrocytes, demonstrate that LPS molecules bearing as few as 5 O-antigen repeats are as efficient as LPS molecules bearing 18–40 O-antigen repeats in activating the AP.

It appears that substantial differences exist between different LPS O-polysaccharides in their capacity to activate complement. Galanos et al demonstrated major differences between the LPS from various *Salmonella* and *E. coli* strains in their ability to deplete CH_{50} activity from serum (42). It appears likely that differences in the degree of LPS aggregation played a role in these findings (43). Likewise, Morrison et al (44) demonstrated a substantial difference in the capacity of LPS from different organisms and of different serotypes to elicit a serum-dependent respiratory burst from neutrophils. The fine specificity of the differential AP-activating capacity of LPS was demonstrated in studies comparing LPS from two *Salmonella* strains varying only in the substitution of abequose for tyvulose (epimers of one another) in the O-antigen repeat unit of the O-polysaccharide (41).

Complement and Bacterial Capsules

For both gram positive and gram negative bacteria, the presence of a capsule is a major determinant of the ability of complement to opsonize the bacterium effectively. This has been studied with a number of gram positive organisms. For some bacteria, such as type III, group B streptococci, the capsular polysaccharides apparently totally inhibit complement activation by the organisms (45). This inhibition occurs because the terminal capsular carbohydrate repeating unit contains a sialic acid. Thus the portion of this streptococcus exposed to the solvent consists entirely of sialic acid. Its removal or modification allows complement activation by the organism, primarily by the AP. The profound inhibitory effect of cell-surface sialic acids has been shown in a number of other systems. For example, on sheep erythrocytes, removal of sialic acid leads to AP activation. Here the sialic acid apparently increases the affinity of factor H for cell-bound C3b, leading to the abolition of further AP activation. A similar mechanism may account for the inhibitory effect of sialic acid on complement activation by group B streptococci; however this has not been formally demonstrated. It is interesting that antibody to the group B

Annual Reviews

capsule allows complement activation by the AP (46). The authors suggest that specific antibody interferes with the inhibitory effect of the sialic acid on AP activation. The bacterial structures that activate the AP in the absence of sialic acid or in the presence of antibody have not been identified but are likely to be cell-wall components. It has been shown that clinical isolates of Type I group B streptococci may activate the CP in the absence of antibody, but the activating molecules remain likewise unidentified (47).

For some gram positive organisms, unlike group B strep, the bacterial capsule does not interfere with AP activation by the cell wall. A variety of techniques, including immunoelectron microscopy (48,49) and preferential enzymatic digestion (50), have been used to demonstrate that complement activation and the deposition of C3b and terminal complement components occur at the bacterial cell wall of *Streptococcus pneumoniae* and *Staphylococcus aureus* below the polysaccharide capsule. This subcapsular complement activation occurs despite the inability of the capsular polysaccharide itself to activate complement. The difference in ability to activate complement between the capsular polysaccharide and the cell-wall has been studied with Type 7 and Type 12 pneumococci. C3b deposited onto the cell wall of either organism has a higher affinity for factor B than for factor H. Thus, in serum, the formation of C3bB, the proenzyme of the alternative pathway C3 convertase is favored at the cell wall and the complement cascade enters a positive feedback loop of amplification of C3 cleavage and C3b deposition. C3b deposited on the pneumococcal capsule has a low affinity for factor H similar to that of cell-wall C3b. However, affinity of capsular C3b for factor B is only about 1/50 of the affinity of cell-wall C3b. Thus, for C3b molecules deposited on the pneumococcal capsule, C3bB formation is inefficient at serum concentrations of factor B, and there is little amplification of C3b deposition (28).

The biological effectiveness of opsonic complement components is critically dependent on their localization on the bacterial surface. The localization of C3b on the cell wall deep to the capsule after opsonization of pneumococci and staphylococci in non-immune serum may largely explain the well-known "antiphagocytic effect" of the polysaccharide capsules. From the perspective of the bacteria, the increased survival advantage of a capsule that does not activate complement may explain the selective pressure that has led to the almost exclusive persistence of pneumococcal capsules that do not activate complement (51).

The interaction of complement with polysaccharide capsules on gram negative bacteria has been less extensively investigated. Certainly, most of the isolated capsules studied do not activate complement efficiently. The K1 capsule of *E. coli* and the type B capsule of *N. meningiditis* consist of a homopolymer of sialic acid and thus represent the prototype of a molecule incapable of activating the AP. It is obvious, as discussed in more detail below, that even the presence of the K1 capsule does completely block subcapsular com

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plement activation by LPS on encapsulated gram negative organisms. Nonetheless, the presence of gram negative bacterial capsules that do not activate complement likely provide the organisms with a physical antiphagocytic barrier, just as occurs with gram positive bacteria (52).

THE EFFECTS OF OPSONIC ANTIBODY AND C-REACTIVE PROTEIN ON COMPLEMENT ACTIVATION BY BACTERIA

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The protective effect to the host of complement activation by invading bacteria is greatly potentiated by the presence of specific antibacterial antibodies. For most virulent organisms, either active or passive immunization of the host before challenge leads to a marked increase in LD_{50}. As mentioned above, specific antibody may actually convert an organism that does not activate complement in non-immune serum, such as a Type III, group B streptococcus, to one that is effectively opsonized by complement activation and C3b deposition. However, an increased rate of complement activation may not be the only mechanism by which organism-specific antibodies affect host defense. Antibody directed against capsular polysaccharides of encapsulated organisms may also have major effects on the localization of complement deposition. In the presence but not the absence of anticapsular antibodies, complement activation occurs on the pneumococcal and staphylococcal capsules, leading to the deposition of C3b onto the capsule (48,53). This leads to much increased efficiency of interaction with the phagocyte C3b receptors and to increased phagocytosis and more effective host defense. This can occur in the absence of a detectable difference in the amount of C3b actually deposited onto the pneumococcus.

The importance of antibody isotype in complement-mediated host defense against bacteria has been examined in only a few circumstances. The relative efficiency of IgG and IgM antipneumococcal capsular polysaccharide antibodies has been examined for complement-dependent in vivo bacterial clearance. In these studies, as few as 100 molecules per organism of anticapsular IgM significantly increased the rates of bloodstream clearance of pneumococci in guinea pigs, whereas 1200–1400 molecules per organism of IgG were required for the same effect. The antibody-dependent increases in bacterial clearance were complement-dependent, and the differences between IgM and IgG in vivo were reflected in their relative abilities to activate complement in vitro (54). Thus, the differences between IgM and IgG in host defense were postulated to reflect directly the differences in their intrinsic abilities to activate complement. This was consistent with what is known about the interaction of the two isotypes with C1, since it has been demonstrated that a single molecule of IgM is sufficient to activate the CP; but aggregates of IgG are required for efficient activation.

Annual Reviews

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An area that has attracted recent interest is the potential role of C-reactive protein (CRP) in host defense. This protein is a 115-kd pentraxin that binds specifically and with high affinity to phosphorylcholine (PC), a major component of cell-wall teichoic acids (55). The binding site for CRP is exposed only in gram positive organisms. The binding of CRP to pneumococci or to any surface containing PC has been shown to lead to CP activation (56). CRP also causes inhibition of intrinsic AP activation by the pneumococcal cell wall (57). CRP-mediated CP activation leads to C3b deposition onto the pneumococcal cell wall, as is the case for AP activation in nonimmune serum (58). The demonstration that CRP activates complement, as well as the remarkable increase in its synthesis during infection, led to a direct exploration of its role in host defense. Passive administration of CRP confers protection on mice from challenge by some encapsulated pneumococci (59,60). While this is of great interest, the mechanism of this protection is unknown, and there is yet no evidence that it depends upon complement activation . That it may have a significant role in host defense is supported by similar protection obtained with antiphosphorylcholine antibodies, which also bind to the pneumococcal cell wall below the capsule (61).

THE ROLE OF COMPLEMENT IN BACTERIAL CLEARANCE FROM THE BLOOD STREAM

The study of host defense against bacteremia has interested immunologists since the early 20th century. The rate of clearance of injected bacteria from the bloodstream of laboratory animals was first studied by Bull in 1916 (62). The major effects of immunization on clearance rates were first demonstrated by Hedley Wright in 1927 (63). At that time the complement cascade was insufficiently understood, and the role of complement in opsonization had not yet been discovered. Hence, it was not until the 1960s and the work of Biozzi (64), Banacerraf (65), and Spiegelberg (66) that the role of complement in the bloodstream clearance of bacteria was examined. In those studies, complement depletion by immune complexes or immunoglobulin aggregates led to decreased bacterial clearance and decreased sequestration of bacteria by hepatic macrophages. Recent studies of the role of complement in host defense against bacteremia have confirmed these observations and have, by use of modern reagents, led to the dissection of the role of complement activation in bacterial clearance. Experimental animals depleted of C3 by cobra venom factor had a marked defect in pneumococcal clearance; the LD_{50} of Type 7 pneumococci was 4 logs lower in these animals than in guinea pigs with normal amounts of C3 both in the presence and absence of antipneumococcal antibody. On the other hand, pneumococcal clearance was similar in normal guinea pig

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and animals with a total genetic absence of C4 (67). Thus in the non-immune animals, complement-mediated host defense against this organism occurs mainly via AP activation. However, C4-deficient guinea pigs do show a suboptimal increase in clearance with IgG anticapsular antibody, and they show no response to IgM. This suggest that anticapsular antibodies confer their protective effect by activation of the CP. Recent studies with three isogenic strains of *Salmonella* varying only in O-polysaccharide structure demonstrate that susceptibility of these isolates to in vitro phagocytosis by macrophage and in vivo clearance is directly related to their capacity to activate the alternative pathway and to consume C3 from serum (41,68-70).

Earlier studies on host defense against bacteremia focused on the organs in which bacteria were cleared from the bloodstream. Studies in both animals and humans demonstrated that the liver and spleen, the so-called splanchnic reticuloendothelial organs, were the major sites of sequestration (71). Although differences in the relative rates of hepatic and splenic phagocytosis could be demonstrated for various organisms, little was known of the differences in opsonization that led to sequestration of bacteria in the liver or the spleen. Recent studies with the pneumococcus have suggested that complement may be a critical determinant of reticuloendothelial organ sequestration (72). Normal animals clear about three times as many radiolabeled pneumococci in the liver as in the spleen. Complement depletion leads to a major shift in the sequestration of organisms from the liver to the spleen. Conversely, anticapsular antibody, regardless of its isotype, causes increased hepatic sequestration. This effect of antibody is, like its ability to accelerate bloodstream sterilization, completely dependent on its ability to activate complement. Thus, hepatic clearance of bacteria in particular seems to be dependent on adequate complement opsonization of the organisms. The requirement for complement opsonization for effective hepatic clearance has been confirmed in vitro for *Salmonella typhimurium* using isolated perfused rat livers (73). Splenic clearance of organisms, on the other hand, continues unabated and may actually increase in animals treated with cobra venom factor. The requirements for entrapment of bacteria by splenic macrophages are clearly different than for Kupffer cells. This difference in phagocytic potency of the liver and the spleen may represent an intrinsic difference in the macrophage populations—i.e. that splenic macrophages may possess receptors for opsonins (as yet unidentified) that are not present on Kupffer cells. On the other hand, it may represent an increased sensitivity of the splenic macrophage to identical opsonins, perhaps because splenic anatomy allows contact between sinusoidal macrophages and blood-borne particles. Recent studies of the clearance of desialated erythrocytes in guinea pigs suggest that small amounts of deposited C3b may be opsonic for splenic macrophages in circumstances in which the particles are not cleared by the liver (74).

Annual Reviews

An interesting corollary of this difference between hepatic and splenic clearance of bacteria is the observation that for pneumococci, increased dependence on splenic clearance correlates with bacterial virulence (75). This was observed despite the fact that the encapsulated pneumococcal isolates tested activated complement equally and had equal numbers of C3b deposited on them during incubation with serum. Since, for each organism, C3b was deposited primarily on the cell wall rather than the capsule in the bloodstream of non-immune guinea pigs, less virulent organisms presumably allowed greater access of the Kupffer cell C3b receptors to cell-wall C3b. This emphasizes the critical nature and potential complexity of the exact architecture of the bacterium it its interactions with host defense systems. As predicted, anticapsular antibody, which led to complement deposition onto the bacterial capsule, abolished differences in clearance rate, sequestration, and virulence among the various pneumococcal strains (75).

Thus far, we have focused on the role of complement in bacterial clearance. However, when complement is activated intravascularly, phlogistic peptides are generated, especially C3a and C5a, which also have important biological consequences. Intravascular C5a leads to neutrophil "activation" and potentially to their increased adherence and aggregation in capillary beds. This is probably a major factor in the neutropenia associated with severe sepsis and is seen in experimental animals injected intravascularly with bacteria. Moreover, C5a-mediated entrapment of neutrophils in capillary beds can lead to degranulation, release of proteolytic enzymes, superoxide radicals, and singlet oxygen, with local tissue destruction (76). This may explain in part the development of the shock lung syndrome that sometimes accompanies bacteremia. The dependence of this damaging chain of events on C5 activation by the invading bacteria has been shown in an animal model (77).

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INVOLVEMENT OF COMPLEMENT IN INTRACELLULAR KILLING OF BACTERIA

Little is understood about the role of complement in the intracellular killing of bacteria once the process of phagocytosis is completed. Leigh et al (78) have made the fascinating observation that human monocytes do not kill phagocytosed *S. aureus, S. epidermidis, or E. coli* efficiently if they are removed from a serum-containing environment. Moreover, extracellular IgG and C3b can restore efficient intracellular killing by the monocytes. This effect of IgG is receptor-mediated, since blockade or removal of C3b receptors and Fc receptors from monocytes abolished the enhancing effect of these proteins. The nature of the signal transmitted by occupancy of these receptors that results in increased monocyte killing of bacteria remains unknown.

Annual Reviews

MECHANISM OF COMPLEMENT-MEDIATED KILLING

Direct killing of gram negative bacteria by serum was the first identified function of the complement system. Since this discovery over 85 years ago, a large body of work has accumulated describing the biochemistry and biology of this process, the serum bactericidal reaction. This subject has been extensively reviewed recently (79,80). We concentrate here on selected aspects of the process, particularly those relating to the mechanism of complement action on bacteria and to the mechanism of bacterial resistance to complement-mediated killing.

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Locus of the Lethal Event

The locus of the lethal event for killing of gram negative bacteria by serum has not yet been determined unambiguously. Feingold, Goldman & Kuritz (81) reported that the initial site of complement action was on the outer membrane but that outer membrane damage in the absence of lysozyme was not synonymous with bacterial killing. Several other lines of evidence also suggest that outer-membrane damage to gram negative bacteria is not lethal per se.

In normal serum a two-step process for serum killing was postulated with serum-mediated damage to the outer membrane followed by subsequent lysozyme digestion of the peptidoglycan. It was appreciated that gram negative organisms may be killed but not lysed in lysozyme-depleted serum. This finding has been documented repeatedly and raises the question of how complement kills organisms in the absence of lysozyme-mediated digestion of the peptidoglycan. Two major possibilities exist. Complement may sequentially damage first the outer membrane and then the inner cytoplasmic membrane; alternatively, complement may damage simultaneously both the cytoplasmic and outer membranes. The latter hypothesis is supported by the demonstration of essentially simultaneous release of periplasmic and cytoplasmic markers from *E. coli* incubated in lysozyme-depleted serum (82). The suggestion was made that bactericidal C5b-9 was deposited at sites of apposition between the outer and inner membrane described by Bayer (83) since it seemed unlikely, based on the dimensions of the gram negative cell wall, that a single C5b-9 complex of length 16 nm could traverse the entire outer membrane (7.5 nm), peptidoglycan (2.5 nm), and periplasmic space (7.5 nm) to span the inner membrane (7.5 nm). Another study did not confirm this finding but instead demonstrated different kinetics of release of periplasmic and cytoplasmic makers from a rough *E. coli* strain incubated in lysozyme-depleted serum (84). Furthermore, neither these latter workers (85) nor others (86) were able to demonstrate C9 on the isolated inner membrane of serum-sensitive organisms after incubation in lysozyme-depleted serum. Since the fate or localization of

Annual Reviews

Bayer's junctions following separation of outer and inner membranes is unknown, these results do not provide information on the Bayer's junctions as sites for deposition of bactericidal C5b-9.

Loss of Cell-Wall Constituents and Effects on Metabolic Pathways

The consequences of complement attack on the gram negative cell wall have been studied thoroughly. Macromolecular phosphorus-containing compounds including predominantly phospholipids and LPS are released from serum-sensitive rough and smooth Enterobacteriaciae incubated in lysozyme-depleted serum (87,88). Specifically, predominantly phosphatidylethanolamine (PE) was released from serum-incubated, intrinsically labelled *E. coli* early in the reaction, although PE accumulation stopped after 10 min. However, the gradual and continued appearance of free fatty acids and lysophosphatidylethanolamine was detected over 60 min, suggesting that degradation of PE had occurred. That the degradation but not release of PE was due to bacterial phospholipase A was shown by similar experiments with a phospholipase A–deficient mutant of *E. coli* B (89,90,91). Of interest, several studies have demonstrated that release of ^{14}C-containing compounds, including phospholipids, does not occur when serum-resistant organisms are incubated in serum. These results support the notion that phospholipid release results from the detergent-like action of the C5b-9 complex on the bacterial cell wall and cytoplasmic membrane. In the absence of effective intercalation of the C5b-9 complex into the bacterial membrane, phospholipid release does not occur.

Serum Treatment and Bacterial Metabolism

Killing of bacteria by the serum complement system is obviously associated with cessation of macromolecular synthesis. Several investigators examining this issue have reported that bacterial killing, as assessed by culture, precedes a decrease or a cessation of RNA, DNA, and protein synthesis by 7, 15, and 30 minutes, repectively. The exact meaning of these results is unclear, however, since bacterial viability by culture reflects only the ability of plated organisms to grow over 16–24 hrs and cannot be used to exclude the possibility that cell death actually occurs after plating by the continued action of already deposited complement components C5b-9. The rapid kinetics of release of intracellular $^{86}Rb^{2+}$ by serum-treated rough strains of *E. coli* with delayed inhibition of RNA, DNA, and protein synthesis argues for rapid cell death as suggested by culture viability results. The state of bacterial metabolism at the time of complement attack influences serum sensitivity. Of particular note are the studies of Taylor & Kroll (84) and of Griffiths (92), who demonstrated that serum killing was inhibitable by uncouplers or inhibitors of oxidative

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phosphorylation such as 2,4-dinitrophenol and cyanide. Taylor & Kroll (84) also demonstrated increased ATP levels in bacteria treated with normal but not dialyzed serum and that the increase in ATP levels as well as restoration of killing occurred if glucose was added to the dialyzed serum. These workers suggested that optimal killing of gram negative bacteria requires bacterially generated ATP. The demonstration that killing is inhibited by inhibitors of protein synthesis such as chloramphenicol suggests that bacterial growth may be necessary for optimal killing, possibly by exposing sites for C5b-9 attack that are not present in stationary-phase organisms.

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Complement Requirements for Serum Killing

Many studies have demonstrated the requirement of complement components through C9 for effective bacterial killing (93,94). Only one study convincingly demonstrates bacterial killing in the absence of C9. Using *Neisseria gonorrhoeae* (GC), Harriman et al showed slow killing of two clinical isolates of GC by the serum of a patient with a congenital absence of C9, as well as by serum immunochemically depleted of C9 (95). Electron-microscopic evidence of spheroplast formation was provided, indicating that the killing sequence was likely related to cell-wall destruction by complement. Killing of other highly complement-susceptible bacteria in the absence of C9 will probably be demonstrated. It is less certain that killing of many strains of bacteria in the absence of C8 and C9 is possible, since we and others have been unable to demonstrate significant killing of even a deep rough strain of *S. minnesota* in C8-deficient sera.

Killing by C5b-9 Alone

Bacterial killing by C5b-9 in the absence of earlier complement components has been examined in several laboratories. Goldman & Austen (96) demonstrated that a strain derived from *E. coli* K12 was killed by C5b6, C7, C8, and C9, but only if the bacteria were pretreated with EDTA or EDTA was incorporated into the reaction mixture. An additional factor isolated from serum, eluting from the gamma and beta regions of an ion exchange resin and having an approximate molecular weight of 100,000, greatly augmented killing, but only when added after completion of C5b-9 formation. We have recently reexamined this issue using the system of acid activation to generate $C5b6^a$-9 from highly purified, high titer complement components C5,C6,C7,C8, and C9 (97). Our results demonstrated nearly 3 log killing of deep rough *Salmonella minnesota* Re 595, 1 log killing of rough *E. coli* J5, but minimal killing of a smooth, encapsulated *E. coli* 0111B4 strain in the presence of isolated $C5b6^a$-9.

Annual Reviews

BACTERICIDAL ANTIBODY

Antibody is required to achieve complement-mediated killing of many bacterial strains. The class and antigenic targets of bactericidal antibody have been extensively investigated. Although natural antibodies in serum may belong to the IgM, IgA, or IgG classes, the predominant natural antibodies to representative enterobacterial species on a weight basis contain IgM, which is 18-fold to 560-fold more active than IgG on a weight basis. The role of IgA in mediating the serum-bactericidal reaction in unclear. Some studies suggest that IgA might mediate the bactericidal reaction, perhaps via the alternative pathway. However, several other studies have failed to demonstrate that IgA can initiate the serum-bactericidal pathway.

The antigenic targets for bactericidal antibodies are diverse and include LPS, outer membrane proteins, capsular polysaccharide, and enterobacterial common Ag. Antibodies directed against pili or flagella are generally ineffective in mediating complement killing, perhaps because complement activation is being initiated at too great a distance from the bacterial outer membrane.

Antibody is often required for killing of gram negative bacteria via the alternative pathway and $F(ab')_2$ but not F(ab′) may be effective. It has been demonstrated that antibody is not functioning primarily to increase the extent of complement activation at the bacterial surface since bacterial killing required IgG even under conditions where equivalent numbers of C5b-9 were deposited on *E. coli* 0111 in the presence and absence of the antibody (97,98).

The bactericidal activity of antibody may be profoundly affected by the complement source. For example, Zollinger & Mandrell (99) have recently demonstrated that human postvaccination antibody to the meningococcal group B polysaccharide was strongly bactericidal with rabbit complement, but had little or no bactericidal activity when tested with human complement. Simultaneous studies with a murine IgM monoclonal directed against the meningococcal B capsule showed excellent bactericidal activity with either rabbit or human complement.

The presence of blocking antibody may interfere with the ability to demonstrate bactericidal activity of complement. This was first illustrated in the studies of Adler (100), who demonstrated that antibody could block the serum-mediated killing of *Salmonella*. Subsequently, Rice (101), Grifiss (102), McCutchan (103), and others have demonstrated the presence of both IgA and IgG-blocking antibodies for killing of *Neisseria gonorrhoeae* and *Neisseria meningitidus*. These antibodies are present in both normal human serum and in convalescent sera from patients infected with these organisms. Neither the mechanism of action nor the target sites for blocking antibody have been unequivocally elucidated in these systems. Rice (101), though, has provided evidence that IgG against gonococcal outer-membrane protein has markedly

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enhanced blocking activity in comparison to normal IgG, and does not react with gonococcal LPS by ELISA.

NONANTIBODY, NONCOMPLEMENT FACTORS REQUIRED FOR COMPLEMENT KILLING

Although studies with purified complement components suggest that killing of some organisms can occur in the absence of antibody, several workers have suggested requirements for additional nonantibody and noncomplement components in complement-mediated bacterial killing. The first such factor was reported by Goldman & Austen (96) as described above. Clas & Loos (38) recently reported that an extra factor from serum was necessary for killing of *Salmonella minnesota* Re 595 by the isolated components of the CP. The extra factor that apparently is required either for C4 binding or for C2 binding or cleavage is a euglobulin with a PI = 6.0; it is heat labile, inactivated by β-glucosidase, pronase, and proteinase K, and has molecular weight of approximately 210,000. The activity of the factor cannot be replaced by either IgG or IgM directed against the organism. No such factor was required in other studies demonstrating antibody-independent CP-mediated killing of *E. coli* J5 (8), suggesting that the factor of Clas & Loos may be specific for *Salmonella* or for the Re chemotype. More recently, Kawakami (104) reported the presence of a 28-kd heat- and 2-mercaptoethanol-labile factor in the serum of normal, nude, and germ-free mice; it reacts specifically with Ra but not Rb, Rc, Rd, or Re chemotype strains of *Salmonella* and killed the bacteria in conjunction with guinea-pig serum. Thus, a variety of nonantibody and noncomplement factors have been described that either enhance or are required for complement-mediated killing of various gram negative bacteria. These factors apparently share little biochemically or functionally with one another and demonstrate considerable organism specificity. More precise elucidation of their mechanism of action is required before general statements are possible regarding the role of extra factors in complement-mediated killing of gram negative bacteria.

BACTERIAL FACTORS ASSOCIATED WITH SERUM RESISTANCE

LPS

The presence of the smooth phenotype—e.g. long polysaccharide side chains in LPS—is the characteristic most closely associated with serum resistance in gram negative bacteria. The initial basis for this conclusion was the observation that rough Enterobacteriaceae, lacking long O-polysaccharide side chains, are susceptible to the serum bactericidal reaction whereas many smooth orga-

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nisms are serum-resistant (105,106). Additional evidence implicates long-chain LPS in serum resistance. Taylor & Robinson (107) demonstrated that transfer of the *rfb* locus, encoding for O-polysaccharide side chains, into a rough mutant of *E. coli* conferred relative complement resistance on the recipient strain. Goldman, Joiner & Leive (manuscript submitted) have shown that a serum-resistant strain of *E. coli* 0111B4, which had been derived from a serum-sensitive parent strain, contained a more extensive coverage of lipid A core subunits and longer polysaccharide side chains than the parent. Smooth LPS alone, however, is not able to mediate serum resistance for some organisms. Taylor (108) demonstrated little correlation between the amount of O-antigen polysaccharide extracted from isolates of *E. coli* from the urinary tract and their serum sensitivity or serum resistance. LPS from some resistant strains contained smaller amounts of O side-chain sugars than sensitive strains of the same O serotype. As the authors pointed out, the extent of substitution of lipid A core units may vary between strains in the face of equivalent amounts of O-polysaccharide. In fact, Grossman, Joiner & Leive (manuscript submitted) have recently demonstrated that subtle changes in the percent substitution of lipid A core molecules with O-polysaccharide and in the average length of the O side chain confer marked changes in serum sensitivity of a strain of *Salmonella montevideo*.

Capsules or Acidic Polysaccharides

A polysaccharide capsule was first associated with resistance to serum killing in the studies of Muschel. However, subsequent studies have yielded highly variable results when attempts were made to correlate K antigens and serum resistance (109,110). For example, transformation of a serum-sensitive rough *E. coli* strain for K27 capsule production did not result in any change in serum sensitivity (111). The largest body of evidence has been accrued with the well studied *E. coli* K1 capsule (112,113). The K1 capsule is identical to the capsule on group B meninogococcus and consists of a polymer of sialic acid. As discussed earlier, this structure is the prototype of a nonactivating surface of the AP since it should facilitate C3b cleavage by factor I. However, in the many investigations that examined serum killing of *E. coli* K1 strains, results vary. In general, the presence of K1 does not substantially impair serum killing. This conclusion is based on comparative studies of K1 and non-K1 isolates from clinical sources and on the failure to correlate amount of K1 with serum resistance. K1 is not produced in strains grown at 22°C, yet these strains retain the same serum-sensitivity profile organisms grown at 37°C which produce K1 normally. However, the most definitive experiments are those using isogenic strains of *E. coli* varying only in K1. Such studies have suggested that K1 partially blocks serum killing. Undoubtedly, the O-serotype of the organism involved, the presence or absence of anti-O antibodies in the serum source, and even the presence of anti-K1 antibodies influences the

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results of serum killing studies. These factors remain largely undefined in the above studies. The one consistent finding in the above reports is the failure of K1 strains to be opsonized for phagocytosis by the alternate pathway, the clear implication being that surface-exposed stable C3b is not deposited on the K1 capsule by AP activation (114).

Protein

A number of workers have shown an association between serum resistance and protein factors in the bacterial cell wall of *E. coli* (115,116). These factors include the 25-kd *tra* T protein, coded for by the R6-5 plasmid and present at approximately 21,000 copies per cell. Many other plasmids, such as R 100/NR 1 and R1, share sequence homology with R6-5 and are likely to confer serum resistance by means of the *tra* T gene product.

Specific forms of the major outer-membrane protein of *Neisseria gonorrhoeae*, PI, have also been associated with serum resistance in GC. For example, transformation of serum-sensitive strains of GC containing a high-molecular-weight PI with DNA from a serum-resistant isolate containing a low-molecular-weight PI resulted in stable serum-resistant transformants containing low-molecular-weight PI (117). Furthermore, a close relationship has been demonstrated between the presence of low-molecular-weight PI in GC and serum resistance. In addition, organisms isolated from cases of disseminated GC and pelvic inflammatory disease commonly show the PI of low molecular weight. However, in other studies the locus encoding for PI was shown to be distinct from but closely linked to loci encoding for serum-antibody-complement (sac) resistance (118,119). The gene products of these loci have not been identified.

Additional recent reports have suggested an association between the presence of specific bacterial outer-membrane proteins and serum resistance in *Yersinia enterocolitica* and *Aeromonas salmonicida* (120). However, no mechanism has been described for conferring serum resistance. The results with *Neisseria gonorrhoeae* reported above provide a note of caution in ascribing a cause and effect role to outer-membrane proteins and serum resistance.

MECHANISM OF RESISTANCE TO COMPLEMENT-MEDIATED KILLING

Extensive studies have defined the antibody requirements (see above) for serum killing and have outlined the bacterial factors associated with resistance to complement attack. The specific mechanism by which complement attack is inhibited or subverted has been less extensively investigated. The central question in this regard is whether complement resistance represents inefficient complement activation or instead represents subversion of the membrane attack

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process at some later step. There is now substantial evidence that the latter mechanism is operative in most circumstances. Many serum-resistant strains are opsonized well in non-immune serum, and C3b is demonstrable on the bacterial surface by immunofluorescence. Fierer (121) demonstrated equivalent deposition of C3 by immunofluorescence on a serum-resistant *E. coli* strain before and after conversion of the strain to a serum-sensitive form with diphenylamine. Reynolds et al (122) demonstrated C3 deposition but were unable to demonstrate C5b on a serum-resistant strain of *Salmonella* following incubation in C6-deficient rabbit serum. This finding was likely due to the instability of C5b binding to target sites and decay in C6 binding capacity of C5b. Ogata & Levine (123) initially reported that equivalent amounts of C3 and C5 were consumed by two *E. coli* strains varying in complement susceptibility due to the presence of the *tra* T protein in one isolate. This group has subsequently shown that C8 and C9 consumption did not differ when the two *E. coli* isolates were compared, suggesting that the relative complement resistance of the *tra* T–containing strain did not represent inability to activate complement through C9 (11).

We have systematically reinvestigated this subject. Initial studies compared the interaction of complement with a smooth, serum-resistant isolate of *Salmonella minnesota* and an isogenic, deep rough (Re), serum-sensitive mutant of the parent strain (125,126). Our experiments indicated that on the sensitive isolate the bulk of the C5b-9 was intimately associated with and probably inserted into hydrophobic portions of the outer membrane. Conversely, although complement was activated efficiently by the resistant isolate, and a C5b-9 complex was formed on the bacterial surface, the complex did not insert into hydrophobic domains of the outer membrane and was released from the surface. The attachment of C5b-9 to several different serum-sensitive rough strains (Rc and Ra chemotype) of *E. coli* and *Salmonella* and to the smooth serum-resistant parental strains was also tested. Results were entirely analogous to those with *S. minnesota* and suggest that the conclusions may apply to unencapsulated Enterobacteriaceae as a group. We have extended these observations by examining the molecular site of complement deposition on serum-resistant isolates of *E. coli* and *Salomonella.* Our experiments to date have demonstrated that the major acceptor site for C3 deposition on unencapsulated serum-resistant strains is the LPS (127). More importantly, C3 appears to attach preferentially to only the longest LPS molecules within the bacterial outer membrane (e.g. those molecules possessing the largest number of O-polysaccharide subunits). By using a strain of *Salomonella montevideo* in which LPS composition could be altered during growth, we have shown that the organism becomes serum-sensitive when the average LPS side-chain length and the percentage coverage of lipid A cores decrease. This conversion to serum sensitivity is accompanied by attachment of C3 to shorter-chain mol-

Annual Reviews

ecules of LPS. We have therefore postulated that in the normal cell, the LPS molecules containing long O-polysaccharide side chains sterically hinder access of C3b to the shorter molecules. When attached to short-chain-length LPS, the C3b provides a focus of attachment for C5b-9 near the outer membrane, facilitating complement-mediated killing.

We have recently turned our attention to the mechanism of serum resistance in *Neisseria gonorrhoeae* (GC) (128), an important biologic problem since strains causing disseminated gonococcal infection (DGI) in humans are almost invariably complement-resistant (129). In contrast to Enterobacteriaceae, GC have not been shown to possess smooth LPS. In GC there is a clear association of serum resistance with a particular form of the principal outer-membrane protein (PI) of this organism. It is also known that serum-resistant GC are not absolutely refractory to serum killing, as is the case with some Enterobacteriaceae, since virtually all resistant organisms can be killed by the addition of sufficient bactericidal antibody. However, some patients develop a blocking antibody that inhibits lysis of a serum-sensitive organism.

We confirmed data indicating equivalent terminal-component consumption and binding when sensitive and resistant strains are compared (130). However, the bound C5b-9 complex is in a different molecular configuration on the surface of resistant GC than on the surface of sensitive organisms or resistant isolates rendered sensitive with bactericidal antibody (128). We have recently found that bactericidal but not nonbactericidal C5b-9 on GC is associated with distinctive surface-iodinatable constituents in the organism and showed by electron microscopy that bactericidal C5b-9 has different associations with bacterial constituents than does nonbactericidal C5b-9.

We have also examined the molecular basis for resistance of gram positive organisms (pneumococci) to complement attack (131). A stable C5b-9 complex forms on the surface of both rough and type-7 *S. pneumoniae* when these organisms are incubated in serum. However, complement-mediated killing of these strains does not occur. It appears that the thick peptidoglycan cell wall of the gram positive organism serves as a physical barrier to C5b-9 disruption of the cytoplasmic membrane, a necessary requirement for serum killing. Thus, although the membrane-attack complex is stably bound to the bacterial wall, the inner-membrane bilayer is not disrupted.

Thus we have already observed three different mechanisms for bacterial resistance to complement-mediated killing: *(a)* shedding of the completed membrane-attack complex from the bacterial surface; *(b)* the formation of noncovalent interactions that prevent the membrane-attack complex from inserting into the bacterial outer membrane; and *(c)* the presence of a thick peptidoglycan layer shielding the vulnerable inner membrane from attack. Our studies have shown that antibody can mediate serum killing by increasing the bactericidal efficiency of C5b-9.

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Literature Cited

1. Atkinson, J. P., Frank, M. M. 1980. *Complement in Clinical Immunology,* ed. C.W. Parker, p. 219. Philadelphia: Saunders
2. Fearon, D. T., Austen, K. F. 1980. Current concepts in immunology—the alternative pathway of complement. *N. Engl. J. Med.* 303:259–263
3. Müller-Eberhard, H. J. 1980. Complement reaction pathways. In *Immunology 80, Progress in Immunology IV,* ed. M. Fougereau, J. Dausset, p. 1981. London: Academic
4. Mayer, M. M., Michaels, D. W., Ramm, L. F., Whitlow, M. B., Willoughly, J. B., Shin, M. L. 1981. Membrane damage by complement. *Crit. Rev. Immunol.* 2:133–37
5. Janatova, J., Lorenk, P. E., Schechter, A. N., Prahl, J. W., Tack, B. F. 1980. Third component of human complement: Appearance of sulfhydryl group following chemical or enzymatic inactivation. *Biochemistry* 19:4471–77
6. Pangburn, M. K., Müller-Eberhard, H. J. 1980. Relation of a putative thioester bond with C3 to activation of the alternative pathway and binding of C3b to biological targets of complement. *J. Exp. Med.* 152:1102–10
7. Berger, M., Gaither, T. A., Frank, M. M. 1981–82. Complement receptors. *Clin. Immunol. Rev.* 1:471–575
8. Biano, C., Nussenzweig, V. 1977. Complement receptors. *Contemp. Top. Molec. Immunol.* 6:145–50
9. Fearon, D. T., Wong, W. W. 1983. Complement ligand-receptor interactions that mediate biological responses. *Ann. Rev. Immunol.* 1:243–71
10. Beller, D. I., Springer, T. A., Schreiber, R. D. 1982. Anti-Mac 1 selectively inhibits the mouse and human type three complement receptor. *J. Exp. Med.* 156:1000–7
11. Sanchez-Madrid, Nagy, J. A., Robbins, E., Simon, P., Springer, T. A. 1983. A human leukocyte differentiation antigen family with distinct subunits and a common B subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKMI/Mac-1) and the p150,95 molecule. *J. Exp. Med.* 158:1785–1803
12. Dana, N., Todd, R. F. III, Pitt, J., Springer, T. A., Arnaout, M. A. 1984. Deficiency of a surface membrane glycoprotein (Mo 1) in man. *J. Clin. Invest.* In press
13. Michl, J., Pieczonka, M. M., Unkeless, J. C., Siverstein, S. C. 1979. Effects of immobilized immune complexes on Fc and complement receptor function in resident and thioglycollate-elicited mouse peritoneal macrophages. *J. Exp. Med.* 150:607–21
14. Petty, J. R., Smith, L. M., Fearon, D. T., McConnell, H. M. 1980. Lateral distribution and diffusion of the C3b receptor of complement HLA antigens and lipid probes in peripheral blood leukocytes. *Proc. Natl. Acad. Sci. USA* 77:6587–91
15. Horwitz, M. A. 1980. The roles of the Fc and C3 receptors in the phagocytosis and killing of bacteria by human phagocytes. *J. Reticuloendothel. Soc.* 28:175–265
16. Mantovani, B., Rabinovitch, M., Nussenzweig, V. 1972. Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin IgG. *J. Exp. Med.* 135:780–92
17. Bianco, C., Griffin, F. M., Silverstein, S. C. 1975. Studies of the macrophage complement receptor. Alteration of receptor function on macrophage activation. *J. Exp. Med.* 141:1278–90
18. Pommier, C. G., Inada, S., Fries, L. F., Takahashi, T., Frank, M. M., Brown, E. J. 1983. Plasma fibronectin enhances phagocytosis of opsonized particles by human peripheral blood monocytes. *J. Exp. Med.* 157:1844–54
19. Griffin, F. M. Jr., Bianco, C., Silverstein, S. C. 1975. Characterization of the macrophage receptor for complement and demonstration of its functional independence from the receptor for the Fc portion of immunoglobulin G. *J. Ex. Med.* 141:1269–77
20. Griffin, F. M. Jr., Griffin, J. A., Leider, J. E., Silverstein, S. C. 1975. Studies on the mechanism of phagocytosis. I. Requirements for circumferential attachment of particle bound liquids to specific receptors on the macrophage plasma membrane. *J. Exp. Med.* 142:1263–82
21. Jack, R. M., Fearon, D. T. 1983. Cytoskeletal attachments of C3b receptor (C3bR) and reciprocal Co-redistribution with Fc receptor (FcR) on human polymorphonuclear leukocytes (PMN). *Immunobiology* 164:258a
22. Nelson, R. A. 1953. The immune adherence reaction: an immunologically specific reaction between microorganisms and erythrocytes leading to enhanced phagocytosis. *Science* 118:733–37
23. Tomasz, A. 1981. Surface components

Annual Reviews

of *Streptococcus pneumoniae*. *Rev. Infect. Dis*. 3:190–211
24. Verbrugh, H. A., Van Dijk, W. C., Peters, R., Van Erne, M. E., Daha, M. R., Peterson, P. K., Verhoef, J. 1980. Opsonic recognition of staphylococci mediated by cell wall peptidoglycan: antibody independent activation of human complement and opsonic activity of peptidoglycan antibodies. *J. Immunol*. 124:1167–73
25. Winkelstein, J. A., Tomasz, A. 1977. Activation of the alternative pathway by pneumococcal cell walls. *J. Immunol*. 118:451–54
26. Winkelstein, J. A., Tomasz, A. 1978. Activation of the alternative complement pathway by pneumococcal cell wall teichoic acid. *J. Immunol*. 120:174–81
27. Wilkinson, B. J., Sisson, S. P., Kim, Y., Peterson, P. K. 1979. Localization of the third component of complement on the cell wall of encapsulated *Staphylococcus aureus*. Implications for the mechanisms of resistance to phagocytosis. *Infect. Immun*. 26:1159–63
28. Brown, E., Joiner, K., Gaither, T., Hammer, C., Frank, M. 1983. The interaction of C3b bound to pneumococci with factor H, factor I and properdin factor B of the human complement system. *J. Immunol*. 131:409–15
29. Lancefield, R. C. 1962. Current knowledge of type-specific M antigens on Group A streptococci. *J. Immunol*. 89:307–13
30. Horwitz, M. A. 1982. Phagocytosis of microorganisms. *Rev. Infect. Dis*. 4:104–123
31. Sjoquist, J., Stalenheim, G. 1969. Protein A from *Staphylococcus aureus*. IX. Complement-fixing activity of protein A-IgG complexes. *J. Immunol*. 103:467–73
32. Peterson, P. K., Verhoef, J., Sabath, L. D., Quie, P. G. 1977. Effect of protein A on staphylococcal opsonization. *Infect. Immun*. 15:760–64
33. Cooper, N. R., Morrison, D. C. 1978. Binding and activation of the first component of human complement by the lipid A region of lipopolysaccharides. *J. Immunol*. 120:1862–69
34. Morrison, D. C., Kline, L. F. 1977. Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS). *J. Immunol*. 118:362–69
35. Tenner, A. J., Ziccardi, R. J., Cooper, N. R. 1984. Antibody independent C1 activation by *E. coli*. *J. Immunol*. In press
36. Betz, S. J., Isliker, H. 1981. Antibody-independent interactions between *Escherichia coli* J5 and human complement components. *J. Immunol*. 127:1748–51
37. Clas, F., Loos, M. 1981. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S and R forms of *Salmonella minnesota*. *Infect. Immun*. 31:1138–44
38. Clas, F., Loos, M. 1982. Requirement for an additional serum factor essential for the antibody-independent activation of the classical complement sequence by gram-negative bacteria. *Infect. Immun*. 37:935–39
39. Pillimer, L., Schoenberg, M. D., Blum, L., Wurz, L. 1955. Properdin system and immunity. II. Interaction of the properdin system with polysaccharides. *Science* 122:5453–57
40. Pangburn, M. K., Morrison, D. C., Schreiber, R. D., Müller-Eberhard, H. J. 1980. Activation of the alternative complement pathway: Recognition of surface structures on activators by bound C3b. *J. Immunol*. 124:977–87
41. Grossman, N., Leive, L. 1984. Complement activation via the alternative pathway by purified *Salmonella* lipopolysaccharide is affected by its structure but not its O-antigen length. *J. Immunol*. 132:376–85
42. Galanos, C., Luderitz, O. 1976. The role of the physical state of lipopolysaccharides in the interaction with complement. *Eur. J. Biochem*. 65:403–8
43. Wilson, M. E., Morrison, D. C. 1982. Evidence for different requirements in physical state for the interaction of lipopolysaccharides with the classical and alternative pathway of complement. *Eur. J. Biochem*. 128:137–41
44. Morrison, D. C. 1983. Bacterial endotoxins and pathogenesis. *Rev. Infect. Dis*. 5:5733–47
45. Edwards, M. S., Kasper, D. L., Jennings, H. J., Baker, C. J., Nicholson-Weller, A. 1982. Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. *J. Immunol*. 128:1278–83
46. Edwards, M. S., Nicholson-Weller, A., Baker, C. J., Kasper, D. L. 1980. The role of specific antibody in alternative complement pathway mediated opsonophagocytosis of Type III, group B streptococcus. *J. Exp. Med*. 151:1275–87
47. Baker, C. J., Edwards, M. S., Webb, B. J., Kasper, D. L. 1982. Antibody independent classical pathway mediated

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Annual Reviews

opsonophagocytosis of type Ia, group B streptococcus. *J. Clin. Invest.* 69:394–404

48. Brown, E., Joiner, K., Cole, R., Berger, M. 1983. Localization of complement component 3 or *Streptococcus pneumoniae:* anticapsular antibody causes complement deposition on the pneumococcal capsule. *Infect. Immun.* 39:403–8
49. Wilkinson, B. J., Kim, Y., Peterson, P. K. 1981. Factors affecting complement activation by *Staphylococcus aureus* cell walls, their components, and mutants altered in teichoic acid. *Infect. Immun.* 32:216–24
50. Winkelstein, J. A., Abramovitz, A. S., Tomasz, A. 1980. Activation of C3 via the alternative complement pathway results in fixation of C3b to the pneumococcal cell wall. *J. Immunol.* 124:2502–6
51. Winkelstein, J. A., Bocchini, J. A., Schiffman, G. 1976. The role of capsular polysaccharide in the activation of the alternative pathway by the pneumococcus. *J. Immunol.* 116:367–70
52. Horwitz, M. A., Silverstein, S. C. 1980. Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by phagocytes. *J. Clin. Invest.* 65:82–94
53. Verbrugh, H. A., Peterson, P. K., Nguyen, B-Y. T., Sisson, S. P., Kim, Y. 1982. Opsonization of encapsulated *Staphylococcus aureus:* The role of specific antibody and complement. *J. Immunol.* 129:1681–87
54. Brown, E., Hosea, S., Hammer, C., Burch, C., Frank, M. 1983. A quantitative analysis of the interactions of antibody and complement in experimental pneumococcal bacteremia. *J. Clin. Invest.* 69:85–98
55. McCarty, M. 1982. Historical perspectives on C-reactive protein, in C-reactive protein and the plasma protein response to tissue injury. *Ann. NY Acad. Sci.* 389:1–10
56. Claus, D. R., Siegel, J., Petras, K., Osmand, A. P. Gewurz, H. 1977. Interaction of C-reactive protein with the first component of human complement. *J. Immunol.* 119:187–92
57. Mold, C., Gewurz, H. 1981. Inhibitory effect of C-reactive protein on alternative complement pathway activation by liposomes and *Streptococcus pneumoniae*. *J. Immunol.* 127:2089–92
58. Holzer, T. J., Edwards, K. M., Gewurz, H., Mold, C. 1983. Binding of C-reactive protein to pneumococcal capsule or cell wall results in differential localization of C3 and stimulation of phagocytosis. *Fed. Proc.* 42:1237–43
59. Mold, C., Nakayama, S., Holzer, T. J., Gewurz, H., Du Clos, T. W. 1981. C-reactive protein is protective against *Streptococcus pneumoniae* infections in mice. *J. Exp. Med.* 154:1703–8
60. Yother, J., Volanakis, J. E., Briles, D. E. 1982. Human C-reactive protein is protective against fatal *Streptococcus pneumoniae* infection in mice. *J. Immunol.* 128:2374–75
61. Briles, D. E., Nahm, M., Schroer, K., Davie, J., Baker, P., Kearney, J., Barletta, R. 1981. Antiphosphoryl choline antibodies found in normal mouse serum are protective against intravenous injection with Type 3 *Streptococcus pneumoniae*. *J. Exp. Med.* 153:694–710
62. Bull, C. A. 1916. Immunity factors in pneumococcus infection in the dog. *J. Exp. Med.* 24:7–12
63. Wright, H. D. 1927. Experimental pneumococcal septicemia and antipneumococcal immunity. *J. Pathol. Bacteriol.* 30:185–252
64. Biozzi, G., Steffel, C. 1961. Role of normal and immune opsonins in the phagocytosis of bacteria and erythrocytes by the reticuloendothelial cells. In *Mechanisms of Cell and Tissue Damage Produced by Immune Reactions,* ed. G. Pierce, P. Miescher, P-249. NY: Gruene and Stratton
65. Benacerraf, B., Sebestyen, M. M., Schlossman, S. 1959. A quantitative study of the kinetics of blood clearance of 32P-labelled *Escherichia coli* and *Staphylococci* by the reticuloendothelial system. *J. Exp. Med.* 110:27–31
66. Spiegelberg, H. L., Miescher, P. A., Benacerraf, B. 1963. Studies on the role of complement in the immune clearance of *Escherichia coli* and rat erythrocytes by the reticuloendothelial system in mice. *J. Immunol.* 90:751–62
67. Brown, E. J., Hosea, S. W., Frank, M. M. 1981. The role of the spleen in experimental pneumococcal bacteremia. *J. Clin. Invest.* 67:975–82
68. Valtonen, M. V. 1977. Role of phagocytosis in mouse virulence of *Salmonella typhimurium* recombinants with O-antigen 6,7 or 4,12. *Infect. Immun.* 18:574–81
69. Liang-Takasaki, C.-J., Mäkelä, P.H., Leive, L. 1982. Phagocytosis of bacteria by macrophages: changing the carbohydrate of lipopolysaccharide alters interaction with complement and macrophages. *J. Immunol.* 128:1229–35
70. Liang-Takasaki, C.-J., Saxen, H., Mäkelä, P. H., Leive, L. 1983. Com-

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Annual Reviews

plement activation by polysaccharide of lipopolysaccharide: An important virulence determinant of salmonellae. *Infect. Immun.* 41:563–69
71. Rogers, D. E. 1960. Host mechanisms which act to remove bacteria from the blood stream. *Bacteriol. Rev.* 24:50–66
72. Brown, E. J., Hosea, S. W., Frank, M. M. 1981. The role of complement in the localization of pneumococci in the splanchnic reticuloendothelial system during experimental bacteremia. *J. Immunol.* 126:2230–35
73. Friedman, R. L., Moon, R. J. 1980. Role of Kupffer cells, complement and specific antibody in the bactericidal activities of perfused livers. *Infect. Immun.* 29:152–57
74. Brown, E. J., Joiner, K. A., Frank, M. M. 1983. Interaction of desialated guinea pig erythrocytes with the classical and alternative pathways of guinea pig complement in vivo and in vitro. *J. Clin. Invest.* 71:1710–19
75. Hosea, S. W., Brown, E. J., Frank, M. M. 1980. The critical role of complement in experimental pneumococcal sepsis. *J. Infect. Dis.* 142:903–9
76. Jacob, H. S., Craddock, P. R., Hammerschmidt, D. E., Moldow, C. F. 1980. Complement-induced granulocyte aggregation: an unsuspected mechanism of disease. *N. Engl. J. Med.* 302:789–94
77. Hosea, S. W., Brown, E. J., Hammer, C., Frank, M. M. 1980. Role of complement activation in a model of adult respiratory distress syndrome. *J. Clin. Invest.* 66:375–82
78. Leigh, P. C. J., van den Burselaar, M. T., van Zwet, T. L., Daha, M. R., van Furth, R. 1979. Requirement of extracellular complement and immunoglobulin for intracellular killing of microorganisms by human monocytes. *J. Clin. Invest.* 63:772–84
79. Inoue, K. 1972. Immune bacteriolytic and bactericidal reactions. In *Research in Immunochemistry and Immunobiology,* 1:177–222, ed. J.B.G. Kwapinski. Baltimore: University Park Press
80. Taylor, P. W. 1983. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiol. Rev.* 47:46–83
81. Feingold, D. S., Goldman, J. N., Kuritz, H. M. 1968. Locus of the lethal event in the serum bactericidal reaction. *J. Bacteriol.* 96:2127–31
82. Wright, S. D., Levine, R. P. 1981. How complement kills *E. coli*. I. Location of the lethal lesion. *J. Immunol.* 127:1146–51
83. Bayer, M. E. 1975. Role of adhesion zones in bacterial cell-surface function and biogenesis. In *Membrane Biogenesis,* ed. A. Tzagoloff, pp.393–427. NY: Plenum
84. Taylor, P. W., Kroll, H.-P. 1983. Killing of an encapsulated strain of *Escherichia coli* by human serum. *Infect. Immun.* 39:122–31
85. Taylor, P. W., Kroll, H.-P. Bhakdi, S. 1983. Killing of *Escherichia coli* LP1092 by human serum. *Immunobiology* 164:304–11
86. Kozone, H., Hone, K., Takeda, J., Kinoshita, T., Inoue, K. 1983. Attack site of complement of gram-negative bacteria. *Immunobiology* 164:257–63
87. Spitznagel, J. K. 1966. Normal serum cytotoxicity for ^{32}P-labeled smooth *Enterobacteriaceae*. II. Fate of macromolecular and lipid phosphorus of damaged cells. *J. Bacteriol.* 91:148–53
88. Wilson, L. A., Spitznagel, J. K. 1971. Characteristics of complement-dependent release of phospholipid from *Escherichia coli*. *Infect. Immun.* 4:23–28
89. Inoue, K., Takamizawa, A.-I., Amano, T. 1974. Chemical studies on damages of *Escherichia coli* by the immune bactericidal reaction. I. Release and degradation of phospholipids from damaged bacteria. *Biken J.* 17:127–34
90. Inoue, K., Yano, K.-I., Amano, T. 1974. Chemical studies on damages of *Escherichia coli* by the immune bactericidal reaction. II. Release of phosphatidylenthalomine from a phospholipase A–deficient mutant of *E. coli* during the immune bactericidal reaction. *Biken J.* 17:135–40
91. Inoue, K., Kinoshita, T., Okada, M., Akiyama, Y. 1977. Release of phospholipids from complement-mediated lesions on the surface structure of *Escherichia coli*. *J. Immunol.* 119:65–70
92. Griffiths, E. 1974. Metabolically controlled killing of *Pasteurella septica* by antibody and complement. *Biochim. Biophys. Acta* 362:598–602
93. Goldman, J. N., Ruddy, S., Austen, K. F., Feingold, D. S. 1969. The serum bactericidal reaction. III. Antibody and complement requirements for killing a rough *Escherichia coli*. *J. Immunol.* 102:1379–87
94. Schreiber, R. D., Morrison, D. C., Podack, E. R., Müller-Eberhard, H. J. 1979. Bactericidal activity of the alternative complement pathway generated from 11 isolated plasma proteins. *J., Exp. Med.* 149:870–82

Annu. Rev. Immunol. 1984.2:461-492. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

Annual Reviews

95. Harriman, G. R., Esser, A. F., Podack, E. R., Wunderlich, A. C., Braude, A. I., Lint, T. F., Curd, J. G. 1981. The role of C9 in complement-mediated killing of *Nisseria*. *J. Immunol.* 127:2386–92

96. Goldman, J. N., Austen, K. F. 1974. Reaction mechanisms of nascent C567 (reactive lysis). II. Killing of a rough form of *Escherichia coli* by C567, C8 and C9. *J. Infect. Dis.* 129:444–50

97. Joiner, K. A., Goldman, R. C., Hammer, C. H., Leive, L., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. VI. IgG increases the bactericidal efficiency of C5b-9 for *E. coli* 0111B4 by acting at a step prior to C5 cleavage. *J. Immunol.* 131:2570–76

98. Joiner, K. A., Goldman, R. C., Hammer, C. H., Leive, L., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. V. IgG and $F(ab')_2$ mediate killing of *E. coli* 0111B4 by the alternative complement pathway without increasing C5b-9 deposition. *J. Immunol.* 131:2563–69

99. Zollinger, W. D., Mandrell, R. E. 1983. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. *Infect. Immun.* 39:25–64

100. Adler, F. I. 1953. Studies on the bactericidal reaction. II. Inhibition by antibody, and antibody requirements of the reaction. *J. Exp. Med.* 70:79–88

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97. Joiner, K. A., Goldman, R. C., Hammer, C. H., Leive, L., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. VI. IgG increases the bactericidal efficiency of C5b-9 for *E. coli* 0111B4 by acting at a step prior to C5 cleavage. *J. Immunol.* 131:2570–76
98. Joiner, K. A., Goldman, R. C., Hammer, C. H., Leive, L., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. V. IgG and $F(ab')_2$ mediate killing of *E. coli* 0111B4 by the alternative complement pathway without increasing C5b-9 deposition. *J. Immunol.* 131:2563–69
99. Zollinger, W. D., Mandrell, R. E. 1983. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. *Infect. Immun.* 39:25–64
100. Adler, F. I. 1953. Studies on the bactericidal reaction. II. Inhibition by antibody, and antibody requirements of the reaction. *J. Exp. Med.* 70:79–88
101. Rice, P. A., Kasper, D. L. 1982. Characterization of serum resistance of *Neisseria gonorrhoeae* that disseminate. *J. Clin. Invest.* 70:157–61
102. Grifiss, J. M., Broud, D. D., Bertram, M. A. 1975. Bactericidal activity of meningococcal antisera: blocking by IgA or lytic antibody in human convalescent sera. *J. Immunol.* 114:1779–84
103. McCutchan, J. A., Katzenstein, D., Norquist, D., Chikami, G., Wunderlick, A., Braude, A. I. 1978. Role of blocking antibody in disseminated gonococcal infection. *J. Immunol.* 121:1884–91
104. Kawakami, M., Ihara, I., Suzuki, A., Harada, Y. 1982. Properties of a new complement-dependent bactericidal factor specific for Ra chemotype *Salmonella* in sera of conventional and germ-free mice. *J. Immunol.* 129:2198–2204
105. Muschel, L. H., Larsen, L. J. 1970. The sensitivity of smooth and rough gram-negative bacteria to the immune bactericidal reaction (34472). *Proc. Soc. Exp. Biol. Med.* 133:345–48
106. Rowley, D. 1968. Sensitivity of rough gram-negative bacteria to the bactericidal action of serum. *J. Bacteriol.* 95:1647–50
107. Taylor, P. W., Robinson, M. K. 1980. Determinants that increase the serum resistance of *Escherichia coli*. *Infect. Immun.* 29:278–80
108. Taylor, P. W. 1976. Immunochemical investigations of lipopolysaccharides and acidic polysaccharides from serum-sensitive and serum-resistant strains of *Escherichia coli* isolated from urinary-tract infections. *J. Med. Microbiol.* 9:405–12
109. Glynn, A. A., Howard, C. J. 1970. The sensitivity to complement of strains of *Escherichia coli* related to their K antigens. *Immunology* 183:331–37
110. Van Dijk, W. C., Verbrugh, H. A., Peters, R., Van der Tol, M. E., Peterson, P. K., Verhoef, J. 1978. *Escherichia coli* K antigen in relation to serum-induced lysis and phagocytosis, *J. Med. Microbiol.* 10:123–28
111. Opal, S., Cross, A., Genski, P. 1982. K antigen and serum sensitivity of rough *Escherichia coli*. *Infect. Immun.* 37:956–60
112. Pitt, J. 1978. K-1 Antigen of *Escherichia coli*. Epidemiology and serum sensitivity of pathogenic strains. *Infect. Immun.* 22:219–24
113. Stephens, P., Huang, S. N-Y., Welch, W. D., Young, L. S. 1978. Restricted complement activation by *Escherichia coli* with the K-1 capsular serotype: A possible role in pathogenicity. *J. Immunol.* 121:2174–82
114. Van Dijk, W. C., Verbrugh, H. A., Van der Tol, M. E., Peters, R., Verhoef, J. 1979. Role of *Escherichia coli* K capsular antigens during complement activation, C3 fixation and opsonization. *Infect. Immun.* 25:603–9
115. Moll, A., Manning, P. A., Timmis, K. N. 1980. Plasmid-determined resistance to serum activity: a major outer membrane protein, the *traT* gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. *Infect. Immun.* 28:359–67
116. Taylor, P. W., Parton, R. 1976. A protein factor associated with serum resistance in *Escherichia coli*. *J. Med. Microbiol.* 10:225–32
117. Hildebrandt, J. F., Mayer, L. W., Wang, S. P., Buchanan, T. M. 1978. *Neisseria gonorrhoeae* acquire a new principal outer-membrane protein when transformed to resistance to serum bactericidal activity. *Infect. Immun.* 20:267–72
118. Cannon, J. G., Lee, T. J., Guymon, L. F., Sparling, P. F. 1981. Genetics of serum resistance in *Neisseria gonorrhoeae:* the sac-1 genetic locus. *Infect. Immun.* 32:547–55
119. Shafer, W. M., Guymon, L. F., Sparling, P. F. 1982. Identification of a new genetic site (sac-3) in *Neisseria gonorrhoeae* that affects sensitivity to normal human serum. *Infect. Immun.* 35:764–73
120. Munn, C. B., Ishiguro, E. E., Kay,

Annual Reviews

120. Munn, C. B., Ishiguro, E. E., Kay, W. W., Trust, T. J. 1982. Role of surface components in serum resistance of virulent *Aeromonas salmonicida*. *Infect. Immun.* 36:1069–75
121. Fierer, J., Finley, F., 1979. Lethal effect of complement and lysozyme on polymyxin-treated, serum-resistant, gram-negative bacilli. *J. Infect. Dis.* 140:581–88
122. Reynolds, B. L., Rother, U. A., Rother, K. O. 1975. Interaction of complement components with a serum-resistant strain of *Salmonella typhimurium*. *Infect. Immun.* 1:944–48
123. Ogata, R. T., Levine, R. P. 1980. Characterization of complement resistance in *Escherichia coli* conferred by the antibiotic resistance plasmid R100. *J. Immunol.* 124:1494–98
124. Binns, M. W., Mayden, J., Levine, R. P. 1982. Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV,I-K94. *Infect. Immun.* 35:654–59
125. Joiner, K. A., Hammer, C. H., Brown, E. J., Cole, R. J., Frank, M. M. 1982. Studies on the mechanism of bacterial resistance to complement-mediated killing. I. Terminal complement components are deposited and released from *Salmonella minnesota* S218 without causing bacterial death. *J. Exp. Med.* 155:797–804
126. Joiner, K. A., Hammer, C. H., Brown, E. J., Frank, M. M. 1982. Studies on the mechanism of bacterial resistance to complement-mediated killing. II. C8 and C9 release C5b67 from the surface of *Salmonella minnesota* S218 because the terminal complex does not insert into the bacterial outer membrane. *J. Exp. Med.* 155:809–15
127. Joiner, K. A., Goldman, R., Schmetz, M., Berger, M., Hammer, C. H., Frank, M. M., Leive, L. 1984. A quantitative analysis of C3 binding to O-antigen capsule, lipopolysaccharide, and outer membrane protein of *E. coli* 0111B4. *J. Immunol.* 132:369–75
128. Joiner, K. A., Warren, K. A., Brown, E. J., Swanson, J., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. IV. C5b-9 forms high molecular weight complexes with bacterial outer membrane constituents on serum-resistant but not serum-sensitive *Neisseria gonorrhoeae*. *J. Immunol.* 131:1443–49
129. Schoolnick, G. K., Buchanan, T. M., Holmes, K. K. 1976. Gonococci causing disseminated gonococcal infection are resistant to the bactericidal action of normal human sera. *J. Clin. Invest.* 58:1163–71
130. Harriman, G. R., Podack, E. R., Braude, A. I., Corbeil, L. C., Esser, A. F., Curd, J. G. 1982. Activation of complement by serum-resistant *Neisseria gonorrhoeae*. Assembly of the membrane attack complex without subsequent cell death. *J. Exp Med.* 156:1235–39
131. Joiner, K. A., Brown, E. J., Hammer, C., Warren, K., Frank, M. M. 1983. Studies on the mechanism of bacterial resistance to complement-mediated killing. III. C5b-9 deposits stably on rough and type 7 *S. pneumoniae* without causing bacterial killing. *J. Immunol.* 130:845–49

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Annual Review of Immunology
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CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey* 1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz* 23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack* 51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson* 67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori* 103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf* 127
REGULATION OF IgE SYNTHESIS, *Kimishige Ishizaka* 159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie* 183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang* 199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober* 219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi* 239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike* 257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton* 283
INTERLEUKIN 2, *Kendall A. Smith* 319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson* 335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT

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INTRODUCTION

This discussion focuses on whether any relationships exist between the patterns of murine B-lymphocyte responses to different sets of stimuli and their potential for expression of Ig[1] heavy-chain isotypes. We also attempt to treat the

[1]*Abbreviations used in this paper:* Ab, antibody; ABC, antigen-binding cell; Ag, antigen; A-MuLV, Abelson murine leukemia virus; ASC, *Ascaris suum;* BA, *Brucella abortus;* BL, bacterial levan; BM, bone marrow; BUdR, 5′-bromodeoxyuridine; C_H, constant region of heavy chain; ConA, concanavalin A; Dex, dextran; DNP, dinitrophenyl; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FcR_α, F_c receptor for IgA; FITC, fluorescein isothiocyanate; FL, fluorescein; Gal, galactose; GlcNAc-GG, *N*-acetylglucosaminyl-coupled goat IgG; H-LPS, haptenated lipopolysaccharide; HRBC, horse red blood cells; Hy, *Limulus polyphemus* hemocyanin; i.g., intragastric; Ig, immunoglobulin; IL-2, interleukin 2; In, inulin; i.p., intraperitoneal; i.v., intravenous; J_H, J sequence of heavy chain; LPS, lipopolysaccharide; 2-ME, 2-mercaptoethanol; NIP, 4-hydroxy-3-iodo-5-nitrophenyl; PC, phosphocholine; PFC, plaque-forming cell; PNA, peanut agglutinin; PnC, heat-killed vaccine of *Streptococcus pneumoniae;* POL, polymerized flagellin; PP, Peyer's patches; PPD, purified protein derivative of tuberculin; PW-LPS, phenol/water extracted lipopolysaccharide; RAM, rabbit anti-mouse; RITC, tetramethylrhodamine isothiocyanate; S_H, switch sequence of heavy chain; sIg, surface immunoglobulin; SPA, Staphylococcal protein A; SRBC, sheep red blood cells; T4, bacteriophage T4; TCA-LPS, trichloroacetic acid-extracted lipopolysaccharide; T_H, helper T cell; $T_{H(Ag)}$, antigen-specific helper T cell; TI, thymus-independent; TD, thymus-dependent; TNP, trinitrophenyl; Tx, thymectomized; TXD, cholera toxoid; TXN, cholera toxin; V_H, variable region of heavy chain; V_L, variable region of light chain; Y, pyrimidine

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Annual Reviews

closely related issue of whether the response of a B cell to a particular set of stimuli includes isotype switching and, if so, whether a particular pattern is more probable, either as revealed by expression of secreted Igs of different isotypes or by concomitant restriction in future isotype potential. In fact, we review the possible interrelationships between the two main bases for classifying B lymphocytes into subpopulations and for organizing these into developmental pathways: (*a*) the qualitative and quantitative surface content of *functional* receptors and signaling ligands that determine a pattern of responsiveness to a given set of external stimuli; and (*b*) the residual potential in the genome for the expression of each of the eight murine C_H isotypes, either by a cell directly or by its descendants that may arise over many generations of clonal outgrowth. We suggest developmental schemes for B-lymphocyte lineages that are most consistent with both of these aspects of differentiation.

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Patterns of Response to Different Sets of External Stimuli

The rather limited repertoire of B-cell responses that is ordinarily resolved for monitoring purposes includes: (*a*) "activation," or the promotion of resting B cells from G_0 to G_1 state; (*b*) "proliferation," or the movement of B cells into S or through M state, depending on the assay used; (*c*) "maturation," or the development of the extensive rough endoplasmic reticulum and other membranous intracellular compartments involved in secretion, along with the synthesis of secretory forms of Ig and its detection within the cell; and (*d*) secretion of detectable amounts of antibody. Evidence for the role of specific antigen in mediating these processes is stronger for some (e.g. activation, proliferation) than for others. Often, some of these somewhat arbitrarily defined steps are not resolved by a particular assay system, such as *a* and *b* or *c* and *d*. For instance, in vitro bulk culture systems usually permit neither clear assignment of response patterns to all stimulable B cells nor a separate assessment of the extent of proliferation versus maturation (clone size versus burst size). One other clearly definable physiologic response B cells can make to external stimuli—the sessile-to-motile transition and cell translocation—is usually ignored in an analysis of response patterns.

C_H *Isotype Switching and Isotype Potential*

Traditionally, cell biologists have defined isotype switching as the display of a new surface or secreted Ig isotype by a B cell or its descendants, different from the isotype originally expressed by that cell or its antecedents. Detection of at least two isotypes, excluding IgD, in the cytoplasm, secretions, or surface of single cells (1–3) has been taken as evidence for transition of a cell or cell line from the expression of one isotype to that of another. The commonness of the switching phenomenon was persuasively demonstrated by the effect of

Annual Reviews

anti-μ administered to chicks (4) and mice (5) during early development to prevent the appearance of not only IgM but all isotypes of Ig. The inference from these observations is that B cells making any IgG, IgE, or IgA isotype have progenitors that first express IgM. Cell biologists' attempts to define isotype potential have involved efforts to determine whether switching is reversible, whether B cells become committed or restricted to expression of a particular C_H isotype or pattern of isotypes, what future capabilities a B cell may retain that can be expressed over many generations of clonal arborization, and how the expression of the switch is regulated.

Early molecular biological evidence for switching came from protein sequence analysis of IgM and IgA molecules that presumably were made by clonally related plasmacytoma lines expressing the same variable region "gene" (6). More recently, molecular genetic analyses of the heavy-chain locus have revealed a complex, two-stage, recombinational process leading to the activation of μ/δ chain gene followed by switching to the expression of other C_H isotypes (see 7). We discuss relevant aspects later. Briefly, one of each V_H, D, and J_H gene segments are recombined with the deletion of intervening DNA sequences. An accurate translocation places the $V_H/D/J_H$ segment 5′ to C_μ and C_δ genes, but separated from them by at least one intron, and allows transcription of this whole region as a polycistronic mRNA. Isotype switching has been correlated with another recombination, involving sites within the introns 5′ to C_H genes, by which the $V_H/D/J_H$ gene is relocated immediately 5′ to a new C_H gene. Probably the most general definition of successful isotype switching by a molecular geneticist is the synthesis of heavy-chain isotype mRNA by a cell or cell line different from the one(s) originally made.

Obviously, a major aim is to reconcile the switching process as a cellular phenomenon with precise information about heavy-chain gene context and content in order to appreciate the distinctions between the events that can occur at the DNA level and their strict consequences and those that often normally occur during clonal outgrowth of B cells, especially those that result in the expression of isotype switching and affect future isotype potential.

In Vitro Culture of Single B-Cell Clones to Assess B-Cell Responsiveness and Isotype Potential

Because the B-cell capabilities of interest—either pattern of responsiveness or C_H isotype potential—are expressed over several or many generations and the precusor cell–product cell relationship must therefore be either directly or statistically established, we here consider mainly clonal B-cell cultures. Occasionally, if some phenomenon relevant to the possible relationship between response pattern and isotype potential has not yet been analyzed in clonal B-cell cultures, we provide data from the bulk culture system that revealed the phenomenon.

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Three main variations of clonal B-cell culturing are now commonly used, and each has certain advantages and limitations for addressing our main issue. The simplest culturing technique involves placing a few (i.e. one to ten) B cells in microculture (10 μl) and stimulating clonal outgrowth with antigens (8,9) or mitogens (9,10), sometimes along with one or several more or less well-defined cytokines and factors (11). Advantages of the system are that secondary effects due to stimulation of bystander cells can be excluded, activation as revealed by increase in cell size can be scored, proliferation through M phase can be evaluated, and the relationship between clone size and burst size of IgM-secreting cells can easily be estimated. The method is appropriate for unfractionated B cells with a high incidence of responsiveness to mitogens (10); but for specific antigen stimulation, a preliminary enrichment for specific B cells is required for workable frequencies of responding microcultures (12,13). This method excludes, almost by definition, the analysis of B cells responding to other intact cells, although this type of culture may eventually be modified to permit examination of one or a few B cells interacting with relatively few T_H cells. At present, 10^5 "filler" cells of various sorts have been added to enriched B cells in microculture to enhance a clonal response by various parameters, but obviously the increase in cell number per culture obviates some of the advantages of the system. So far, isolated B-cell clones developing in response to TD antigens in the absence of other intact cells have not been obtained. Finally, the regular expression of secreted antibody other than IgM by these isolated B cell clones has not been detected.

A second method for culturing B cell clones involves the distribution of mitogen- or antigen-responsive cells by the limiting dilution procedure (14) among minicultures (0.1–0.2 ml) containing irradiated or nonirradiated syngeneic, allogeneic, or xenogeneic filler cells (15–17). The filler cells are added to provide specific T_H cells and/or antigen nonspecific stimuli and to generally "condition" the medium. Of course, preconditioned medium and/or better-defined cytokines and factors may also be added to the B cell and filler cells. Generally, such B-cell clonal minicultures are more robust than cultures without filler cells; develop in response to mitogens, TI, and TD antigens; and may express IgM along with other isotypes. Presently, T_H cell lines and cloned T_H cells are being used to provide antigen-specific fillers. Disadvantages of this culturing procedure often include a low signal-to-noise ratio due to cross-reacting antibodies or PFC that are generated if antigen-specific B cells are not enriched to exclude irrelevant B cells. Further limitations are that blast cell formation reflecting activation is obscured, proliferation through M phase cannot be analyzed, and clone size versus burst size cannot be easily estimated. Further, it has yet to be established that B cells previously stimulated to switch from IgM expression in vivo can respond in clonal miniculture, although very

Annual Reviews

recent findings for IgA-bearing murine B cells suggest that they can respond in a large-scale (1–2 ml) bulk culture (18).

The third principal method currently used for growing B-cell clones is based on culturing of splenic fragments in vitro following adoptive transfer of limiting numbers of B cells (19). The recipients are generally lethally irradiated to minimize the background contributions of the endogenous B cells. If the recipients are primed in advance with a "carrier" protein, their irradiated splenic fragments can contain nonlimiting numbers of T_H cells that stimulate the clonal outgrowth of B cells specific for any hapten, if it is also added to the culture as a covalent substituent of the appropriate carrier protein. Otherwise, nonimmunized, irradiated recipients can be used to provide splenic fragments for adoptively transferred B cells responsive to Type 2 antigens (20,21). The major advantage of this rather cumbersome culturing procedure is that of all the methods it appears to provide better growth conditions for the enlargement of clones of antibody secreting cells and for the display of any or all murine isotypes. B cells stimulated to proliferate and switch in vivo from IgM expression can be stimulated in splenic fragment cultures to again proliferate and generate clones expressing IgG, IgE, and/or IgA isotypes (22). Some of the disadvantages of this assay are the same as those for miniculturing—extraneous B cells cause background antibody secretion, activation is obscured, proliferation cannot be easily estimated, and hence burst size can only be inferred from output of antibodies. However, the amounts of specific antibody secreted by single clones are often quite high relative to the background Ig. A more serious disadvantage of the system is the low in vivo lodging frequency in the spleen of the adoptively transferred B cells (4–10%), which necessitates the use of large numbers of test cells, reduces the accuracy of a generally determined lodging frequency for a particular, small subset of B cells, and necessitates sizeable correction factors for estimating frequencies.

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C_H ISOTYPE SWITCHING AND RESTRICTION ACCOMPANYING B-CELL RESPONSES TO MITOGENS/ANTIGENS AND THEIR DEVELOPMENTAL PROCESSES

In order to follow changes in isotype potential of B cells and the development of populations with differing requirements for activation, proliferation, and secretion, it is necessary to identify the subpopulations with differing response patterns. The definition of such B-cell subpopulations is ultimately dependent upon the presence and amount of cell-surface receptors or recognition molecules for mitogen/antigen, cytokines, and regulatory T-cell receptors. Presumably, intracellular second messengers are coordinately regulated with the

Annual Reviews

appearance of the cell-surface molecules that initiate a response upon interaction with an extracellular stimulus. In practice, the delineation of functional B-cell subsets is usually determined by responsiveness to a particular molecular form of antigenic/mitogenic determinants. Although this criterion for responsiveness should be largely valid as one means of defining B-cell subsets because correlations do exist between sensitivity to form of antigen and the other stimuli that trigger a full response, the approach is limited by the extent to which these latter stimuli are optimized in the scoring system. Since most analyses of isotype switching undertaken at the clonal level have used B cells defined in response pattern by sensitivity to a particular form of mitogen/antigen, we continue to categorize the observations according to this criterion. Where possible, we add or infer other aspects of the response pattern based on the known developmental stage of the B-cell donor or the dominant surface phenotype of the cell, based on markers, receptors, or recognition molecules. Unfortunately, not many of these surface molecules have been assigned a functional role in any of the forms of B-cell response. An obvious ambiguity arises from the use of form of antigen/mitogen to define response pattern if members of a subset so categorized could respond to more than one form of these, provided each was accompanied by the appropriate stimulatory "package" of other cytokines, factors, and cells. The likelihood of overlap in B-cell subsets is discussed below where relevant.

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C_H *Isotype Switching May Be Expressed During the Transition from Pre-B to B Cells and May Occur in Pre-B Cells*

Some time ago, double fluorescent Ab analysis for surface Igs showed that neonatal spleens contain some B cells that can co-express one of the IgG or IgA isotypes *along* with IgM (3). Recently, a dynamic analysis of neonatal liver cells from euthymic and athymic mice has been carried out over four days of in vitro culture (23). Whereas the initial content of Ig-bearing cells is very low (<1%), it rises in absolute terms more than 20-fold as IgM-bearing cells appear. Varying but low (1.5–5.2%) proportions of these also acquire one of the four IgG isotypes without co-expression of IgD. The absolute recoveries of each class of switched B cell are about the same whether its origin was athymic or euthymic mouse liver. Thus, it appears that the expression of C_H isotype switching can occur in very early B cells in the absence of T cells, without deliberate antigenic stimulation and with no co-expression of IgD as they arise from the pre-B cells that are abundant (5–15%) in neonatal liver. The relationship of antigen/mitogen stimulation to the switching process cannot be rigorously decided since fetal calf serum and 2-ME were present in the medium. Further, the functionality of these switched B cells is untested, and so it is not known whether they become capable of any response pattern

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to a given set of stimuli. However, some of these early B cells may be expected to respond to LPS by proliferating and secreting IgM (24,25). Further, early B cells similarly defined have been analyzed by TD splenic fragment culturing, and almost all clonal responses include IgM, with or without other isotypes (26). Since about one quarter of the pre-B cells gaining IgM after four days of culture also express an IgG isotype, these cells either do not respond in the TD assay or have not yet become restricted in isotype potential through C_μ gene deletion. Finally, it is difficult to exclude a role for cell division in the isotype switching process by this analysis of the pre-B to B-cell transition in vitro, which certainly can occur without mitosis (23). This is so because the C_H isotype switch could have already occurred during the known cell divisions of pre-B cells (27). In fact, there is some support for this possibility from studies of A-MuLV transformed lines derived from infected, newborn mice (28). Two separate sublines from such transformations have been identified that have begun expressing cytoplasmic $\gamma 2b$ chain, although their parent lines expressed μ chain or no heavy chain, respectively. These switched sublines also display C_μ gene deletion and retention of both C_κ genes in the embryonic context. Thus, ostensible pre-B cells can display C_μ gene deletion and C_H isotype switching. The implications of these findings for C_H isotype restriction or commitment in normal B-cell development remain to be determined—i.e. whether switched pre-B cells could normally become functional, responsive B cells.

C_H Isotype Switching May Occur During Polyclonal Stimulation with Thymus-Independent Mitogens Such As Lipopolysaccharide

Bacterial lipopolysaccharide (LPS) has long been employed as a polyclonal stimulus of the whole range of murine B-cell responses in order to circumvent the difficulties in quantitative analysis posed by low frequencies of any particular set of Ag-stimulable cells without resorting to their enrichment. Among the responses stimulated by LPS is maturation of B blasts to IgM secretion (29). In 1975 Kearney & Lawton (30) also detected the expression of cytoplasmic IgG1 or IgG2 in day 4–5 bulk cultures (1×10^6 cells/ml) of LPS-stimulated B cells from several tissues by staining with fluorescent anti-isotypes. Few B blasts matured to express cytoplasmic IgA. Again using LPS-stimulated bulk cultures, IgG was found to be synthesized and secreted, although its overall yield appeared to depend on cell density and the presence of additives such as 2-ME and FCS (31–33). However, LPS-induced IgG expression was not dependent on accompanying T cells since cultures from athymic mice or T_x, X-irradiated, BM-restored mice also generated IgG-producing cells (30–33). Time-course studies indicate that LPS-stimulated B cells making the secretory form of IgG reach a maximum number in bulk cultures about

Annual Reviews

1–2 days (day 5–6) after those making IgM (3,33), suggesting the possibility of a precursor-product relationship. This notion is supported by the detection of some plasmablasts containing both IgM and some IgG isotype in their cytoplasm in LPS-stimulated cultures (3,34). Additional inferences from LPS-induced bulk cultures concerning the switch from IgM to IgG expression are that IgG expression accompanies several rounds of proliferation (32,35) and that it occurs asymmetrically during mitosis and karyokinesis (36). There is a direct relationship between generation of IgG-secreting cells and DNA synthesis. Titration of such DNA inhibitors as thymidine or hydroxyurea into cultures indicates that development of cells secreting IgG is more sensitive to these inhibitors than the development of cells making IgM (32,35). Cytochalasin B was used to block cytokinesis but not karyokinesis beginning on day 2 after LPS-stimulated expression of IgM and IgG by splenic cells. The proportion of plasmablasts containing IgG1 or IgG2a along with IgM rose to 60–80% by day 3–4 compared with about 25% of such cells in cultures lacking the drug (36). Most of this increase was contributed by bi- and multinucleated cells. The inference from these observations is that the gene rearrangements leading to switching occur during division and only affect the transcription potential of one of each pair of productive sister chromatids. Presumably bi- or multi-nucleated cells retain the capacity to continue to transcribe μ-chain and γ-chain mRNA and accumulate products in their cytoplasm because of an active gene for each in separate nuclei; successful cell division accompanied by decay of mRNA makes double expression of two heavy chains in the cytoplasm of each daughter more transient. Presumably, if switching occurred before S/M phase, each daughter's nucleus would synthesize the new γ-chain mRNA, and its product would coexist as transiently with the declining mRNA for μ-chain in bi- and multi-nucleated cells as in mononucleated cells in cultures without the drug. The assumptions concerning the timing of the gene rearrangements leading to switching vis-à-vis the cell cycle in these elegant experiments are principally that the inhibitor does not affect the development and functioning of the processes leading to Ig secretion and that the behavior of plasmablasts is typical of that of dividing lymphoblasts, which may be undergoing gene rearrangements leading to switching without expression of cytoplasmic or secreted Ig product. Evidence that cytochalasin B did not interfere with the secretory process for Igs has been presented (see 36), and the high proportion (>10%) of switched plasmablasts make it unlikely that they reveal an esoteric switching mechanism different from that used by nonsecretory lymphoblasts.

Currently, limiting dilution of LPS-responsive B cells in cultures containing growth-supporting filler cells has proved most informative about intraclonal isotype switching and expression induced by mitogens. Andersson and his co-workers first defined the components of a culture system, including opti-

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mized concentrations of 2-ME, FCS, and 3 × 10^6 nonirradiated rat thymus filler cells in 1 ml, which they propose permits growth and maturation of Ig-secreting cells from every LPS-stimulable B cell (17,37). Depending on the donor mouse strain, as many as one in six to one in three splenic B cells generates productive clones (17,38). Initially, using an indirect PFC assay with SPA and either anti-μ or anti-γ1 +t γ2a, about one tenth of the clones making IgM on day 5 were found to give rise to cells secreting IgG by day 6–7 (37). More recently, simplified microcultures (10 μl), lacking both filler cells and conditioned medium, have been developed that support proliferation of single B cells responsive to the apparently synergistic stimuli given by optimized concentrations of LPS and dextran sulfate (9,10). These also give high cloning efficiency and have the advantage of easily permitting visual estimation of growth and clone size over time by microscopy. However, the yield of hapten-specific Ig-secreting cells is lower than that obtained using specific Ag plus conditioned medium, and the frequency of clones giving IgM PFC is less than one third that obtained when thymus fillers are added (9). Thus, most recent analyses of LPS-induced isotype switching and secretion of Ig have utilized miniculture (0.2 ml) including 6 × 10^5 rat thymus fillers and optimal concentrations of FCS, 2-ME, and LPS alone (39,40).

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CHARACTERISTICS OF THE SUBSET OF B CELLS RESPONSIVE TO LPS, INCLUDING THOSE THAT GENERATE CLONES DISPLAYING ISOTYPE SWITCHING LPS-responsive B cells bear surface IgM and are likely capable of continuing transcription of the complex μ-chain gene. Addition of anti-μ chain to cultures for as little as 30 min before removal by washing and addition of LPS ablates the development of both IgM- and IgG-secreting clones (33). Anti-γ1 + γ2a only inhibits the appearance of IgG-secreting cells and must be present throughout the culturing period. Further, B cells positively selected by either FACS or panning techniques for those cells bearing IgM could account for the full range of responsiveness to LPS shown by unfractionated B cells, including the generation of cells secreting IgG or bearing IgA (41,42). Conversely, B cells negatively selected by FACS for absence or near absence of sIgM gave much-reduced responses to LPS (41), and those positively selected by panning for sIgA- or sIgG-positive cells were refractory to stimulation (42). That the precursor cells of all clones that eventually express any one or a mixture of IgG, IgA, or IgE isotypes can continue to synthesize and process mRNA for the secreted form of IgM is supported by direct observation and by the statistical argument that IgM-secreting cells are present in all clones making any other isotype(s) (37,39).

Cells with the potential to become responsive to LPS and give rise to IgM- and IgG-secreting clones have been obtained from fetal liver as early as 13 days after fertilization (33), although culture in vitro for extra days roughly

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equivalent to the number remaining until birth is required for clonal display of Ig isotypes equivalent to those shown by adult B cells (24,33). The B cells with potential to become LPS-responsive arise in fetal liver, presumably from rapidly dividing progenitors of pre-B cells (27), and then decline perinatally (24). A reciprocal rise in such B cells around birth occurs in perinatal spleen although their origin via migration of cells from fetal liver has never been established (24). In the days following birth there is a steady rise in both the yield of IgM-secreting cells from neonatal spleen and the DNA synthetic response following LPS stimulation (25). It is not clear whether increased proliferation, followed by higher clonal burst of Ig-secreting cells, reflects the appearance of a distinct subpopulation of B cells or the acquisition of increased responsiveness to particular signals. A dissociation between the response to a proliferation signal and the induction of Ig secretion has been suggested (25). Further, B cells expressing both sIgM and high levels of sIgD rise in proportion shortly after birth (43,44), and the presence of sIgD has been associated with the continued potential to proliferate in response to a variety of stimuli, including LPS (45,46). Unfortunately, a molecule that acts as a functional LPS receptor and that could serve as a marker for a responsive subset of B cells has not yet been defined to general satisfaction (47). Comparisons between congenic LPS-responder and nonresponder B cells suggest that both show indistinguishable saturable binding of LPS and that the genetic defect is expressed at a subsequent stage of the stimulatory process (48). A similar receptor-independent refractoriness to LPS may be displayed by B cells that have switched to IgG1 or IgA (42,49). Thus, we presently cannot define a distinctive surface phenotype for the subset of B cells that responds to LPS (17).

Physiologic characteristics of the LPS-responsive B-cell subset likely include a rapid turnover rate with constant renewal (38,50), although the various dissimilar methods for estimating half-life give values ranging from 1 day (38) to 1–2 weeks (50). The rapid turnover of LPS-responsive cells is consistent with two other kinds of observations. First, deliberate in vivo immunization with either thymus-dependent or thymus-independent (Type 1 or Type 2) antigens fails to appreciably raise the frequencies of LPS-stimulable B cells that give rise to clones secreting the appropriate specific antibodies (51; B. Pike, personal communication; J. Cebra, unpublished observations). Second, B cells from thoracic duct lymph that are LPS-responsive are only about one eighth as frequent as from spleen (52); and the former tissue, along with peripheral lymph nodes, is known to be rich in long-lived, recirculating B cells responsible for immunologic "memory" (50,53). Presumably some B cells that arise following in vivo stimulation by antigen are refractory to LPS, and at least some of these have undergone isotype switching (see below).

Annual Reviews

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PATTERNS OF ISOTYPES SECRETED BY B-CELL CLONES STIMULATED BY LPS A comprehensive estimation of the frequencies of LPS-stimulated B-cell clones arising in miniculture with filler cells was made for those secreting IgM, IgG1, IgG2, IgG3, and IgA using reverse plaquing with SPA and specific anti-isotypes (39). The frequencies of the expression of other secreted isotypes relative to IgM was 7% for IgG1, 39% for IgG2a/IgG2b, 41% for IgG3, and about 1% for IgA. These relative frequencies were about the same for all lymphoid tissues tested, including spleen, mesenteric lymph node, bone marrow, and thoracic duct lymph, irrespective of whether the donors were athymic or euthymic mice (39,52). Thus, although absolute frequencies of responsive B cells varied 4–8-fold depending on the strain and tissue source, the stimulable cell populations each seemed to have the same range of isotype potential expressed in the same proportions. Further, LPS-responsive cells arising in athymic mice did not appear altered in their isotype potential by their development in the absence of functional T cells. Solid-phase immunoassay at a sensitivity similar to that used to examine intraclonal diversification with respect to isotype in thymus-dependent and thymus-independent antigen-stimulated B-cell clones (20,21,54) has now been applied by Coutinho & Forni to the analysis of Ig secreted by single, LPS-stimulated clones grown in miniculture (40). Culture supernatants were divided to assay for any and all murine isotypes except IgD. Initial deductions were that: (*a*) IgM-producing clones could give rise to expression of each other isotype with different probabilities; (*b*) up to 80% of all IgM-producing clones express switching; (*c*) IgG3 or IgG2b appear most commonly and about three to four times more frequently than IgG1 or IgG2a; (*d*) IgE expression is uncommon (2%) and IgA is even more rarely detected. Of some interest is that IgG1 was detected at about twice the frequency by immunoassay as previously by reverse PFC assay (39,40). As has been found previously for clones stimulated by TD or TI antigens (20,21,54,55), the LPS-induced, IgM-secreting clones coexpress from one to five other isotypes. The probability of multiple isotype expression by clones decreases with increasing numbers detected. The expression of the two most prevalent non-IgM isotypes—IgG3 and IgG2a—were found to be significantly associated; clones secreting one of these have a higher probability of producing the other than do clones not making the former. Conversely, secretion of both IgE and IgA by the same clone was never detected, although their secretion was rare enough to preclude any generalization about switching mechanisms. Otherwise, the frequencies of each isotype were analyzed in relation to all others coexpressed by clones in an attempt to discern some pattern for the switching process relative to the known C_H gene order (56). The expression of the IgG isotypes seemed to fall into a pattern in that the closer an expressed C_H gene was to the C_μ gene the more likely was the clone to secrete also

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products of the other 3′ C_H genes; the further an expressed C_H gene was from the C_μ gene the more likely its clone secreted products of intervening C_H genes. However, attempts to interpret these observations in terms of a simple increase in probabilities of switches with distance of C_H genes from C_μ occurring as single, isolated $C_\mu \rightarrow C_X$ events during intraclonal differentiation are discouraged by the possibility that consecutive switches occur within the same B-cell line of a clone and by the conspicuous failure of IgG1, IgE, and IgA secretion to fit into the overall pattern. In fact, an intriguing aspect of this analysis (40) was the finding of LPS-blasts bearing two different sIgG isotypes or one cytoplasmic IgG isotype and another sIgG isotype that was the product of a 5′ C_H gene. These observations raise the possibility that switches occur without their detection by secreted product and support the hypothesis that consecutive switches occur in the same B-cell line (57). Finally, evidence is accumulating that expression of IgG1 and IgA during LPS stimulation of the responsive subset of B cells has special requirements.

Initial observations indicated that the distribution of IgG isotypes secreted was distinctly different depending on whether LPS alone or TNP-specific helper T-cell lines were used to polyclonally stimulate lightly trinitrophenylated splenic B cells: LPS-responsive cells gave mostly IgG3- and IgG2-secreting cells while those responding to T_H cells mostly made IgG1 and IgG2 (58). That the same responsive B cells generated plasmablasts displaying different patterns of secreted IgG isotypes depending on the nature of the stimuli was shown by recovering blasts after 48-hr exposure to one sort of stimulus and then reexposing them to the other (59). Conditioned media from T_H-cell lines reactive with minor histocompatibility antigens or LPS alone were also used to restimulate LPS-blasts at 48 hr of culture. Media from two cell lines were compared, both of which stimulated continued proliferation of LPS blasts and one of which was known to increase IgM PFC per culture. This latter conditioned medium increased the frequency of clones secreting IgG1 about 7–8-fold compared with the use of LPS alone for continued stimulation while the former did not. Significantly, both conditioned media as well as continuation of LPS resulted in a marked rise in sIgG1-positive cells compared with unstimulated blasts in bulk culture, but only the medium known to induce maturation of IgM plasmablasts also generated cells secreting IgG1 and IgG3; continued LPS stimulation yielded mostly IgG3-secreting cells (60). Thus, it appeared that the T_H maturation factor acted mainly on B cells that had randomly switched to sIgG1 expression, although all growth-promoting stimuli appeared to increase the proportion of such cells. An enrichment of sIgG1 and sIgG1$^+$ LPS blasts by FACS also supported the hypothesis that the latter cells are the targets of the T_H maturation factor. However, a careful analysis of membrane versus cytoplasmic IgG isotypes generated in bulk culture by LPS blasts restimulated by LPS or T_H cells also revealed a small but significant

Annual Reviews

number of cells with sIgG3 and cytoplasmic IgG1, further suggesting that the growth-promoting activity of T_H cells could facilitate *consecutive* switches and activation of C_γ genes (49).

The other example of modulation of isotype expression by LPS-stimulated B cells concerns the differential generation of sIgA-bearing cells in bulk culture in the presence of a ConA-propagated T-cell line from Peyer's patches (PP) but not in the presence of an equal number of fillers contributed by a ConA-propagated T-cell line from spleen (42,61). Both lines have typical T_H-cell markers plus surface I-A/E; additionally the PP line bears FcR_α (61). Expression of IgA by proliferating murine B cells stimulated by LPS is a notoriously rare event (30,40). Only $sIgM^+$ cells are induced to proliferate and/or show changes in isotype expression by LPS, with or without the filler T_H cells; $sIgA^+$ or $sIgG^+$ B cells, positively selected after removal of $sIgM^+$ cells, are not. The main finding is that the PP T_H line induces a rise to over 40% $sIgA^+$ cells in PP $sIgM^+$ B cells and to about 9% in splenic B cells over the five days in the presence of LPS (42). The splenic T_H-cell line is relatively inert, although its presence with LPS is sometimes accompanied by a modest (1.5–2.0-fold) rise in the number of $sIgG^+$ cells. No appearance of cells with cytoplasmic IgA accompanied any set of stimuli. Although the stimulated bulk cultures increased about 3-fold over five days it is difficult to rule out selection, either for survival or replication, since isolated, LPS-stimulated B-cell clones can easily expand 60–100-fold in this time (10,17). Further, since PP B cells respond to the stimulus of LPS plus PP T_H cells far differently from splenic B cells (42% vs 9% becoming $sIgA^+$), it is difficult to rule out an inherent difference in the potential of the B cells. Strober and his co-workers (42) suggest that many PP B cells may already be "partially switched." Possibly these cells have already completed their switch to activate the α-chain gene but still remain partially responsive to LPS. After all, the responsive B cells were positively selected for sIgM and may have borne too little sIgA to have been killed by anti-α plus complement. Thus, the PP T_H line may indeed play a role in the maintenance and development of $sIgA^+$ cells in culture, but we are not persuaded that the PP T_H cell line itself induced the switch.

Finally, although LPS is considered a B-cell mitogen, we conclude this section with a few further observations suggesting that full expression of all secreted isotypes indicative of an LPS-reactive B cell may be dependent on growth, differentiation, and maturation factors that remain uncharacterized. Whether any of these may be isotype-specific is conjectural. Nevertheless, the cultures most supportive of B-cell clonal outgrowth and expression contain copious filler cells—often nonirradiated rat thymocytes (17,40). Goodman & Weigle recorded the potentiation of LPS-induced PFC by addition of murine splenic T cells to purified B cells (62,63). More recently, others have reported that conditioned media from some murine hybridomas and T-cell lines (64),

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or from ConA-induced rat spleen cells after the removal of IL-2 (65), could potentiate the secretion of IgG or IgA, respectively, from LPS-stimulated bulk cultures of purified B cells. So far, no special effects of additives to culture on expression of IgE by LPS-stimulated murine B cells have been shown. However, all these observations suggest that the display of the entire range of isotype potential by LPS-reactive B cells may be dependent on a complex set of stimuli not always present in clonal cultures. Indeed, if hapten-specific B cells, enriched by plating on haptenated gelatin (12), are stimulated in microculture (9,10) with LPS plus dextran sulfate or with LPS alone, the resulting B-cell clones, proliferating in the absence of any other cells, rarely if ever secrete detectable IgG or IgA isotypes (J. Cebra, unpublished observations), although they frequently express IgM (9).

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MODELS FOR ISOTYPE SWITCHING DURING RESPONSE OF MURINE B CELLS TO LPS AND THE STATUS OF MOLECULAR GENETIC ANALYSIS OF THE PROCESS It seems that the subset of B cells reactive to LPS and displaying the full spectrum of responses (i.e. activation, proliferation, maturation, and secretion) is totipotent with respect to isotype potential. The responsive B cells appear to be capable of generating cells that secrete IgM and also progeny expressing and secreting any of the other isotypes. The observations are consistent with random isotype switches in the direction of activation of $5' \rightarrow 3'$ C_H genes. The development of B cells with this full range of isotype potential seems independent of antigen stimulation—it occurs beginning with 13-day fetal liver cells (33)—and of T-cell influences—athymic mice generate the same sort of B cells (30–33). Clearly, intraclonal differentiation occurs with respect to expression of non-IgM isotypes. A rough probability for secretion of IgG isotypes can be perceived, decreasing with the distance of the C_γ gene from C_μ gene. The overall process viewed at the cell biologic level could be described as random switching with unequal probabilities. Sequential switches could further modify overall probabilities for expression of a particular IgG isotype. Aside from raising the concern over the possible discrepancy between switching at the genetic level and at the level of either expressed sIg or secreted Ig, the observations concerning IgE or IgA expression from LPS-responsive clones seem uninformative about the switching process.

Molecular analysis at the DNA level has been meager for normal B cells involved in the switching process. However, most analyses have been of B cells before and after their response to LPS stimulation. Normal IgM-bearing B cells were positively selected by FACS and their DNA was compared with that from mouse embryos by restriction endonuclease mapping (66). The Kpn 1 and Eco R1 enzymes were used for selective cleavage to analyze for the juxtaposition of V_H and C_μ genes, which is accompanied by deletion of sequences between the V_H/D gene and one of the J_H sequences closely linked with the

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C_μ gene (67,68). This process precedes normal isotype switching to yield active heavy-chain genes. The findings indicated that $V_H/D/J_H/C_\mu$ or $D/J_H/C_\mu$ recombinational events are not restricted to the expressed chromosome but may proceed consecutively on both chromosomes (66). Normal splenic cells were then cultured with LPS for four days, gently fixed, stained for cytoplasmic IgM, and separated by FACS to yield IgM plasmablasts (69). Their DNA was compared with that of embryonic DNA using the restriction endonucleases Eco R1 and Bam H1 as probes. Compared with those of B lymphocytes or embryonic cells, at least 25% of the C_μ genes were further altered in context and had undergone another rearrangement involving the loss of an EcoR 1 site normally present between the J sequences and the C_μ gene (69). Since this event accompanies normal proliferation and maturation of B cells, it may play a role in regulating the development of secretory plasma cells or in influencing the probability of isotype switching. Recently, day-6 and day-9/10 IgG3-containing blasts were isolated from LPS-stimulated cultures by FACS, and their DNA was analyzed for content of C_μ genes (70). By day 6 after stimulation, IgG3-containing blasts had lost, on average, half of their complement of C_μ genes. Surprisingly, similar switched cells sampled at the later time showed appreciable loss of the remainder of their C_μ genes. These observations are consistent with the deletion of C_μ gene during isotype switching of normal B cells as suggested by a model proposed by Honjo & Kataoka based on analysis of DNA from plasmablasts (71). Further, this process may continue on both homologous chromosomes, although only one heavy-chain locus is expressed.

Does Isotype Switching Occur During Responses to Submitogenic Doses of Type 1 Antigens?

Formally, there may seem to be little difference between the stimulation of B cells with mitogens, such as LPS, and with submitogenic doses of those bearing antigenic determinants, such as endogenous O-antigenic determinants or chemically coupled haptens. This would presumably be so if the full range of clonal responses were due only to mitogen interacting with cell-surface molecules followed by subsequent intracellular events leading to all other modes of response, and if the Ig receptor for antigen served only to concentrate or "focus" the Type 1 antigen on the cell surface (72). The previous section documents that the display of the full range of isotype potential by LPS-stimulated B-cell clones, especially of secreted product Igs, is mediated by a much more complex sets of signals. Some of these other stimuli may well require the interaction of mitogenic concentrations of LPS with filler cells for their production and release to act on B-cell clones (17,63). Thus, although we can find little information concerning isotype switching as revealed by

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expression of sIg or cytoplasmic/secreted Ig by single B-cell clones, it may be informative to review Type 1 antigen responses briefly.

Fidler found that both athymic and euthymic mice, immunized with low doses (1 μg) of TNP-LPS, displayed equivalent serum and PFC responses entirely consisting of IgM anti-TNP (73). The response in vivo was maximal at day 5 and there was no evidence of polyclonal stimulation of antibody synthesis. No evidence of priming for a secondary response was found, and all second responses were also exclusively of IgM antibodies. Subsequently, Minami found that mice injected with LPS made significant amounts of a then recently described isotype of antibodies, IgG3, specific for the endogenous O-antigenic determinants (74). It was considered possible that previous attempts to detect isotype switching upon stimulation with haptenated-LPS (H-LPS) had failed to employ reagents that could score IgG3 antibodies. Since then, several groups have used either TNP-LPS (100 μg) or PC-LPS (5 μg) to immunize mice and have found, along with IgM antibodies, a significant IgG3 serum and PFC response (75,76). Often some IgG2 is also produced, but usually little IgG1 antibody can be detected. Thus, it appears that submitogenic doses of H-LPS can stimulate isotype switching in vivo as revealed by secretion of non-IgM antibodies, but there is a caveat. The LPS probably exists in the bacterial outer membrane in close association with lipids and with proteins variously known as "endotoxin protein," "lipid-A-associated protein," and "porin" (77–79). LPS preparations are usually contaminated with these proteins. Preparation of LPS by the trichloroacetic acid (TCA) method generally leaves 5–15% protein, while the phenol/water (PW) method usually gives a product that is <1% protein. Hepper and co-workers (80) observed that a primary in vivo response to PW-LPS consisted exclusively of IgM PFC, while the primary response to TCA-LPS showed a much larger number of IgM PFC and some IgG PFC. Both kinds of LPS preparations gave IgM and IgG PFC as secondary responses, although the response to TCA-LPS was greater. Truffa-Bachi and his co-workers (81,82) went to considerable lengths to render the PW-LPS used to make TNP-LPS free of protein and to control for *specific* T_H-cell influence of the response. Nevertheless, they found that secondary stimulation with their TNP-LPS (5 μg) did yield both IgM and IgG PFC and serum antibodies in C57BL/6 mice. Priming with the homologous "carrier" LPS alone did not lead to IgG PFC on challenge with TNP-LPS, and priming with TNP-conjugates made with a different serotype of LPS was also effective in giving secondary IgG PFC on challenge with the original conjugate (81,82). However, this secondary IgG response to submitogenic doses of protein-free H-LPS was relatively rare in mouse strains and somewhat peculiar to C57BL/6 mice (81). Finally, in vivo priming with fluorescein-LPS (FL-LPS) led to increased IgM PFC on secondary challenge with either FL-LPS, FL-poly-

Annual Reviews

merized flagellin (POL), or FL-*Brucella abortus* (BA), suggesting cross-priming by FL-LPS of B cells sensitive to either Type 1 or Type 2 antigens (83).

Jacobs & Morrison were able to induce IgM PFC responses in vitro using exceedingly low doses (1 ng) of TNP-LPS in bulk cultures of spleen cells (10^7/ml) from either normal or thymectomized, irradiated, bone marrow–reconstituted donors (84). Since mild alkaline hydrolysis of TNP-LPS, known to cleave away only the mitogenic lipid A moiety, rendered the antigen inactive, they inferred that its mitogenic effect played a role in inducing the PFC response. Using limiting dilution of splenocytes in minicultures, frequencies of IgM PFC^+ clonal precursors were determined that were reactive with TNP-LPS, TNP-Ficoll, TNP-dextran, TNP-erythrocytes, and pairs of these haptenated carriers (85). These frequencies suggested considerable overlap in the subsets of B cells responsive to Type 1 and TD antigens and less overlap between the Type 1 and Type 2 stimulable sets. Other attempts to analyze subsets of B cells responsive to these different types of antigen agree that H-LPS and H-BA, both Type 1 antigens, stimulate the same subset; but the analyses are less easily reconciled concerning other possible cross-responsiveness (83, 85, 86). One broad generalization that may be compatible with most of the diverse observations is that considerable overlap exists between B-cell subsets responsive to Type 1 antigens and those responsive to other forms in unprimed spleen. After in vivo priming with one form of the antigenic determinant or another, the subsets show less cross-responsiveness.

Clones have been generated from single, antigen-specific B cells in microculture by stimulation with submitogenic concentrations of H-LPS or H-BA in the absence of filler cells (87) or in the presence of very few (20–30) WEHI-3 filler cells (13). A significant proportion of these clones secrete specific IgM antibodies, but their possible switching to expression of other isotypes was not tested. The overall frequencies of FL-specific B cells responding to a variety of Type 1 and Type 2 forms of FL-antigens is consistent with considerable congruence of the B-cell populations responding to each in the absence of filler cells and with the premise that distinctions between these two types of antigen may fade at the single cell/clone level (87). Recent analyses for secretion of any IgG or IgA isotypes by IgM-producing clones derived from single, antigen-specific B cells indicated that such a display of switching is rare if it occurs at all (J. Cebra, unpublished observations). Thus, the B cell responsive to submitogenic doses of H-LPS or H-BA remains less well-defined than that reactive with mitogenic levels of LPS in the various culture systems at their present state of optimization. The target B cell clearly has the potential to proliferate and generate cells secreting IgM. In vivo studies suggest a potential for expression of other isotypes, but other stimuli accompanying submitogenic doses of H-LPS or H-BA that effect secretion of non-IgM iso-

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types have not yet been successfully employed at the clonal level. Analysis of switching at the level of sIg has just begun for clones generated by H-LPS or H-BA, and preliminary data, shown in Table 1, indicate switching of substantial proportions of clonal progeny to expression of sIgG1 or sIgA.

C_H *Isotype Switching Accompanies Responses to Type 2 Antigens*

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The weakly mitogenic Type 2 antigens stimulate the expression of the heavy-chain class switch after in vivo immunization, although various investigators report predominantly IgM responses with low or variable detection of IgG PFC (88,89), while others detect significant IgG responses based on molecular size of antibody (90) or its ability to adsorb to SPA (91). The use of isotype-specific antisera allowed the detection of antigen-specific IgG or IgA isotypes from mice immunized with various Type 2 antigens by the facilitation of PFC (75,92–94) or radioimmunoassay (95,96). Antibodies of the IgG3 isotype dominate the IgG response in immune serum from mice immunized with some Type 2 antigens (95,96). This isotype is rare in murine serum. Thus a major population of non-IgM plasma cells may often be undetected owing to the inability of polyspecific xenosera raised to murine immunoglobulins to react with it.

In bulk cultures immunized with dextran, Kagnoff and co-workers (94,97) have detected PFC secreting IgA. This response is observed only in the presence of T cells or soluble T-cell products and is more pronounced in cultures from aged animals. Mosier et al report the response to TNP-Ficoll in vitro to give IgG PFC, although the IgG3 component of this response was not specified (98).

Table 1 Surface immunoglobulin detected by direct immunofluorescence on clones stimulated with FL-Ficoll or FL-LPS[a]

Surface isotype	Stimulating antigen	% Cells recovered
sIgG1[b]	FL-Ficoll[c]	9.3 (n = 97)
sIgG1	FL-LPS	10.3 (n = 116)
sIgA	FL-Ficoll	22.5 (n = 111)
sIgA	FL-LPS	49.5 (n = 105)
sIgG3	FL-Ficoll	6.4 (n = 113)
sIgG2b	FL-Ficoll	6.2 (n = 179)

[a]Cells were pooled from wells of 10 μl cultures where approximately two thirds of the wells had clones
[b]Immunofluorescent reagents were directly coupled RITC-RAM γ3, γ1, γ2b, and α.
[c]Concentration of FL-Ficoll = 10 ng/ml; FL-LPS = 1 μg/ml.

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SWITCHING IN B-CELL CLONES AFTER STIMULATION WITH ANTIGENS Although Type 2 antigens have been used to stimulate clonal development of B cells, the ability of these clones to secrete multiple isotypes has only been analyzed by a few laboratories. TNP-Ficoll, bacterial levan (BL), and a heat-killed vaccine of the rough strain of *Streptococcus pneumoniae* R36A (PnC) stimulate B cells at limiting dilution in the presence of accessory cells in an irradiated spleen fragment to secrete multiple isotypes in vitro (20). The predominant isotypes detected by radioimmunoassay are IgM and IgA. In response to BL or PnC, IgG appeared in 67 and 60% of the clones, respectively. IgG2 was the predominant IgG isotype secreted in cultures stimulated with BL, while IgG3 was dominant in responses to PnC. Cultures stimulated with TNP-Ficoll had detectable IgG2 in approximately 10% of positives; the bulk of the response was IgM or IgA. Mongini et al have reported a similar analysis of B-cell clones stimulated with TNP-Ficoll (21,99). Using nu/nu recipient mice for splenic fragment culturing, B cells with or without added T cells were scored for the production of anti-TNP antibodies of all isotypes except IgD. TNP-Ficoll-stimulated clones secreted immunoglobulins of all isotypes, with IgM, IgG3, and IgA appearing in the majority of clonal cultures. Their analysis showed that the frequency of secretion of various IgG isotypes correlated with the 5′ to 3′ gene order; and in clones positive for any given IgG, secretion of the isotypes whose genes lie 5′ in the heavy-chain gene complex was frequently observed. The frequency of clones secreting IgE or IgA exceeded the frequency of all IgG isotypes except IgG3, and a high probability of co-expression of 5′ encoded C_H gene products was not observed in clones producing IgA.

The different patterns of IgG isotype expression reported for TNP-Ficoll responsive clones may reflect the technical differences between the assays. Factors such as the strain of mice, euthymic versus athymic recipients, the sensitivity or specificity of the radioimmunoassays, the molecular characteristics of the antigen, or the culturing conditions may contribute. However, clonal analysis of B cells in euthymic mice (20) reveals two general patterns of isotype expression in response to Type 2 antigens. The response to TNP-Ficoll only elicits small numbers of clones secreting easily detectable IgG isotypes. These differences may reflect differences in the antigenic/mitogenic composition of such antigens prepared from microorganisms, the hapten density on the carrier molecule, or the level of endotoxin contamination. Alternatively, the patterns of isotypes secreted in response to BL or PnC may reflect a more differentiated state of B cells reactive with these determinants acquired by environmental stimulation. These antigenic specificities have been determined to be environmental by criteria previously described (100). Although the clones that respond to BL or PnC in vitro secrete IgM as a product, indicating that their precursors retained an active C_μ gene, their ability to secrete IgG isotypes may depend on environmental priming in mucosal fol-

Annual Reviews

licles to render their member progeny susceptible to maturation/secretion signals after switching to IgG. It is interesting to note that cells enriched by binding to haptenated gelatin dishes and stimulated with FL-Ficoll in microcultures, in the absence of filler cells or conditioned medium, form clones of cells and generate IgM PFC (87). Although no switching has been detected in these cultures by PFC or radioimmunoassay (J. Cebra, P. Schweitzer, unpublished observations), preliminary evidence shows that non-IgM isotypes are detectable by immunofluorescence on the surface of daughter cells from these clones as shown in Table 1. Cell populations derived from clones generated in response to FL-Ficoll and analyzed by direct immunofluorescence for sIgG3, sIgG2b, or sIgA show approximately 25–40% of cells bearing non-IgM isotypes at day 4. This population consists of predominantly IgA-bearing cells (22.5%), with smaller percentages of IgG-bearing clonal daughters. The analysis of single clones indicates that all clones are not homogeneous, and not all clones switch to a given isotype (data not shown). Likewise, clonal daughters of cells stimulated by FL-LPS have 10% $sIgG1^+$ and 49.5% $sIgA^+$ cells.

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CHARACTERISTICS OF THE RESPONSIVE B-CELL SUBSET The phenotype of the B-cell subset that responds to Type 2 antigens may reflect the developmental stage of these cells and may indicate their accessory cell requirements or their potential to express immunoglobulins as secreted products. The subset of cells that give rise to PFC after in vitro immunization is defined by the anti-Lyb5 and anti-Lyb7 antisera. This population may also bear Lyb3 determinants [reviewed in (101)].

Treatment of normal, adult spleen cells with anti-Lyb5 plus complement diminishes the response to TNP-Ficoll and TNP-Hy in vitro, and administration of the anti-Lyb7 serum to cultures immunized with TNP-Ficoll reduces the PFC response 70–80% but does not affect the response to TNP-Hy (102). The inability to identify the molecule(s) with which these antisera react or their roles in the immune response makes it impossible to designate either marker as exclusive for the Type 2 responsive cells. If $Lyb5^+$ cells responsive to TNP-Ficoll and TNP-Hy are overlapping subsets, then the blocking of the Type 2 response by the anti-Lyb7 serum indicates that there is an obligatory requirement for the Lyb7 determinant(s) to function as receptor or signal for the maturation to PFCs. These interactions may not be required for the response to TNP-Hy. The characteristics of these antisera make it impossible to sort for these populations in order to answer these questions directly. The presence of the Lyb3 marker on the Type 2 responsive subset is implied by the absence of $Lyb3^+$ cells in the CBA/N mouse (103) and its late appearance in ontogeny.

The ontogenic appearance of responsiveness to Type 2 antigens correlates with the appearance and increase of the $Lyb3^+$ and $Lyb5^+$ cells at approximately three weeks of age. Mosier et al (104) stimulated spleen cells from

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mice of various ages with TNP-Ficoll and reported that the number of PFC generated in vitro reached adult levels at 3–4 weeks of age. This lack of neonatal responsiveness is not likely to be caused by the immaturity of accessory-cell populations because irradiated spleen cells from adult animals do not increase the number of PFC generated from neonatal B cells. This late-appearing population of cells also bears the sIgM < IgD phenotype; sIgM > sIgD cells remain after treatment of adult cells with anti-Lyb5 and complement (105). Eldridge et al (106) sorted normal spleen cells on the basis of sIgM density. Cells expressing low sIgM densities responded to TNP-Ficoll, in vitro, while the population with a high density of sIgM did not respond to TNP-Ficoll with the generation of PFC. High densities of IgM are present on a high proportion of neonatal B cells (43) and on CBA/N splenic B cells (106).

In contrast to the Lyb3, 5, and 7 markers and sIg phenotype, all of which suggest that the Type 2 antigen–responsive cells are found in a late-appearing B-cell subset, surface I-A/E densities are found to be low on cells responsive to TNP-Ficoll. Surface I-A/E densities are also low on neonatal B cells, and their density rises to adult levels at approximately eight weeks (107). B cells from CBA/N animals also show this increase in surface I-A/E density with age, but fewer arise that bear low-to-intermediate densities of I-A/E. The B cells that respond to TNP-Ficoll in vitro can be killed by anti-I-A/E plus complement (108). Greenstein et al (109) subsequently used FACS to divide B cells into Ia^{high} and Ia^{low} populations. Cultures stimulated with TNP-BA or TNP-SRBC gave equivalent PFC responses from each population. TNP-Ficoll, however, stimulated a response only in the Ia^{low} population of B cells.

Thus, the Type 2 antigens stimulate a late-appearing subset of B cells. Eldridge et al have identified a population of B cells that bear low densities of surface IgM in PP of CBA/N × BALB/c (*xid*/Y) mice (106). These $sIgM^{low}$ cells can respond to TNP-Ficoll in vitro to generate PFC. Since PP are the site of antigen sampling from the gut, and are the proposed site of antigen-driven B-cell differentiation (100), this observation indicates that antigen may have a role in driving B cells to more responsive states.

REGULATION OF THE EXPRESSION OF INTRACLONAL SWITCHING The pattern of isotypes secreted in response to Type 2 antigens in vitro may be modulated by the presence or activation of T cells. The removal of viable T cells from the cell inoculum before stimulation with BL in splenic fragment culture led to a decrease in the number of positive clonal cultures that had detectable IgG2 and IgG3 (20). Similarly, stimulation of bystander T cells in cultures responding to PnC or TNP-Ficoll led to an increase in the frequency at which IgG isotypes are detected in culture supernatants. The frequency of responsive cells did not change significantly (20,110), and the effects of T cells on these clonal responses were not dramatic.

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Mongini et al (21,99) have analyzed the effect of T cells upon clone: responding to TNP-Ficoll in athymic mice. When viable T cells are include(in the donor inoculum, clones responding to TNP-Ficoll showed enhance(expression of switching. This was observed as an increase in the number o clonal cultures that secreted more IgG isotypes. They propose that the T-cel effects are not directed at IgG2-bearing cells but act to enhance the probabilit' of a switch to these isotypes. In addition, an increase in the number of clone that secreted only IgA was observed with a concomitant decrease in IgM plu IgA producers. Along with this increase in frequency of clones secreting onl' IgA, the average amount of IgA secreted in the presence of T cells was highe than that observed in their absence. This effect has also been reported for ' cells on the TD clonal response to inulin-Hy and cholera toxoid (111). Th regulation of non-IgM isotypes in clonal microcultures appears to be controlle(or modulated by T cells as well. Cells selected by binding to hapten-gelati plates and stimulated in vitro with the Type 2 antigens, haptenated POL, o haptenated Ficoll form direct plaques (87) and secrete IgM antibodies detect able by radioimmunoassay (J. Cebra, P. Schweitzer, unpublished observa tions). In the absence of filler cells, secretion of non-IgM isotypes was a rar event. The addition of 10^5 thymus fillers to clones responding to haptenate(POL or haptenated Ficoll in microcultures results in 5–15% of productiv clones secreting detectable IgG and/or IgA isotypes by radioimmunoassay (J Cebra, unpublished observations).

OVERLAP OF SUBSETS RESPONSIVE TO TYPE 2 ANTIGENS AND TD ANTIGEN. Controversy exists over the identity or extent of overlap of the cell popula tion(s) that respond to Type 2 and TD antigens. B cells, when stimulated b' a mixture of TD and Type 2 antigens in batch culture or miniculture, hav been reported to give superadditive responses when challenged with PnC plu Pc-Hy (16), TNP-T4 plus TNP-Hy, or DNP-POL plus TNP-Hy (112). Fo these cultures to show anything more than additivity, there must exist eithe interactions between the antigenic forms, which may stimulate another, sepa rate population of B cells, or bystander effects that may not be detectable whe the TD carrier alone is added with the Type 2 antigen. For example, a B cel responsive to the TI form of the antigen may not be subject to bystander effect of the TD carrier in the absence of linked recognition. Thus, new population may be stimulated; or, under different antigenic challenge, the characteristic of the response may be changed (e.g. a larger burst size by responsive clones) The population subject to these effects and the magnitude of the change become critical in the interpretation of these data to determine the extent o overlap of the Type 2 and TD responsive cells, when B cells are not limited

Limiting dilution analysis of the responsive populations has proved equiv ocal. Using the Type 2 and TD form of the phosphocholine determinan

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Quintáns & Cosenza (16) observed strictly additive frequencies using limiting dilution analysis of unfractionated splenic B cells in microculture. These cultures included 5 × 10^4 unirradiated and 5 × 10^4 irradiated cells from mice immunized with Hy. Likewise, Lewis & Goodman, using TNP-Ficoll and TNP-HRBC, reported an additive frequency of cells that give rise to direct PFC (85). Using similar culture methods, but utilizing hapten-gelatin enriched cells in the presence of Hy-specific helper T cell lines, Scott and co-workers (83) found the opposite. When FL-POL and FL-Hy are used there is considerable overlap in the population of cells that respond to either antigen as judged by nonadditivity in the frequency of responding cells. Experiments addressing this same question utilizing splenic fragment culture have also generated conflicting data. Fung & Köhler have reported a superadditive response to the C-polysaccharide from *Streptococcus pneumoniae* R36A and PC-Hy (110). The superadditive response revealed an incremental population that roughly equaled the frequency of C-polysaccharide responsive cells. Additivity was not observed by Hurwitz et al (20). Using the same haptenic system, although the Type 2 antigen was presented as a heat-killed vaccine of *S. pneumoniae*, the frequency of PC-specific cells that responded to PnC plus PC-Hy was the same as the frequency responsive to either antigen alone.

Utilizing antigen-specific suicide with BUdR plus ultraviolet irradiation, Scott and co-workers demonstrated an overlap in the populations of cells responsive to different FL-antigens. Antigenic stimulation and suicide in bulk cultures and subsequent rechallenge in miniculture revealed largely overlapping populations responsive to Type 1, Type 2, and TD antigens (113). Stimulation of cells with the haptenated forms of polymerized flagellin, LPS, Ficoll, PPD, *Brucella abortus*, or burro red blood cells, led to the diminution of the subsequent response to any of the other haptenated antigens by 60–90%.

On the basis of these data, the existence of one or two populations of cells responsive to the Type 2 or TD form of the antigen remains controversial. However, in light of the data presented above and those discussed in the next section, a given B-cell subset defined by response pattern may occur at different stages of a linear path of B-cell differentiation. Depending on the extent of previous antigenic exposure through deliberate or environmental stimulation, the observed populations may show more or less overlap. The results of these analyses also depend upon the ability of a given assay system to provide optimal stimulation for all responsive B cells at different stages of differentiation. For instance, Gorczynski & Feldmann have separated B cells on the basis of their sedimentation velocity and compared the populations responsive to Type 2 and TD antigens from naive and deliberately primed animals (114). Responsive cells from mice immunized with TNP-Hy, separated by velocity sedimentation and stimulated with DNP-POL or TNP-HY, were found in dif-

Annual Reviews

ferent populations if IgM PFC were scored. For naive mice, such separation of responsive cells reactive with either DNP-POL, DNP-dextran, or DNP-levan versus TNP-Hy or TNP-SRBC, was not observed. The precursors that generated IgG PFC in vitro were found in similar populations regardless of previous antigenic exposure.

IN VIVO PRIMING WITH TYPE 2 ANTIGENS FOR SUBSEQUENT TD RESPONSES: FREQUENCY AND ISOTYPE POTENTIAL OF THE PRIMED CELLS The effect of antigenic stimulation by Type 2 antigens upon subsequent responses by TD stimulable B cells may indicate the relationship between the populations of Type 2 and TD responsive cells. Previous in vivo immunization with TNP-Ficoll before transfer of spleen cells to irradiated recipients leads to a 2–4-fold increase in the PFC response when challenged with TNP-Hy (115). The mechanism of this priming has been investigated by the analysis of precursor frequencies and isotype potentials of cells responsive to TD antigens after in vivo immunization with Type 2 antigens. TNP-Ficoll immunization of neonatal animals leads to an increase in the PFC response upon subsequent in vitro stimulation with DNP-Hy or TNP-BA (116). Limiting dilution analysis showed a 10-fold increase in the frequency of TNP-BA-responsive cells and no increase in the burst size of responsive clones. Presumably the increased TD response is also due to an increased precursor frequency. This priming occurred in the absence of an in vivo PFC response to TNP-Ficoll. Shahin & Cebra similarly immunized newborn mice with bacterial levan and prematurely increased the frequency of cells responsive to the TD form of the β2→1 fructosyl determinant on inulin-Hy (117). All of the resulting clones expressed IgM, with or without other isotypes. Immunization of adult mice with TNP-Ficoll stimulates an increase in the frequency of cells responsive to TNP-Hy when challenged in carrier-primed splenic fragments at limiting dilution. A 3.8-fold higher precursor frequency was found in euthymic mice that had been immunized three weeks prior to analysis (111). Others have reported a 5-fold increase in DNP-Hy responsive cells from athymic mice immunized with DNP-Ficoll four to six weeks earlier (118), although the precursor frequency in primed euthymic mice was only slightly elevated at this time. The clones from cells of euthymic animals previously immunized with DNP-Ficoll showed no change in the frequency of IgG1 secretion, although previously immunized athymic animals showed an increase in the frequency of IgG1 secreting cells from 38 to 84%. Likewise, the analysis of IgG3, IgG2, and IgA secreted by clones from B cells of euthymic mice showed no changes in incidence after immunization with the Type 2 antigen (111).

Type 2 antigen priming for subsequent rechallenge with the *same* antigen has rarely shown an increase in antigen-sensitive cells or the generation of populations of memory cells (82; P. Schweitzer, unpublished observations),

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though transient "priming" may occur (83,119). The population of cells responsive to Type 2 antigens may be considered nonswitched in that IgM is secreted from clonal cultures of cells from unprimed or environmentally primed populations. As discussed above, in vivo immunization with TNP-Ficoll or bacterial levan expands a population of cells responsive to TD forms of the antigen. Whether this expansion occurs through proliferation of cells responsive to TD antigens, or movement of cells, without proliferation, from a state responsive to Type 2 antigen to a developmental state susceptible to stimulation with the TD antigen, or a combination of both processes, this population of TD antigen-sensitive cells shows little evidence of altered isotype potential. A small increase in the proportion of cells that respond to DNP-Hy in an allogeneic environment and secrete IgG1 is observed after DNP-Ficoll immunization in euthymic mice (118), indicating that more subtle developmental changes may be occurring in the absence of restriction in isotype potential. These subtle changes may be a consequence of changes in the density of surface receptors, such as I-A/E determinants, which correlate with the ability to secrete IgG1 in the milieu of an irradiated allogeneic spleen fragment (120). The ability to secrete other IgG isotypes in response to Type 2 antigens may also be dependent upon the developmental stage of the responsive B cells, which may determine the ability of such B cells to interact with accessory cells or receive the required signals to mature and secrete IgG antibodies. The driving forces for stimulating these developmental events by Type 2 antigens may be dependent upon the site of the antigenic stimulation. Microenvironments may differ in the GALT, BALT, or spleen, where B cells may have different developmental fates when stimulated by acute or chronic exposure to Type 2 antigens.

C_H-Isotype Switching and Apparent Restriction in Isotype Potential in B Cells During Their Response to Thymus-Dependent Antigens

Ever since the "carrier-effect" was first elucidated (121), in which antigen-primed T cells synergize an antibody response by B cells specific for determinants attached to the same "carrier" antigen, the expression of IgG antibodies has been taken as one parameter of T-B interaction. Subsequently, the T_H dependence of the expression of a switch by responding B cells to secretion of particular isotypes such as IgG1 (122,123), IgE (124,125), and IgA (123,126) has been supported by now classical approaches using thymectomy and/or adoptive transfer of appropriately primed T- and B-cell mixtures.

Subsequent to the detection of single plasma cells secreting both IgM and IgG antibodies following an in vivo response by rats to a thymus-dependent antigen (1,2), the first convincing demonstration that proliferating B cells could undergo intraclonal differentiation leading to the expression of multiple

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isotypes—IgG1, IgM, and IgA—was by Gearhart and her co-workers (55). The splenic-fragment, T-dependent culture system was employed; specificity for hapten, isoelectric focusing, and idiotype analysis were used to support the clonality of the B cells contributing to the secretion of mixtures of isotypes. Later, allotype analysis of two different isotypes, IgA and IgG1, was applied to clones derived from allotype heterozygous B cells making both isotypes to demonstrate that the same single heavy-chain locus allele remained active during intraclonal switching (57). Such clones, making anti-PC antibodies, were dispersed at different times after antigen stimulation in an effort to detect any pattern of switching or the occurrence of successive switching. Clonal daughters were identified by staining cells for antigen bindingwith a rhodamine conjugate. Switches in isotype expression were detected as the dual expression of IgM and IgA or of IgG1 and IgA in single, clonally related plasmablasts by using a mixture of a fluorescein conjugate reactive with one isotype and a tritium-labeled diagnostic antibody reactive with the other. Analysis was restricted to clones expressing at least some IgM along with the other two isotypes in an effort to compare progeny derived from precursor cells that were initially at the same stage of differentiation with respect to isotype potential. Some clonal daughters were found expressing only IgM + IgA or only IgM + IgG1. These pairings were consistent with direct $V_H/C_\mu \rightarrow V_H/C_\alpha$ and $V_H/C_\mu \rightarrow V_H/C_{\gamma 1}$ switches. The possibilities that *successive* switches could occur in the same cell line of an expanding clone was also suggested by observations that (*a*) some of the plasmablasts from clones secreting IgM contained both IgG1 and IgA, and (*b*) double producers expressing both IgM and IgA decreased in frequency with time after stimulation while cells expressing both IgG1 and IgA increased in frequency (57). Documentation of the intraclonal diversification process occurring in T-dependent clones developing in splenic fragments has since been extended to show that any one or mixture of the known mouse isotypes of antibody can be secreted by single clones, including IgG2a, IgG2b, IgG3, and IgE (20,54,126–128).

RANGE OF ISOTYPES DISPLAYED BY THYMUS-DEPENDENT CLONES REFLECTS CHANGES IN THE POPULATION OF CLONAL PRECURSORS DURING NEONATAL DEVELOPMENT AND UPON NATURAL OR DELIBERATE IN VIVO PRIMING BY ANTIGENS Comparisons of isotypes expressed by thymus-dependent B-cell clones of many different specificities—anti-DNP, anti-TNP, anti-NIP, and anti-In—generated in splenic-fragment cultures from neonatal (three day to three week) and adult splenocytes show that a conspicuous proportion of the former (35–63%) secrete only detectable IgM while the proportion of such clones from adult cells is about 20–30 percentage points lower (117,129–132). A number of observations are consistent with the hypothesis that the precursors of such clones are B cells at an early stage of development that are prevalent in the

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perinatal mouse but that also occur in adult bone marrow and spleen. For instance, resolution by FACS of neonatal and adult spleen cells into sIg^- cells (which include pre-B cells in transition to antigen-sensitive B cells that can eventually generate antibody-secreting clones) and sIg^+ cells (already at the B lymphocyte stage) yielded percentages of clones only secreting IgM for sIg^-:sIg^+ cells of 50:29 and 60:28 for the younger and older cell sources, respectively (131). Several findings support the IgM clonal precursors' arising recently from pre-B cells: They are especially prevalent in large, rapidly sedimenting cell fractions from adult spleen and bone marrow and from neonatal spleen; and such cells, as a population, divide more frequently and take longer to generate clones making detectable IgM (132,133). Fetal liver and spleen, taken at 17–19 days of gestation and assayed for clonal precursors directly or after 3–5 days of organ culture, also give rise to a majority of clones only secreting detectable IgM (26).

The next most prevalent phenotype observed among anti-DNP clones derived from B-cell sources considered to be rich in cells at early stages in development is the secretion of IgM together with one or more IgA or IgG isotypes. In general, clones coexpressing IgM and IgA tend to predominate from fetal liver and spleen, but these diminish proportionally after birth of the spleen donor and then seem most prevalent among clones derived from the sIg^- population (26,131). Culture of fetal liver or spleen for 3–5 days results in the appearance of precursors that generate a modest proportion of clones secreting IgM with IgG isotypes (26). Precursors giving such clones making IgM and IgG isotypes, often along with IgA, continue to rise proportionally within their specific, antigen-sensitive population after birth until their clones give the dominant phenotype of those secreting anti-DNP derived from non-immunized, adult spleen (131).

Another characteristic of B cells at an early stage in development is their sensitivity to tolerization by haptenated proteins in the absence of competent, specific T_H cells. Exposure of specific neonatal B cells at limiting dilution in splenic fragments to haptenated IgG is especially effective at rendering them refractory to subsequent stimulation by the same hapten coupled to hemocyanin in the presence of specific T_H cells (130). Fetal liver and spleen cells and neonatal (three day) spleen cells are very susceptible to tolerization and, among the latter cells, the sIg^- fraction is extremely sensitive (131). The only cell fraction from adult spleen easily tolerized is an analogous sIg^- population. Thus, given a similar tissue-source distribution of precursors that give rise to clones only secreting IgM it is perhaps not unexpected that these appear to account for almost all of those cells susceptible to tolerization (130,131), although not all cells in this class are rendered refractory (131). Of course, the proportion of precursors giving clones expressing non-IgM isotypes are relatively enriched by the tolerization process and are likely unaffected. Thus,

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it appears that one subset of B cells at an early stage of development is responsive to TD antigens and T_H cells, has recently undergone a transition from sIg^- to sIg^+, is readily tolerized, and generates clones that only secrete detectable amounts of IgM. It remains to be determined whether these clones undergo isotype switching without display of secreted non-IgM products. Factors from T cells that stimulate secretion of IgG have been described (49,60,134), and neonatal B-cell populations have been shown to yield increasing numbers of secretory plasma cells with age of neonatal donor upon polyclonal stimulation by T_H-type cells (25). Perhaps resistance to tolerization and competence to express isotype switching by secretion are gained in tandem during the differentiation process.

The preceding analyses apply to B cells of specificities such as anti-DNP, anti-TNP, anti-NIP, and anti-FL. The frequencies of TD clonal precursors of these specificities among neonatal, conventionally reared, young adult, and germ-free, young adult splenic B cells is roughly the same—40–50 per 10^6 B cells (132,135). However, other specificities of B cells from neonatal mice, such as anti-PC, anti-In, and anti-$\alpha 1 \rightarrow 6$ Dex, appear to occur at frequencies responsive to TD stimuli in splenic fragments too low to be comprehensively evaluated with respect to isotype potential (117,136,137). However, a sharp, spontaneous rise in anti-PC precursors from about 2.8 to 15–20 per 10^6 B cells occurs between day 9 and day 14 of neonatal life (136). Similarly anti-In responsiveness (138) and clonal precursors (117) increase between three and five weeks of life as does anti-$\alpha 1 \rightarrow 6$ Dex responsiveness by three months of life (137). In the case of splenic B cells from young adult mice that were not deliberately immunized, the class of anti-In and anti-PC clonal precursors most prevalent were those generating TD clones secreting IgM along with one other or a mixture of IgG and IgA isotypes (117,127,139). Thus upon late expansion, TD-reactive splenic B cells of these specificities generate clones that display the same dominant phenotype as found among clones from young adult, splenic anti-DNP B cells, although the frequencies of this latter specificity remain about the same from birth (131,135). One possible explanation for the observed transition in the splenic B-cell compartment in the weeks after birth is that natural priming by environmental mitogens/antigens both mediates the gain in competence by B cells to display clonal isotype switching and substantially increases the frequencies of some specificities of B cells—the latter being an accepted parameter of priming defined at the cellular level. Such a process could either act alone on the B-cell compartment, simply by selectively driving clonal expansion, or act competitively along with T_H cells against a natural process of tolerization (130,131). The suggested roles for natural, environmental stimulation were given some support by the findings upon clonal analysis of Peyer's patch (PP) B cells of various specificities (126,139). Peyer's patches were known to be richer than other lymphoid tissue

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in B cells with the potential to generate IgA plasma cells (140,141). The findings upon analysis of single clones from PP cells explained this bias and showed that 40–60% of clones of specificities such as anti-PC, anti-In, and anti-βGal exclusively expressed IgA while clones of other specificities, such as anti-DNP and anti-TXD, gave a mixture of isotype displays similar to those given by clones from splenocytes from the same non-immunized donors (111,126,139). These data are consistent with the selective priming of PP cells by naturally occurring antigens in the gut and the subsequent restriction in isotype potential of specific B cells to IgA expression.

Deliberate priming with TD antigens by different routes increases frequencies of specific TD clonal precursors and apparently restricts isotype potential in some of these. Parenteral priming in vivo with DNP-protein conjugates increased the frequency of specific splenic clonal precursors about 2–3-fold (19). Subsequently other, more striking examples of expansion by antigen of In-specific (117) and TXN-specific (142) TD precursors by 10–100-fold have been observed. Generally, the lower the initial frequencies of specific B cells in unprimed mice the more dramatic the increase, which may persist for weeks (142). Table 2 shows some particularly marked increases in specific splenic clonal precursors upon various modes of in vivo priming. In our view, the TD-specific B-cell compartment, as assayed in splenic-fragment culture, is the most expandable of B-cell subsets responding to the different forms of mitogen/antigen, and such perturbations are the most persistent.

Another fairly consistent perturbation in the specific TD splenic B-cell population associated with in vivo administration of antigen parenterally is the shift towards higher proportions of derivative clones secreting one or several IgG isotypes, often with IgA, but in the absence of detectable IgM (see Table 2). Certainly, clones making IgG1 and/or IgA without IgM are present among those derived from neonatal or young adult non-immunized spleen (26,133), but the frequency of these expressing IgG2 (126) as well as the proportion expressing any or all IgG, IgE, and IgA isotypes in the absence of IgM rises roughly with the rise in absolute frequencies of specific cells after priming (117,128,142). It seems likely, in view of correlations between surface phenotype and isotype potential to be considered below, that at least some of the shift towards production of only IgG and IgA isotypes is due to a restriction in isotype potential of the primed clonal precursors.

While deliberate parenteral priming often leads to precursors restricted to IgG, IgE, and/or IgA expression, certain acute or chronic antigenic stimulation of the gut mucosa, such as intraduodenal injection of TXN (142) or intragastric delivery of embryonated *Ascaris* eggs (128), leads to the development of a large proportion of specific precursors in PP that give clones exclusively expressing IgA secretion (see Table 2). We have operationally defined such B cells as "committed to IgA" in that they arise during in vivo mucosal stimu-

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Table 2 Frequency of specific splenic B cells responsive to TD antigens rises and isotype potential changes following in vivo priming with TD antigens by different routes

	Immunologic state of donor mice; non-immunized (non) or immunized in vivo with:									
	non	GlcNAc-GG	non	TXN	non	TXN	ASC	PC-Hy,ASC	ASC	PC-HY,ASC
Specificity	anti-GlcNAc		anti-TXN				anti-PC			
Immunologic route		i.p.[a]		i.d.[b]		i.d.[b]	i.g.[c]	i.p.,i.g.[d]	i.g.[c]	i.p., i.g.[d]
B-cell source	Sp	Sp	Sp	Sp	PP	PP	Sp	Sp	PP	PP
Frequency/10^6 B cells	2	92	1–3	96	1–3	37	4	60	0.7	62
Number of clones	21	77	19	23	20	57	31	29	7	9
Percentage of clones secreting:										
Some M	100	64	58	30	75	19	61	48	62	44
G ± A, no M	0	32	36	52	10	42	26	48	42	22
Some A	10	10	21	56	20	68	39	41	71	78
A only	0	1	5	17	10	39	10	7	14	33
Time (wks)		4		2		2	4	7	4	12

[a]C3H/HeN mice given 3 weekly intraperitoneal (i.p.) injections 1 μg *N*-acetyl glucosaminyl (GlcNAc) coupled goat IgG (GG).
[b]BALB/c mice given one intraduodenal (i.d.) dose of 10 μg cholera toxin (TXN).
[c]CBA/N × BALB/c F_1 ♂ mice given 10^4 embryonated *Ascaris* (ASC) eggs intragastrically (i.g.).
[d]Mice infected with ASC as above, but 4 weeks following injection of 1 μg of PC-coupled Hy on 3 μg alum i.p.

Annual Reviews

lation with antigen and are capable of a further in vitro clonal expansion to yield copious and exclusive IgA secretion over 10–15 days (142). A similar generation of specific, anti-hapten, IgA-committed cells has been reported in PP following intraduodenal priming with haptens coupled to TXN that still retains its tissue-binding properties (143). Many lines of evidence suggest that these IgA-committed B cells are generated in and not collected by PP (142,144). One of the most recent and convincing is the finding of a large number of $sIgA^+$ B cells among the dividing population with a germinal center cell marker (PNA^+) in PP (145). However, the functional properties of these cells remain untested. Priming by the respiratory route in mice with TXN (144) or *Mycoplasma pulmonis* (F. Rose, unpublished) has not been effective at generating IgA-committed clonal precursors, but the latter, chronic stimulation does lead to a significant number of cells that give rise to clones exclusively secreting IgG1 or IgG2. In vivo stimulation by any route leads to wide dissemination of the primed state among all lymphoid tissues and the prolonged appearance of specific precursors in peripheral blood (142,144; F. Rose, unpublished). Peripheral-blood/thoracic-duct B cells are known to include a pool of long-lived, recirculating cells that could account for dissemination of primed cells and immunologic "memory" expressed at sites distant from those initially exposed to antigen (50,144,146). However, if gut mucosal priming is successful in generating IgA-committed cells in PP, a gradient of frequency of such cells that declines the more distal the lymphoid tissue seems to persist for weeks (128,142,144) against a background of rather uniform equilibration of all other specific B cells.

Since we have suggested that natural priming by environmental antigens in the gut may play a role in elevating and maintaining the steady-state frequencies of B cells of some specificities and of regulating their mix of isotype potentials in various tissues, we next consider a few pertinent analyses of gnotobiotic mice before and after deliberate colonization with various enteric bacteria. If mice are maintained germ-free for up to one year, the frequencies of their anti-In and anti-PC B cells in spleen and mesenteric lymph nodes remain at about ~2–3 and ~5 per 10^6 (100,127,147), levels comparable to those found in neonatal mice before the spontaneous rise, which are ~1 and ~3 per 10^6, respectively (117,136). Levels of these specificities in PP remain at ~10 per 10^6 under germ-free conditions (100,127). When these mice were monoassociated with *Proteus morganii,* an occasional gut commensal isolated from mice, or colonized with Schaedler's enteric bacteria, a group of commensals normally living in the gut of healthy mice, and either maintained in isolators or moved to an exposed, conventional environment, the frequencies of In- and PC-sensitive B cells rose steadily in all tissues (100,127,147). The rise was especially pronounced among PP cells, where cells of these specificities reached levels of 80–90 per 10^6 within 5–8 months after

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colonization and transfer to an exposed environment. Further, upon colonization the proportion of B cells that generated clones expressing only IgM or some IgM with other isotypes diminished relative to those in neonates. Concomitantly, B cells that gave rise to clones making IgG isotypes ± IgA but without IgM became more prominent. The most striking occurrence upon colonization was the establishment of a gradient of PC- and In-reactive B cells committed to IgA, with a frequency highest in PP and decreasing in more distal lymphoid tissues. Thus, the perturbations in the B-cell compartment achieved by colonizing germ-free mice appear to correspond to various ones achieved by deliberate mucosal or parenteral administration of antigens and to duplicate ones that occur "spontaneously" at different times during neonatal life in an unprotected environment.

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CHARACTERISTICS OF THE RESPONSIVE B-CELL SUBSETS The surface marker that has been most closely correlated with Ig isotype potential is the presence of sIg itself and the isotype(s) of this sIg. This should not be unexpected, since detection of sIg may simply be a more sensitive assay for the switch than analysis for cytoplasmic or secreted Ig, and may allow the detection of a switch at an earlier stage of the process of maturation from lymphocyte to plasma cell. Splenic cells from neonates (three days) were separated into sIg^+ and sIg^- fractions by FACS; the latter fraction, containing cells in transition from pre-B to B cells, gave the highest proportion of clones exclusively secreting IgM anti-DNP (131). A similar analysis of $sIgD^+$ and $sIgD^-$ fractions, separated by FACS from 2.5-week spleens that contain roughly equal proportions of the two types of B cells, showed that the latter gave the highest proportion of anti-DNP clones only making IgM—45 vs 16% (148). Thus, it appears that the inability to express switching from IgM during clonal outgrowth in vitro is a characteristic of B cells recently arisen from pre-B cells and of the IgD^- population that is more prevalent in neonates but that ordinarily becomes a minority as the IgD^+ cells increase (43). Attempts were then made to resolve non-immune and primed adult splenic cells into $sIgM^+$, $sIgM^-$, $sIgG^+$, and $sIgG^-$ fractions by FACS and to test their potential for isotype expression of anti-DNP (149). Distinctions among the various fractions, especially from unprimed cells, were not very clear, perhaps owing to pre-B/B-cell transitions occurring in the fractions that were expected to be $sIgM^-$ and owing to cytophilic IgG acquired passively in vivo. However, the vast majority of anti-DNP cells from non-immunized and most from primed spleen were $sIgM^+$, and most of these gave clones expressing multiple isotypes including IgM. Of relevance was the finding that $sIgM^-$ cells from primed fractions seldom gave clones expressing IgM, an observation suggesting isotype restriction had occurred during in vivo immunization. In another study, PP from non-immunized mice and splenocytes from mice parenterally immunized with PC-hemocyanin were

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cultured to remove cytophilic antibodies and then separated by FACS into $sIgM^+,IgD^+$ or $sIgM^-,IgD^-$ and $sIgG1,2,3^+$ and $sIgG1,2,3^-$ fractions, respectively (22). In agreement with the previous study (149), the $sIgM^+,IgD^+$ fraction from PP accounted for most of the clones that expressed anti-PC IgM, including those that switched to other IgG and IgA isotypes. The $sIgM^-,IgD^-$ fraction gave the clones making only anti-PC IgA or IgGs without IgM that occurred in the unfractionated population. Of the fractions from primed spleen, the $sIgG^+$ cells gave mostly clones making anti-PC IgGs without IgM while the $sIgG^-$ fraction accounted for most of the clones making some IgM. These data more clearly support isotype restriction occurring during natural or deliberate in vivo priming, its early reflection in sIg isotype, and its extension to the clonal progeny of switched B cells. These observations are consistent with those concerning $sIgG2^+$ and $sIgG1^+$ anti-DNP memory B cells, enriched by FACS and transferred into irradiated recipients (150). The $sIgG2^+$ fraction adoptively transferred mostly on IgG2 PFC response while the $sIgG^-$ fraction accounted for most of the IgG1 response. The 10% most intensively positive sIgG1 cells transferred mostly an IgG1 PFC response, suggesting that these cells did not often switch to IgG2 isotype expression over the seven-day test period. However, the $sIgG^-$ population (dullest 80%) was equally effective at transferring an IgG1 PFC response and it also accounted for most of the IgG2 PFC response given by the unfractionated splenocytes. Thus, this fraction of primed cells may undergo further in vivo switching. When the in vivo priming process was followed sequentially, the development of memory—i.e. the potential to adoptively transfer high-affinity anti-DNP responses—was correlated with the increasing absence of sIgD from specific cells, presumably reciprocally to the increasing presence of sIgG on these cells (151). A similar disappearance of sIgD from antigen-binding cells (ABC) was detected along with the appearance of sIgG after in vivo immunization, although most of the ABC displayed IgM before and after priming (152). Attempts have been made to correlate the continued presence of $sIgD^+$ on DNP-primed B cells with retention of long-term proliferative potential ("self renewal") using intermediate and secondary hosts for adoptive transfer assays (45). It will be of interest to analyze carefully the sIg isotype of those B cells responsible for the expansion of "memory" anti-DNP cells in one of the few in vitro miniculture systems purported to demonstrate this phenomenon leading to IgG PFC (153).

Another group of surface markers operationally present on most but not all B cells and appearing on increasingly more splenic cells in parallel with the rise in B cells during neonatal life are controlled by the I-region genes and expressed as I-A/E (154). A suggestion of haplotype restriction in the TD expression of the IgM to IgG1 switch was given by the finding that splenic cells from non-immunized mice would score as clonal precursors for anti-DNP at much lower frequencies in carrier-primed allogeneic spleens than in similar syngeneic ones and then only express IgM in the former spleens while many

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expressed both IgM and IgG1 in the latter (120). Curiously, after in vivo priming, anti-DNP cells scored about as well in both sorts of splenic fragments and their clones expressed the switch about equally well in either culture. A subsequent finding was that negative selection with anti-I-A plus complement abolished almost all anti-DNP precursors from primed spleen and most but not all such specific cells from non-immunized spleen (155). Clones generated by the surviving, operationally I-A negative, unprimed B cells only made IgM. Their precursors are recovered among the large and medium lymphocytes by sedimentation (133).

In vivo priming to generate anti-DNP cells that cross-score in allogeneic, carrier-primed splenic fragments and express the IgM to IgG1 switch could be effected with TD antigens (DNP-Hy) but not TI antigens (DNP-Ficoll); this phenomenon occurred in euthymic but not in athymic mice (118). Athymic mice primed with either sort of antigen showed increases in frequencies of specific cells and in proportions giving clones making IgG1 in syngeneic splenic fragments similar to those found for euthymic mice, although the former did not develop circulating antibodies to DNP-Hy. All these observations suggest a role for I-A/E in the expression of the switch as revealed by secreted IgG1 antibodies. Perhaps most, if not all, I-A/E-negative cells are coincident with those B cells that have recently arisen from pre-B cells and are negative or have low sIgD expression and can only generate IgM-producing clones. A second feature revealed by these studies involves the expression of a switch to secretion of IgG1 in allogeneic scoring fragments. The apparent dependence of this phenomenon on in vivo priming in the presence of competent T_H cells and circulating IgG1 antibodies may relate to the special thymus dependency of IgG1 secretion noted previously (49,58–60,122,123). It is not yet clear whether the apparent acquisition of competence to display the IgM to IgG1 switch in allogeneic scoring fragments reflects a change in B-cell potential or results from a special T_H-cell potential in the donors of the test inocula. It is of interest that co-expression of IgA with IgM occurred irrespective of whether TD or TI antigens were used for priming, athymic or euthymic mice were used as donors, and allogeneic or syngeneic splenic fragments were used to culture clones (155). Finally, the studies further support the development of isotype restriction in B cells of athymic mice stimulated with TD antigens. Other, in vivo studies had suggested that the acquisition of memory B cells with IgG potential did not require functional T cells (156–158). Recently, intraduodenal priming of athymic mice with TXN has been shown to lead to IgA-committed B cells in PP (111), and intratracheal infection of such mice with *M. pulmonis* leads to B cells giving clones only making IgG isotypes (F. Rose, unpublished). Thus, isotype switching and restriction in TD B cells appear to proceed in the absence of functional T cells.

A final set of surface markers that seem to correlate with B-cell respon-

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siveness to various forms of antigen are those called Lyb3, 5, and 7. The proportion of B cells bearing these markers rises over the first few weeks of life along with the potential to respond to Type 2 antigens (43). The CBA/N mouse, with an X chromosome–linked immunodeficiency (*xid*), lacks this subset of B cells, as do F_1 *xid*/Y hemizygous male mice, and these cannot respond to Type 2 antigens. Recently, B cells from such mice as well as the $Lyb5^-$ population from phenotypically normal F_1 *xid*/X mice have been analyzed for TD responsiveness by culture in splenic fragments (128,159,160). The defective mice are known not to display circulating anti-PC antibodies upon in vivo stimulation with the PC-determinant on any form of carrier, either TD or TI, although it is not yet clear how the *xid* mutation limits TD responsiveness of only certain specificities (159,161). The unresponsiveness seems to reflect the status of precursors in vivo since *xid*/X mice show normal levels of TD PC-specific B cells and *xid*/Y mice have none detectable while both sexes display normal and equivalent frequencies of TNP-specific cells (160). The intriguing part of the observations is that the entire complement of PC-responsive cells in the F_1 females is eliminated by anti-Lyb5 plus complement while the residual $Lyb5^-$ cells still account for half the TNP-responsive cells. Immunization of both sexes of F_1 mice with PC-Hy leads to detectable frequencies of PC-responsive cells in *xid*/Y males that are $<10\%$ of those in *xid*/X females (128,160) and these are, of course, resistant to anti-Lyb5. Strikingly, about 10% of the PC-responsive cells of the primed females are now also $Lyb5^-$. These findings, taken with other observations that *xid*/Y and *xid*/X mice display quantitatively and qualitatively equivalent in vivo antibody responses upon tertiary challenge with TD PC-Hy and develop similar frequencies of PC-responsive cells as detected in vitro (128,159), provide persuasive indirect evidence of the existence of a subset of $Lyb5^-$ B cells in both *xid*/Y and normal mice that do not develop into antibody-secreting clones in splenic fragment culture. The possible role of this subpopulation in mounting IgE responses is discussed below.

ROLE OF T CELLS IN THE DISPLAY OF ISOTYPES BY TD CLONES The potential of T_H cells with antigen specificity to initiate a responsive B cell to generate a clone secreting any or all isotypes has been supported by a variety of limiting dilution assays in microculture (162), miniculture (163), and splenic-fragment culture (164,165; D. Zimmerman, manuscript in preparation). Heterogeneous $T_{H(Ag)}$ cells (162), $T_{H(Ag)}$ cell lines (165; D. Zimmerman, manuscript in preparation), and cloned $T_{H(Ag)}$ cells (163,165) have all been used to limit B-cell cultures statistically to a single effective cell interaction that leads to a single B-cell clone. Since the limiting doses of adoptively transferred $T_{H(Ag)}$ cells ranged from 10^5 to 10^7 prior to splenic-fragment culture and from 10^4

Annual Reviews

to 10^5 directly introduced into micro- or miniculture, one cannot exclude further interactions of the clonal B-cell progeny during the proliferation and secretion phases. All of these observations supported the conclusions that $T_{H(Ag)}$ cell lines and clones initiated the development of clones secreting any and all isotypes, alone or in mixtures, including IgE. The isotype display primarily reflected the inherent isotype potential of the B-cell precursors (D. Zimmerman, manuscript in preparation). For instance, T_H lines resulted in many PP clones' secreting only IgA while the splenic B-cell clones they stimulated mostly made mixtures of isotypes. Cloned T_H cells generally gave patterns of B-cell clonal isotype expression similar to those initiated by their parent cell lines (165). However, the cloned T_H cells tended to stimulate B-cell clones that displayed fewer isotypes, on average, than those given by their parent lines, which in turn gave B-cell clones making fewer isotypes than clones developed by an excess of heterogeneous T_H cells (165). Thus, the possibility remains open that subtle specificity differences among $T_{H(Ag)}$ cells and/or the interaction of more than one T_H cell with a developing B-cell clone can influence the diversity and pattern of its isotype expression. We know of few clear-cut examples of the systematic influence of a particular set of T_H cells on isotype expression, especially at the clonal level (see 167). One possible case is the failure of Hy-primed, *xid*/Y splenic fragments to allow the expected expression of secreted IgG3 by anti-DNP clones from *xid*/X, unprimed B cells (168). The clones developed from this same population or from *xid*/Y B cells in *xid*/X fragments showed about the same proportion secreting IgG3 (11–15%). These observations suggest that there is a deficiency in *xid*/Y $T_{H(Hy)}$ cells, perhaps attributable to their having developed in mice having subnormal levels of IgG3. Another example of $T_{H(Ag)}$ cells of defined specificities being associated with distinctive patterns of isotype expression has been given by McGhee and his co-workers (169). They have grown T_H cell lines and their clones from PP after gut mucosal stimulation of the donor mice with erythrocyte antigens. These T_H clones are antigen-specific, Lyt-1^+, Thy 1.2^+, and FcR_α^+. The many clones fall into two broad groups on the basis of their activity when added to bulk cultures of PP or splenic B cells (2.5×10^6 cells per 0.25 ml). One set of these T_H cells, when added to bulk cultures (5×10^4 cells per well) along with specific erythrocyte antigen, allowed the development of substantial numbers of only IgM and IgA PFC. The other set resulted in the development of IgM, IgG1, IgG2, and IgA PFC. The difference in their fine specificities is unknown and both sets bear $sFcR_\alpha$. Recently, B cells bearing sIgA have been identified as the target cells of the potentiation of IgA PFC given by the T_H cells of the set that selectively stimulates IgM and IgA PFC (18). In both these examples the mode of action of the $T_{H(Ag)}$ cells is unknown. However, a presumption of antigen ("carrier") specificity is not unreasonable. It remains to be determined to what extent factors such

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as specific isotype binding elements of T cells, documented for rat FcR_ϵ (170), and other T-cell factors that appear to result selectively in maturation of cells switched to IgG after LPS-stimulated proliferation (60,64,134) may be differentially expressed by $T_{H(Ag)}$ cell lines to modulate their effects on the isotypes secreted by B-cell clones.

PATTERNS OF ISOTYPES SECRETED BY TD CLONES AND POSSIBLE MODELS FOR ISOTYPE SWITCHING AND ISOTYPE RESTRICTION DURING CLONAL PROLIFERATION As previously described, switching may result in expression of a new sIg isotype in the absence of secretion. This process may be modulated by T cells and factors they produce. Nevertheless, it may be informative to seek insights into switching and its restriction among the patterns of isotypes expressed by TD clones, since the positive display surely reflects the minimal isotype potential of the clonal precursors, and often certain systematic absences of isotype(s) from the display of a subset of clones occurs under culture conditions permitting the secretion of the same isotype(s) by other clones developing in similar splenic fragments. This type of analysis of clonal phenotype has mainly been useful to rule out any necessarily predetermined route of switching by a subset of B cells leading to expression of a particular isotype. The negative aspects of isotype patterns—the isotype not expressed—have been used to infer isotype restriction and isotype commitment as a consequence of the switching process. Finally, the disproportionately large fraction of clones from splenic-fragment culture making some IgA, together with the subset making exclusively IgA (which is much larger than any other subset making a single isotype—except for the B cells that make IgM at an early stage of development), has been used to argue that switching to IgA is a terminal productive event, finally committing a B cell to expression of a sole isotype while leaving its proliferative potential intact.

The most comprehensive analyses of most or all murine isotypes produced by TD clones have been made by Teale and co-workers (54,165) and by our own group (100,111,126–128,139,144,147). These studies have shown that clones may express any single isotype or mixture of isotypes up to the entire group of seven. Tables 3 and 4 simplify and summarize some of these comprehensive analyses of almost 1000 clones of three specificities—anti-PC, anti-In, and anti-DNP—in order to highlight certain features of the data:

(*a*) About 31–38% of all anti-In and anti-PC clones derived from nonimmunized but presumably naturally primed B cells express some IgM. A considerably higher proportion of anti-DNP clones from unprimed cells make some IgM (85%), but upon immunization of the donors the frequency falls to 61%. Most of these clones making IgM also switch to the expression of other isotypes.

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Table 3 Proportion of B-cell clones of different specificities secreting each heavy-chain isotype and different multiplicities of isotypes[a]

Specificity	Number of clones analyzed	Proportion of clones secreting (%):									Proportion of clones secreting following number of isotypes (%):						
		M-only	M	G3	G1	G2b	G2a	E	A	A-only	1	2	3	4	5	6	7
Anti-PC	190	3	38	27	17	10[c]			90	43	48	31	12	7	2		
Anti-In	213	2	31	25	39	43			71	23	36	27	23	11	3		
Anti-PC[b]	109	5	66	25	29	17			83		12	51	21	12	4		
Anti-In	164	3	40	33	51	56			63		20	35	28	13	4		
Anti-DNP (nonprimed)	125		85	7	61	22	18	16	59		30	24	27	12	4	3	0
Anti-DNP (primed)	163		61	10	85	42	32	16	59		9	19	31	25	9	5	1

[a]Data for anti-In and anti-PC clones from (100,127,147) and for anti-DNP from (165).
[b]Same data as above except that all clones making only IgA have been removed from consideration.
[c]IgG2a and IgG2b not resolved.

Annual Reviews

Table 4 Coexpression of IgG3 or IgE with other isotypes[a]

	Anti-In or Anti-PC (94 clones) IgG3 with:		Anti-PC (93 clones) IgE with:	
Other clonal products	Number	Percentage	Number	Percentage
Alone	10	11	6	6
IgM only	7	7	8	9
IgA only	18	19	6	6
IgGX only	6	6	29	31
IgM + IgA	9	10	6	6
IgM + IgGX	1	1	8	9
IgA + IgGX	25	27	22	24
IgM + IgA + IgGX	18	19	7	8
No IgM	59	63	64	69
Some IgM	35	37	29	31
Some IgG3			16	17
Some IgA	70	74	41	44
Some IgG1	34	36	60	64
Some IgG2	44	47	22	24

[a]Data from (100,127,128,147).

(*b*) All of the IgG isotypes are expressed at roughly similar levels by anti-PC (25–43%) and anti-In (10–17%) clones. These levels rise appreciably if the data are adjusted to exclude clones exclusively making IgA. Among the anti-DNP clones, IgG1 tends to be the predominant IgG isotype and IgG3 the most seldom expressed of all isotypes. Upon immunization of donors, the proportion of clones given by their B cells that make IgG2 conspicuously rises, as noted previously (126).

(*c*) IgA is by far the most common isotype coexpressed with others in switching clones making anti-In or anti-PC. It is also prevalent in switching clones from primed and unprimed anti-DNP B cells although IgM and IgG1 are about as common or slightly more so.

(*d*) A substantial proportion of the anti-In and anti-PC clones exclusively express IgA (23% and 43%, respectively, documented in Table 3). This is not the case for anti-DNP clones (126,139), which do not appear to develop from specific precursors under the same intensity of environmental stimulation as the other two specificities. Nevertheless, in the absence of deliberate in vivo priming, no other isotype is expressed singly by clones at frequencies even close to those for IgA. For instance, IgE is the solely detected isotype by only 0–0.5% of anti-DNP or anti-PC clones from unprimed B cells or those scored after primary immunization (54,128). The IgG3 isotype is the sole product of only about 2% of anti-In or anti-PC clones of 494 analyzed. Other than exclusive IgM production by clones from B cells at an early stage in development

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(26,131), no isotype is so frequently the sole product of clones from B cells taken from non-immunized mice as is IgA.

(*e*) The prevalence of multiple isotype secretion by clones generally falls off with the number of isotypes detected, but if sole IgA expression is excluded, it is more common to find at least two isotypes secreted by clones than a single one (see also 165). This observation applies both to clones expressing some IgM and to those secreting no detectable IgM. Since many of these latter likely arise from pre-switched precursors (22,149), the conclusion is that B-cell clones developing from such cells continue to switch, leading to intraclonal diversification and secretion of IgG, IgE, and IgA isotypes in the absence of IgM.

(*f*) Another kind of information available from analysis of isotypes produced by clones is the association of one isotype with another that is coexpressed, and Table 4 shows the patterns of coexpression of other isotypes with both IgG3 and IgE. Almost a hundred clones making each of these isotypes have been analyzed. The 94 IgG3-producing clones represent the 19% of 494 anti-In and anti-PC clones of B cells from mice colonized with gut flora but not otherwise deliberately immunized. The 93 IgE-producing clones have an anti-PC specificity and mostly come from B cells of mice given a special, secondary in vivo priming of a sort known to favor an IgE response. This priming was necessary to obtain sufficient numbers of IgE-producing clones—up to 30–50%–for this type of analysis and may have biased the results by minimizing coexpression of IgM. Nevertheless, the data should still be informative about the other isotypes that can be coexpressed with IgE by clones from a B-cell population with a high propensity for IgE expression (128). The main observations from Table 4 in common between the patterns of coexpression for IgG3 or IgE isotypes are that their singular expression or coexpression with IgM alone was rare and that two thirds of the clones producing each of these particular isotypes did not make detectable IgM at all. Of course, this latter finding could relate to the similar status of the precursor B-cell populations with respect to natural (IgG3) or deliberate (IgE) priming but still indicate no greater linkage between IgG3 and IgM expression than for IgE. Otherwise, both of the isotypes being considered frequently occur in switching clones with at least two other isotypes (47–56%). The IgG3 was most frequently coexpressed with at least some IgA (74%) while the IgE, perhaps as a reflection of deliberate priming of precursors, was more commonly found with either IgG isotypes alone (31%) or at least some IgG1 (64%). However, expression of IgE with IgA alone was detected (6%) and coexpression with at least some IgA was not uncommon (44%). These generalizations concerning IgE coexpression with other isotypes are consistent with those for anti-DNP clones from unprimed B cells, <10% of which made some IgE (54).

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The coexpression patterns of IgG3 and IgE were particularly singled out for analysis here because apparent preferential switching from IgM to IgG3 (75) and from IgM to IgE (171,172), following particular regimens for in vivo stimulation with antigen, have provided the main bases for a branched model for B-cell differentiation. According to this model, separate populations of B cells arise, while still capable of synthesis and translation of μ-chain mRNA, that are predestined to undergo particular routes of switching to, and expression of, one other particular isotype, such as IgG3, IgE, or IgA (173,174). The data in Table 4 cannot rule out the existence of such predetermined subsets but are not compatible with a branched model for differentiation with respect to isotype potential for those specific B cells that are prevalent in non-immunized mice, increase in frequency substantially upon deliberate in vivo priming, and are capable of considerable TD antigen–driven clonal expansion over 10–15 days in splenic-fragment culture with accompanying antibody secretion. Before putting forward a simple working model for the normal switching process during B-cell differentiation, which we believe is consistent with data for TD-responsive B cells, it may be useful to point out the major differences between patterns of isotype expression by clones driven by TD-antigen and those stimulated by LPS (40): (*a*) the rarity of IgA expression by LPS-clones and its commonness in TD-clones (Table 3), and (*b*) the relatively low frequency of IgG1 secretion by LPS clones compared to those stimulated in a TD-culture system. These quantitative differences are likely due to culture conditions and limitations by the necessary stimuli for secretion of particular isotypes, as has been shown for LPS clones making IgG1 (49) and suggested for those expressing IgA (42,65). Most other differences between the isotype patterns given by the two types of clones can be attributed to the lack of responsiveness to LPS by B cells that have switched from IgM expression to that of another isotype.

Figure 1 shows a schematic diagram of the simple stochastic model for linear pathways of B-cell differentiation with respect to isotype potential. We originally suggested this model for TD B cells (147,175) as an extension of the then recently deduced organization of C_H genes and an allelic deletion model to explain C_H-gene doses in plasmacytoma lines (71). The model is "linear" since no B cell still able to express its C_μ gene is committed to any particular route of switching. Rather, restriction in isotype potential occurs randomly, during clonal outgrowth via successive switching of a $V_H/D/J_H$-gene segment to C_H genes in their known order 5′-C_μ-$C_{\gamma 3}$-$C_{\gamma 1}$-$C_{\gamma 2b}$-$C_{\gamma 2a}$-C_ϵ-C_α (56). The model, incorporating the notion of successive switching, assumes this process is irreversible and thus favors a process of deletion of intervening C_H genes (71) rather than one of sister chromatid exchange (176). The switching process can occur in a stepwise fashion as found for rare switches in

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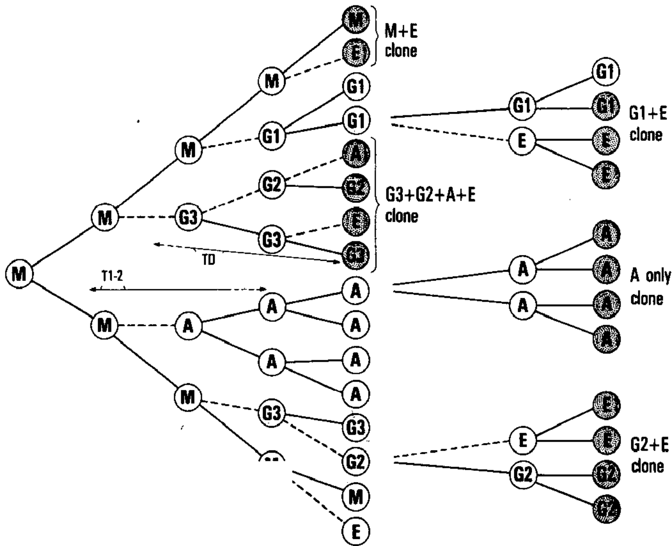

Figure 1 This is a schematic diagram depicting the outgrowth of a clone in vivo after stimulation of a single B cell. Each isotype switch represents the isotype potential of that cell and its progeny to the newly expressed isotype and other 3′ C_H genes. The diagram illustrates how IgE can be coexpressed with any one or a mixture of isotypes during the natural development of clonally related B cells. For instance, the three subclones depicted at the right could have been independently developed in vitro from clonally related precursors that had arisen and switched in vivo after antigenic stimulation. Two of these subclones coexpress an IgG isotype along with IgE, and the third, generated by a cell restricted to exclusive IgA expression through terminal differentiation upon switching to the 3′ C_H gene, C_α, gives rise to a clone making only IgA. Hatched cells represent secretory plasma cells. [Adapted from (111)]

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plasmacytoma lines (177), or by recombination to more distant C_H genes—i.e. direct $V_H/D/J_HC_\mu \rightarrow V_H/D/J_H/C_\alpha$ switching, as suggested by the common occurrence of clones making only IgM and IgA from neonatal cells (26,131) and molecular genetic data from permanent cell lines (28,67,178). Though the switching process is postulated to be random, the probabilities for each particular switch may be different. For instance, these probabilities may reflect the degree of homology between two sets of switch sequences (7) and/or the intervals between C_H genes in the DNA sequences (56). The essence of the model is that B cells may undergo isotype switching in vivo to restrict their isotype potential while retaining the capacity for another round of TD clonal outgrowth in vitro and consecutive switching to diversify clonal isotype expression. Thus, with consecutive switching in the same B-cell line possible, cells could have arrived at the fourth generation shown in the figure in vivo and then, upon sampling and culture in splenic fragments, could have gen-

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erated clones displaying a mixture of isotypes as shown in the fifth and sixth generations in the scheme. The figure depicts how IgE coexpression with any other isotype can commonly occur as observed (Table 4) during clonal outgrowth. The figure also illustrates how B cells may become committed to IgA expression as a terminal, productive event upon switching to the most 5′ C_α gene while retaining the capacity for further clonal outgrowth and exclusive IgA secretion. The possibilities of consecutive and irreversible switching within single B-cell lines of a clone and its necessary termination only after a switch to the C_α gene would account for two conspicuous features of the data given in Table 3: the occurrence of IgA as both the most commonly coexpressed isotype in switching clones and the most common isotype to be solely expressed by clones. The model is deficient in being unable to account for the restriction of isotype secretion to a single IgG or IgE isotype, often that one expressed as sIg (150,172,173; see Table 3), without making further ad hoc assumptions. Explanations for such discrepancies would be that IgA secretion was not tested (150) or that a shift to the expression of the selected form of any particular isotype may preclude switching (69,172,173).

Finally, the "linearity" of differentiation with respect to isotype potential, shown in Figure 1, need not correspond with a "linearity" in differentiation with respect to responsiveness. One possible explanation for the apparent complete dissociation between the switching process and expression of IgE secretion may be the existence of two B-cell subsets, which may switch to activation of C_ϵ gene with similar probabilities but may differ in their responsiveness to signals leading to secretion of its product (127,128).

Unresolved Issues Concerning C_H Isotype Switching and Restriction in Normally Developing B Lymphocytes That May Be Susceptible to Analyses by Techniques of Molecular Biology

Molecular geneticists have made impressive progress in elucidating the order and context of the murine C_H genes (56) as well as the two types of somatic recombinational events necessary for the successful activation of a productive heavy-chain gene (see 7). The first recombinational event involves the joining of V_H-, D-, and J_H-gene segments, with the deletion of DNA sequences intervening between the sites of translocation (67,68). Since the particular combinations of V_H-, D-, and J_H-gene segments may vary as well as the exact sites of recombination, which occur in regions that will be transcribed and translated, the process can create many variants of the V_H/D/J_H-exon for possible use in an active heavy-chain gene. The second type of recombinational event is different in that it involves switching of the V_H/D/J_H-exon from its position adjacent to but separate from the C_μ gene to a position adjacent to another C_H gene by a recombinational event involving sites in the flanking region 5′ to

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C_μ gene and in the flanking region 5′ to the relevant C_H gene. No variations in the $V_H/D/J_H$-exon or the C_H-exons are introduced directly by the process, and a new heavy-chain gene with the same newly acquired variable region is now activated for transcription. Most of what we know about these processes has come from analysis of DNA from the heavy-chain locus of plasmacytoma cells, and certainly the information is valid in indicating the sorts of rearrangements and deletions that can occur. However, all of the data are not yet easily interpretable in terms of the most probable genetic changes to occur during normal B-cell development, especially the period of 5–12 divisions of clonal proliferation after mitogen or antigen stimulation leading to secretion of multiple isotypes. For instance, some data (71, 179–181) have been interpreted to indicate that switching is accompanied by deletion of C_μ and other C_H genes 5′ to the newly expressed C_H gene and that such a recombinational process proceeds at the nonexpressed, homologous C_H-gene locus as well. Other data from analysis of a particular IgG1 secreting plasmacytoma (MC101) have been taken by one of these same groups to suggest that switching may occur by unequal crossing over between sister chromatids (176). Other data that do not fit a simple scheme for normal B-cell differentiation seem to indicate that switching may occur consecutively (177,182) and in the 5′ to 3′ order of C_H genes (71,177). These contrast with analyses that seem to support direct switching from C_μ to any other C_H gene (67,183) and "back switching" in an order the reverse of 5′ to 3′ (182). Some of the principal questions perplexing the cell biologist today involve determining which, if any, of these recombinational events is "normal" or common in the clonal development of a stimulated B lymphocyte. Probably such determinations will involve analysis (*a*) of the C_H-gene content and context of B cells expressing a particular isotype and (*b*) of clonally related B cells expressing different isotypes. One approach to obtain sufficient B cells expressing a particular isotype for analysis of C_H genes is to enrich by FACS (66,69,70,174); another is to immortalize the normal chromosome with the expressed C_H locus by incorporating it into hybridoma cell lines (66). Such information may complement cell biological approaches to answering those main questions about normal B-cell differentiation with respect to isotype potential which include: (*a*) is isotype switching dependent upon or facilitated by cell division; (*b*) is apparent isotype switching reversible; (*c*) does isotype switching occur as single C_μ-to-C_H recombinational events or can it take place in a consecutive, stepwise fashion; and (*d*) is switching random though possibly of unequal probability depending on the particular switch or can its route be influenced by external stimuli?

Since these questions are interrelated—ascertaining which of the many recombinational events known to occur in the complex heavy-chain locus commonly take place during normal B-cell development will help to clarify most or all—we consider them together. Certainly switches in expression of

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C_H isotype are commonly observed during B-cell proliferation (21,30,35,40,57). If isotype switching commonly occurs by unequal crossing-over during sister chromatid exchange then the process is by necessity linked to DNA replication (176). Further, no C_H genes will be lost by deletion and the subsequent division will be asymmetric in that the two daughter cells will have different contents of some C_H genes and express different isotypes. Thus, given equal potential of all daughter cells to continue to divide, the relative content of C_H genes in a clone may be expected to stay the same over the period of growth. This postulate may be directly testable by pooling individual clones known to have undergone extensive switching for DNA analysis. Of course, the daughter cells gaining in C_H genes will acquire the potential for apparent "back-switching" since they may gain additional copies of some C_H genes on the productive chromosome. It may be possible to compare C_H-gene dose in normal, productive chromosomes after their introduction into hybridoma lines, particularly if the permanent cell parent lacks that particular C_H gene as the SP2/0 line lacks C_μ genes (66). After all, half of the daughters of a switching division should gain C_H genes if sister chromatid exchange is the principal mechanism for recombination, and thus incorporation of their productive heavy-chain locus into hybridomas should be a relatively frequent event. However, analyses of C_H-gene content in plasmacytoma (71,179–181), hybridomas (66), and switched LPS-blasts (70) suggest that C_H-gene loss is a common event during switching. These findings support a looping-out and deletion model for switching (71). Although a cell biologic study of inhibiting cytokinesis but not karyokinesis supports the asymmetry of the switching division (36) and hence sister chromatid exchange, it is also consistent with looping-out and deletion on one of the two sister chromatids during division—or a copy-choice process during DNA replication of the new chromatid. In any of these cases we would expect a link between switching and cell division. Deletion of C_H genes would provide a clear mechanism for isotype restriction and preclude back-switching. Another mechanism that may allow for some limited back-switching or coexpression of IgM with another IgG, IgE, or IgA isotype would be the transcription of polycistronic mRNA and control of its processing, a possibility that may apply to a plasmacytoma that has a $C_{\gamma 2b}$ gene rearrangement just 3′ to C_μ in the absence of C_δ, $C_{\gamma 3}$, and $C_{\gamma 1}$ genes (184) and to sIgE-bearing B lymphocytes from SJA mice (174). Although isolation of presumed polycistronic mRNA has not been achieved, in situ hybridization techniques (185) may eventually be applicable to normal single cells to seek continued μ-chain mRNA in cells expressing other isotypes.

The problem of whether switching commonly occurs during intraclonal differentiation by single C_μ-to-C_H switches or by consecutive switches may be more difficult to evaluate quantitatively. There is cell biologic evidence that the former (23,26,30) as well as the latter (49,57,177,182) can occur.

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One somewhat laborious approach may be to generate hybridomas from related cells of switching B-cell clones, expand the active C_H locus by gene cloning, and analyze for portions of switched sequences other than of S_μ from intervening C_H genes that may have been carried into the S_x region of the newly expressed C_x genes by consecutive switching (176,183).

Finally, a decision about whether switching from C_μ to another C_H gene is a random event (possibly of variable probability depending on the C_H genes involved) or can be proscribed by other cell differentiation processes (perhaps initiated by outside stimuli) will probably depend on identifying the recognition elements that are primarily responsible for particular switches. One point of view is that close analogs of the heptanucleotide sequence YAGGTTG, occurring just 5′ to all S_H regions, and more distant analogs as short as tetranucleotides within S_H regions act together with the oft-repeated 5-base sequences GAGCT and GGGGT in the S_μ and their more distant analogs in other S_H regions to align two different C_H genes prior to effective $V_H/D/J_H$ recombination, often at a point just 3′ to the YAGGTTG analogs (186,187). The groups determining these S_H sequences have pointed out closer overall homology between S_μ and $S_{\gamma 3}$, S_ϵ, and S_α than between S_μ and $S_{\gamma 1}$, $S_{\gamma 2a}$, and $S_{\gamma 2b}$. These subtle differences certainly could set different probabilities for each particular switch while allowing the overall process to be random. Another point of view is that homologous sequences within the S_H regions are neither frequent nor comparable enough to allow for stable interactions between S regions acting on their own (183). However, such interactions may be stabilized by a series of proteins that each have a specificity for a particular S region. Pairing of such "switch proteins" could stabilize interactions between switch regions. Of course, such proteins may have the enzymic activities of a recombinase enzyme or form subunits of such enzymes. Obviously, control of "switch protein" expression offers a specific means to direct the switch. A search for such controlling elements with specificity for particular S-region DNA sequences is one approach to identifying a mechanism for directing the switch.

CONCLUSIONS

B-lymphocyte differentiation can be resolved into processes that occur on three levels. The first is their diversification with respect to ligand-binding specificity, based on expression of one particular pair of V_H and V_L polypeptides as part of their Ig receptor for antigen. This process depends on successful $V_H/D/J_H$ and V_L/J_L recombinational events and leads to transcriptionally active μ/δ and L-chain genes, respectively. Completion of the first part of this process and the expression of cytoplasmic μ chain identifies a cell as a pre-B cell, while subsequent completion of the second part of this process and the expres-

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sion of sIg identifies a cell as a B lymphocyte and sets its specificity for antigen binding.

Differentiation of murine B lymphocytes on two other levels—(*a*) by C_H isotype switching, and (*b*) by changes in response pattern to sets of external stimuli—has been used to divide them into subsets and place these in proposed developmental pathways. Though these processes appear to proceed in parallel, the evidence suggests they need be neither temporally linked nor associated at all. However, these two processes do act together to determine the overall functional potential of B lymphocytes.

C_H isotype switching can occur during the pre-B to B-cell transition and may even occur during the generation of pre-B cells. At these stages of development the switching process is almost certainly independent of antigen and T cells. The function of these cells, switched at early stages, is unknown. Isotype switching is commonly observed during clonal proliferation of B cells in response to LPS, Type 1, Type 2, or TD antigens. The display of isotypes as products secreted by single B-cell clones in vitro varies with the form of mitogen/antigen stimulus and the accompanying "package" of growth, differentiation, and maturation stimuli. For instance, clones secreting IgM plus any or all other isotypes arise in response to LPS or Type 2 antigens, but IgA is rare after LPS stimulus and IgE after either sort. Both sorts of clones may evidence isotype switching at the level of sIg expression without secretion of the corresponding isotype. Thus far, single clones growing in vitro in response to submitogenic doses of Type 1 antigens have only been shown to undergo isotype switching at the level of sIg. Progenitors of most if not all of the preceding three types of clones retain the potential to express secreted IgM and may represent overlapping subsets of B cells. The basis for the failure of pre-switched B cells with restricted isotype potential to generate clones in vitro to LPS or Types 1 and 2 antigens is unknown. Finally, B-cell clones developing in response to TD antigens secrete any or all isotypes in vitro. B cells that appear to have many different response patterns generate clones in a TD assay, for instance: (*a*) early B cells just arising from pre-B cells whose clones only secrete IgM; (*b*) B cells with restricted isotype potential from donors naturally or deliberately primed by antigens in vivo; and (*c*) B cells apparently committed to the expression of only the IgA isotype. It is not clearly established whether B cells that have previously switched in response to LPS or Types 1 and 2 antigens gain the potential to generate clones in response to TD antigens.

The overall display of isotypes by clones in vitro is consistent with a random switching process with recombinational events of unequal probability that is dependent on cell division and leads progressively to restriction in isotype potential. Molecular geneticists have documented a variety of recombinational events that can occur at the C_H-gene locus during switching. Certainly the

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content and context of C_H genes should be predictive of B-cell isotype potential. It remains to be determined which of these changes at the DNA level commonly occur during normal B-cell development. Cell biologists have described displays of sIg and secreted Ig isotypes that accompany clonal responses to given sets of stimuli. Now both the molecular nature of each stimulus and the basis of the cellular response to it must be elucidated. These two approaches should complement each other to facilitate understanding of how isotype switching processes and changes in response patterns act together to determine the overall functional potential of B cells.

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Literature Cited

1. Nossal, G. J. V., Szenberg, A., Ada, G. L., Austin, C. M. 1964. Single cell studies on 19S antibody production. *J. Exp. Med.* 119:485
2. Nossal, G. J. V., Warner, N. L., Lewis, H. 1971. Incidence of cells simultaneously secreting IgM and IgG antibody to sheep erythrocytes. *Cell. Immunol.* 2:41
3. Kearney, J. F., Cooper, M. D., Lawton, A. R. 1976. B cell differentiation induced by lipopolysaccharide. IV. Development of immunoglobulin class restriction in precursors of IgG-synthesizing cells. *J. Immunol.* 117:1567
4. Kincade, P. W., Lawton, A. R., Bockman, D. E., Cooper, M. D. 1970. Suppression of immunoglobulin G synthesis as a result of antibody-mediated suppression of immunoglobulin M synthesis in chickens. *Proc. Natl. Acad. Sci. USA* 67:1918
5. Manning, D. D., Jutila, J. W. 1972. Immunosuppression in mice injected with heterologous anti-immunoglobulin antisera. *J. Immunol.* 108:282
6. Wang, A. C., Wilson, S. K., Hopper, J. E., Fudenberg, H. H., Nisonoff, A. 1970. Evidence for control of synthesis of the variable regions of the heavy chains of immunoglobulins G and M by the same gene. *Proc. Natl. Acad. Sci. USA* 66:337
7. Honjo, T. 1983. Immunoglobulin genes. *Ann. Rev. Immunol.* 1:499
8. Vaux, D. L., Pike, B. L., Nossal, G. J.V. 1981. Antibody production by single, hapten-specific B lymphocytes: An antigen-driven cloning system free of filler or accessory cells. *Proc. Natl. Acad. Sci. USA* 78:7702
9. Pike, B. L., Vaux, D. L., Nossal, G. J. V. 1983. Single cell studies on hapten-specific B lymphocytes: Differential cloning efficiency of cells of various sizes. *J. Immunol.* 131:554
10. Wetzel, G. D., Kettman, J. R. 1981. Activation of murine B lymphocytes. III. Stimulation of B lymphocyte clonal growth with lipopolysaccharide and dextran sulfate. *J. Immunol.* 126:723
11. Pike, B. L., Vaux, D. L., Clark-Lewis, I., Schrader, J. W., Nossal, G. J. V. 1982. Proliferation and differentiation of single hapten-specific B lymphocytes is promoted by T-cell factor(s) distinct from T-cell growth factor. *Proc. Natl. Acad. Sci. USA* 79:6350
12. Nossal, G. J. V., Pike, B. L. 1978. Improved procedures for the fractionation and in vitro stimulation of hapten-specific B lymphocytes. *J. Immunol.* 120:145
13. Snow, E. C., Vitetta, E. S., Uhr, J. W.

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1983. Activation of antigen-enriched B cells. I. Purification and response to thymus-independent antigens. *J. Immunol.* 130:607
14. Quintáns, J., Lefkovits, I. 1973. Precursor cells specific to sheep red cells in nude mice. Estimation of frequency in the microculture system. *Eur. J. Immunol.* 3:392
15. Pike, B. L. 1975. A microculture method for the generation of primary immune responses in vitro. *J. Immunol. Meth.* 9:85
16. Quintáns, J., Cosenza, H. 1976. Antibody response to phosphorylcholine in vitro. II. Analysis of T-dependent and T-independent responses. *Eur. J. Immunol.* 6:399
17. Andersson, J., Coutinho, A., Lernhardt, W., Melchers, F. 1977. Clonal growth and maturation to immunoglobulin secretion in vitro of every growth-inducible B lymphocyte. *Cell* 10:27
18. Kiyono, H., Phillips, J. O., Colwell, D. E., Michalek, S. M., Koopman, W. J., McGhee, J. R. 1984. Murine Peyer's patch T cell clones: Fcα receptors regulate T and B cell collaboration for IgA responses. *Nature*. In press
19. Klinman, N. R. 1972. The mechanism of antigenic stimulation of primary and secondary clonal precursors. *J. Exp. Med.* 136:241
20. Hurwitz, J. L., Tagart, V. B., Schweitzer, P. A., Cebra, J. J. 1982. Patterns of isotype expression by B cell clones responding to thymus-dependent and thymus-independent antigens in vitro. *Eur. J. Immunol.* 12:342
21. Mongini, P. K. A., Paul, W. E., Metcalf, E. S. 1982. T cell regulation of immunoglobulin class expression in the antibody response to trinitrophenyl-Ficoll. *J. Exp. Med.* 155:884
22. Gearhart, P. J., Cebra, J. J. 1981. Most B cells that have switched surface immunoglobulin isotypes generate clones of cells that do not secrete IgM. *J. Immunol.* 127:1030
23. Calvert, J. E., Kim, M. F., Gathings, W. E., Cooper, M. D. 1983. Differentiation of B lineage cells from liver of neonatal mice: Generation of immunoglobulin isotype diversity in vitro. *J. Immunol.* 131:1693
24. Melchers, F., Andersson, J., Phillips, R. A. 1976. Ontogeny of murine B lymphocytes: Development of Ig synthesis and of reactivities to mitogens and to anti-Ig antibodies. *Cold Spring Harbor Symp. Quant. Biol.* 41:147
25. Pettersson, S., Pobor, G., Coutinho, A. 1982. Ontogenic development of B cell reactivities to cooperative cell signals: dissociation between proliferation and antibody secretion. *Eur. J. Immunol.* 12:653
26. Teale, J. M., Mandel, T. E. 1980. Ontogenetic development of B lymphocyte function and tolerance susceptibility in vivo and in an in vitro fetal organ culture system. *J. Immunol.* 151:429
27. Osmond, D. G., Nossal, G. J. V. 1974. Differentiation of lymphocytes in mouse bone marrow. II. Kinetics of maturation and renewal of antiglobulin-binding cells studied by double labeling. *Cell. Immunol.* 13:132
28. Akira, S., Sugiyama, H., Yoshida, N., Kikutani, H., Yamamura, Y., Kishimoto, T. 1983. Isotype switching in murine pre B cell lines. *Cell* 34:545
29. Melchers, F., Andersson, J. 1973. Synthesis, surface deposition and secretion of immunoglobulin M in bone marrow–derived lymphocytes before and after mitogenic stimulation. *Transplant. Rev.* 14:76
30. Kearney, J. F., Lawton, A. R. 1975. B lymphocyte differentiation induced by lipopolysaccharide. I. Generation of cells synthesizing four major immunoglobulin classes. *J. Immunol.* 115:671
31. Melchers, F., Coutinho, A., Heinrich, G., Andersson, J. 1975. Continuous growth of mitogen-reactive B lymphocytes. *Scand. J. Immunol.* 4:853
32. Zauderer, M., Askonas, B. A. 1976. Several proliferative phases precede maturation of IgG-secreting cells in mitogen-stimulated cultures. *Nature* 260:611
33. Andersson, J., Coutinho, A., Melchers, F. 1978. Stimulation of murine B lymphocytes to IgG synthesis and secretion by the mitogens lipopolysaccharide and lipoprotein and its inhibition by anti-immunoglobulin antibodies. *Eur. J. Immunol.* 8:336
34. Pernis, B., Forni, L., Luzzati, A. L. 1976. Synthesis of multiple immunoglobulin classes by single lymphocytes. *Cold Spring Harbor Symp. Quant. Biol.* 41:175
35. Gronowicz, E. S., Doss, C., Schröder, J. 1979. Activation to IgG secretion by lipopolysaccharide requires several proliferation cycles. *J. Immunol.* 123:2057
36. Van der Loo, W., Gronowicz, E. S., Strober, S., Herzenberg, L. A. 1979. Cell differentiation in the presence of cytochalasin B: Studies on the "switch" to IgG secretion after polyclonal B cell activation. *J. Immunol.* 122:1203
37. Andersson, J., Coutinho, A., Melchers, F. 1978. The switch from IgM to IgG secretion in single mitogen-stimulated B-cell clones. *J. Exp. Med.* 147:1744
38. De Freitas, A. A., Coutinho, A. 1981.

Annual Reviews

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Very rapid decay of mature B lymphocytes in the spleen. *J. Exp. Med.* 154:994
39. Benner, R., Coutinho, A., Rijnbeek, A.-M., Van Oudenaren, A., Hooijkaas, H. 1981. Immunoglobulin isotype expression. II. Frequency analysis in mitogen-reactive B cells. *Eur. J. Immunol.* 11:799
40. Coutinho, A., Forni, L. 1982. Intraclonal diversification in immunoglobulin isotype secretion: an analysis of switch probabilities. *EMBO J.* 1:1251
41. Severinson Gronowicz, E., Doss, C., Assisi, F., Vitetta, E. S., Coffman, R. L., Strober, S. 1979. Surface Ig isotypes on cells responding to lipopolysaccharide by IgM and IgG secretion. *J. Immunol.* 123:2049
42. Kawanishi, H., Saltzman, L. E., Strober, W. 1983. Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA cells in vitro. *J. Immunol.* 157:433
43. Mosier, D. E., Zitron, I. M., Mond, J. J., Ahmed, A., Scher, I., Paul, W. E. 1977. Surface immunoglobulin D as a functional receptor for a subclass of B lymphocytes. *Immunological Rev.* 37:89
44. Yuan, D., Vitetta, E. S. 1978. Cell surface immunoglobulin. XXI. Appearance of IgD on murine lymphocytes during differentiation. *J. Immunol.* 120:353
45. Vitetta, E. S., Forman, J., Kettman, J. R. 1976. Cell surface immunoglobulin. XVIII. Functional differences of B lymphocytes bearing different surface immunoglobulin isotypes. *J. Exp. Med.* 143:1055
46. Zan-Bar, I., Strober, S., Vitetta, E. S. 1979. The relationship between surface immunoglobulin isotype and immune function of murine B lymphocytes. IV. Role of IgD-bearing cells in the propagation of immunologic memory. *J. Immunol.* 123:925
47. Forni, L., Coutinho, A. 1978. An antiserum which recognizes lipopolysaccharide-reactive B cells in the mouse. *Eur. J. Immunol.* 8:56
48. Watson, J., Kelly, K., Whitlock, C. 1980. Genetic control of endotoxin sensitivity. In *Microbiology—1980*, ed. D. Schlessinger, p. 4. Washington DC: Am. Soc. Microbiol.
49. Forni, L., Coutinho, A. 1982. The production of membrane or secretory forms of immunoglobulins is regulated by C-gene-specific signals. *Nature* 299:173
50. Sprent, J., Basten, A. 1973. Circulating T and B lymphocytes of the mouse. II. Lifespan. *Cell. Immunol.* 7:40
51. Bernabé, R. R., Coutinho, A., Martinez-Alonzo, C., Cazenave, P.-A. 1981. Immune networks. Frequencies of antibody- and idiotype-producing B cell clones in various steady states. *J. Exp. Med.* 154:552
52. Freitas, A. A., Coutinho, A. A. 1980. Characterization of mouse thoracic duct B lymphocytes. I. Evidence of functional heterogeneity. *Eur. J. Immunol.* 10:772
53. Elson, C. J., Jablonska, K. F., Taylor, R. B. 1976. Functional half-life of virgin and primed B lymphocytes. *Eur. J. Immunol.* 6:634
54. Teale, J. M., Liu, F.-T., Katz, D. H. 1981. A clonal analysis of the IgE response and its implications with regard to isotype commitment. *J. Exp. Med.* 153:783
55. Gearhart, P. J., Sigal, N. H., Klinman, N. R. 1975. Production of antibodies of identical idiotype but diverse immunoglobulin classes by cells derived from a single stimulated B cell. *Proc. Natl. Acad. Sci. USA* 72:1707
56. Shimizu, A., Takahashi, N., Yaoita, Y., Honjo, T. 1982. Organization of the constant-region gene family of the mouse immunoglobulin heavy chain. *Cell* 28:499
57. Gearhart, P. J., Hurwitz, J. L., Cebra, J. J. 1980. Successive switching of antibody isotypes expressed within the lines of a B-cell clone. *Proc. Natl. Acad. Sci. USA* 77:5424
58. Martinez-Alonso, C., Coutinho, A., Augustin, A. A. 1980. Immunoglobulin C-gene expression. I. The commitment to IgG subclass of secretory cells is determined by the quality of the nonspecific stimuli. *Eur. J. Immunol.* 10:698
59. Martinez-Alonso, C., Coutinho, A. 1982. Immunoglobulin C-gene expression. III. Possible induction of specific genetic events in activated B lymphocytes by the polyclonal stimuli driving clonal expansion. *Eur. J. Immunol.* 12:502
60. Coutinho, A., Pettersson, S., Ruuth, E., Forni, L. 1983. Immunoglobulin C gene expression. IV. Alternative control of IgG_1-producing cells by helper cell-derived B cell-specific growth or maturation factors. *Eur. J. Immunol.* 13:269
61. Kawanishi, H., Saltzman, L. E., Strober, W. 1982. Characteristics and regulatory function of murine Con A-induced, cloned T cells obtained from Peyer's patches and spleen: Mechanisms regulating isotype-specific immunoglobulin production by Peyer's patch B cells. *J. Immunol.* 129:475
62. Goodman, M. G., Weigle, W. O. 1979. T cell regulation of polyclonal B cell responsiveness. I. Helper effects of T cells. *J. Immunol.* 122:2548

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63. Goodman, M. G., Weigle, W. O. 1979. T cell regulation of polyclonal B cell responsiveness. II. Evidence for a deficit in T cell function in mice with an X-linked B lymphocyte defect. *J. Immunol.* 123:2484
64. Isakson, P. C., Puré, E., Vitetta, E. S., Krammer, P. H. 1982. T cell-derived B cell differentiation factor(s). Effect on the isotype switch of murine B cells. *J. Exp. Med.* 155:734
65. Kawanishi, H., Saltzman, L., Strober, W. 1983. Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissue. II. Terminal differentiation of postswitch sIgA-bearing Peyer's patch B cells. *J. Exp. Med.* 158:649
66. Hurwitz, J. L., Coleclough, C., Cebra, J. J. 1980. C_H gene rearrangements in IgM-bearing B cells and in the normal splenic DNA component of hybridomas making different isotypes of antibody. *Cell* 22:349
67. Davis, M. M., Calame, K., Early, P. W., Livant, D. L., Joho, R., Weissman, I. L., Hood, L. 1980. An immunoglobulin heavy-chain gene is formed by at least two recombinational events. *Nature* 283:733
68. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W., Tonegawa, S. 1980. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. *Nature* 286:676
69. Hurwitz, J. L., Cebra, J. J. 1982. Rearrangements between the immunoglobulin heavy chain gene J_H and C_μ regions accompany normal B lymphocyte differentiation in vitro. *Nature* 299:742
70. Radbruch, A., Sablitzky, F., Rajewsky, K. 1984. C_μ gene deletion and isotype switching in LPS-activated B cells. *Adv. Immunol.*
71. Honjo, T., Kataoka, T. 1978. Organization of immunoglobulin heavy chain genes and allelic deletion model. *Proc. Natl. Acad. Sci. USA* 75:2140
72. Coutinho, A., Möller, G. 1973. B cell mitogenic properties of thymus-independent antigens. *Nature New Biol.* 245:12
73. Fidler, J. M. 1975. In vivo immune response to TNP hapten coupled to thymus-independent carrier lipopolysaccharide. *Cell. Immunol.* 16:223
74. Matuhasi, T., Usui, M., Minami, M. 1976. Easily inducible M-component-like IgG3 antibody to LPS in mice. *J. J. Exp. Med.* 46:415
75. Slack, J., Der Balian, G. P., Nahm, M., Davie, J. M. 1980. Subclass restriction of murine antibodies II. The IgG plaque-forming cell response to thymus independent Type 1 and Type 2 antigens in normal mice and mice expressing an X linked immunodeficiency. *J. Exp. Med.* 151:853
76. Köhler, H., Smyk, S., Fung, J. 1981. Immune response to phosphorylcholine. VIII. The response of CBA/N mice to PC-LPS. *J. Immunol.* 126:1790
77. Goodman, G. W., Sultzer, B. M. 1979. Characterization of the chemical and physical properties of a novel B-lymphocyte activator, endotoxin protein. *Infect. Immun.* 24:685
78. Izui, S., Morrison, D. C., Curry, B., Dixon, F. J. 1980. Effect of lipid A-associated protein and lipid A on the expression of lipopolysaccharide activity. I. Immunological activity. *Immunology* 40:473
79. Goldman, R. C., White, D., Leive, L. 1981. Identification of outer membrane proteins, including known lymphocyte mitogens, as the endotoxin protein of *Escherichia coli* O111. *J. Immunol.* 127:1290
80. Hepper, K. P., Garman, R. D., Lyons, M. F., Teresa, G. W. 1979. Plaque-forming cell response in BALB/c mice to two preparations of LPS extracted from *Salmonella enteritidis*. *J. Immunol.* 122:1290
81. Motta, I., Portnoï, D., Truffa-Bachi, P. 1981. Induction and differentiation of B memory cells by a thymus-independent antigen, trinitrophenylated lipopolysaccharide. *Cell. Immunol.* 57:327
82. Colle, J.-H., Motta, I., Truffa-Bachi, P. 1983. Generation of immune memory by haptenated derivatives of thymus-independent antigens in C57BL/6 mice. I. The differentiation of memory B lymphocytes into antibody-secreting cells depends on the nature of the thymus-independent carrier used for memory induction and/or revelation. *Cell. Immunol.* 75:52
83. Pillai, P. S., Scott, D. W., Piper, M., Corley, R. B. 1982. Effect of recent antigen exposure on the functional expression of B cell subpopulations. *J. Immunol.* 129:1023
84. Jacobs, D. M., Morrison, D. C. 1975. Stimulation of a T-independent primary anti-hapten response in vitro by TNP-lipopolysaccharide (TNP-LPS). *J. Immunol.* 114:360
85. Lewis, G. K., Goodman, J. W. 1977. Carrier-directed anti-hapten responses by B-cell subsets. *J. Exp. Med.* 146:1
86. Tittle, T. V., Rittenberg, M. B. 1980. IgG B memory cell subpopulations: Differences in susceptibility to stimulation

Annual Reviews

Annu. Rev. Immunol. 1984.2:493-548. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

by TI-1 and TI-2 antigens. *J. Immunol.* 124:202
Pike, B. L., Nossal, G. J. V. 1984. A reappraisal of "T-independent" antigens. I. Effect of lymphokines on the response of single adult hapten-specific B lymphocytes. *J. Immunol.* In press

88. Briles, D. E., Davie, J. M. 1975. Clonal dominance. I. Restricted nature of the IgM antibody response to Group A streptococcal carbohydrate in mice. *J. Exp. Med.* 141:1291
89. Klaus, G. G. B., Humphrey, J. H. 1974. The immunological properties of haptens coupled to thymus-independent carrier molecules. I. The characteristics of the immune response to dinitrophenyl-lysine-substituted pneumococcal polysaccharide (SIII) and levan. *Eur. J. Immunol.* 4:370
90. Rüde, E., Wrede, J., Gundelach, M. L. 1976. Production of IgG antibodies and enhanced response of nude mice to DNP-AE-dextran. *J. Immunol.* 116:527
91. Sarvas, H. O., Aaltonen, L. M., Peterfy, F., Seppälä, I. J. T., Mäkelä, O. 1983. IgG subclass distributions in anti-hapten and anti-polysaccharide antibodies induced by haptenated polysaccharides. *Eur. J. Immunol.* 13:409
92. Barthold, D. R., Prescott, B., Stashak, P. W., Amsbaugh, D. F., Baker, P. J. 1974. Regulation of the antibody response to Type III pneumococcal polysaccharide. III. Role of regulatory T cells in the development of an IgG and IgA antibody response. *J. Immunol.* 112:1042
93. Sharon, R., McMaster, P. R. B., Kask, A. M., Owens, J. D., Paul W. E. 1975. DNP-Lys-Ficoll: A T-independent antigen which elicits both IgM and IgG anti-DNP antibody secreting cells. *J. Immunol.* 114:1585
94. Kagnoff, M. F., Arner, L. S., Swain, S. L. 1983. Lymphokine-mediated activation of a T cell-dependent IgA antipolysaccharide response. *J. Immunol.* 131:2210
95. Perlmutter, R. M., Hansburg, D., Briles, D. E., Nicolotti, R. A., Davie, J. M. 1978. Sublcass restriction of murine anticarbohydrate antibodies. *J. Immunol.* 121:566
96. Mongini, P. K. A., Stein, K. E., Paul, W. E. 1981. T cell regulation of IgG subclass antibody production in response to T-independent antigens. *J. Exp. Med.* 153:1
97. Trefts, P. E., Rivier, D. A., Kagnoff, M. F. 1981. T cell-dependent IgA anti-polysaccharide response in vitro. *Nature* 292:163
98. Mosier, D. E., Johnson, B. M., Paul, W. E., McMaster, P. R. B. 1974. Cellular requirements for the primary in vitro antibody response to DNP-Ficoll. *J. Exp. Med.* 139:1354
99. Mongini, P. K. A., Paul, W. E., Metcalf, E. S. 1983. IgG subclass, IgE, and IgA anti-trinitrophenyl antibody production within trinitrophenyl-Ficoll-responsive B cell clones. Evidence in support of three distinct switching pathways. *J. Exp. Med.* 157:69
100. Cebra, J. J., Gearhart, P. J., Halsey, J. F., Hurwitz, J. L., Shahin, R. D. 1980. Role of environmental antigens in the ontogeny of the secretory immune response. *J. Reticuloendothel. Soc.* 28:61s
101. Scher, I. 1982. CBA/N immune defective mice; evidence for the failure of a B cell subpopulation to be expressed. *Immunol. Rev.* 64:117
102. Boswell, H. S., Ahmed, A., Scher, I., Singer, A. 1980. Role of accessory cells in B cell activation. II. The interaction of B cells with accessory cells results in the exclusive activation of an Lyb5$^+$ B cell subpopulation. *J. Immunol.* 125:1340
103. Huber, B., Gershon, R. K., Cantor, H. 1977. Identification of a B-cell surface structure involved in antigen-dependent triggering: Absence of this structure on B cells from CBA/N mutant mice. *J. Exp. Med.* 145:10
104. Mosier, D. E., Mond, J. J., Goldings, E. A. 1977. The ontogeny of thymic independent antigen responses in vitro in normal mice and mice with an X-linked B cell defect. *J. Immunol.* 119:1874
105. Ahmed, A., Scher, I., Sharrow, S. O., Smith, A. H., Paul, W. E., Sachs, D. H., Sell, K. W. 1977. B-lymphocyte heterogeneity: Development and characterization of an alloantiserum which distinguishes B-lymphocyte differentiation alloantigens. *J. Exp. Med.* 145:101
106. Eldridge, J. H., Kiyono, H., Michalek, S. M., McGhee, J. R. 1983. Evidence for a mature B cell subpopulation in Peyer's patches of young adult *xid* mice. *J. Exp. Med.* 157:789
107. Mond, J. J., Kessler, S., Finkelman, F. D., Paul, W. E., Scher, I. 1980. Heterogeneity of Ia expression on normal B cells, neonatal B cells, and on cells from B cell-defective CBA/N mice. *J. Immunol.* 124:1675
108. Mond, J. J., Sehgal, E., Sachs, D. H., Paul, W. E. 1979. Expression of Ia antigen on adult and neonatal B lymphocytes responsive to thymus-independent antigens. *J. Immunol.* 123:1619
109. Greenstein, J. L., Lord, E. M., Horan,

Annual Reviews

Annu. Rev. Immunol. 1984.2:493-548. Downloaded from arjournals.annualreviews.org by HINARI on 08/27/07. For personal use only.

P., Kappler, J. W., Marrack, P. 1981. Functional subsets of B cells defined by quantitative differences in surface *I*-A. *J. Immunol.* 126:2419
110. Fung, J., Köhler, H. 1980. Immune response to phosphorylcholine. VII. Functional evidence for three separate B cell subpopulations responding to TI and TD PC-antigens. *J. Immunol.* 125:640
111. Cebra, J. J., Cebra, E. R., Clough, E. R., Fuhrman, J. A., Komisar, J. L., Schweitzer, P. A., Shahin, R. D. 1983. IgA commitment: Models for B cell differentiation and possible roles for T cells in regulating B cell development. *Ann. NY Acad. Sci.* 409:25
112. Jennings, J. J., Rittenberg, M. B. 1976. Evidence for separate subpopulations of B cells respondng to T-independent and T-dependent immunogens. *J. Immunol.* 117:1749
113. Scott, D. W., Alexander, C. 1980. B-cell subsets responsive to fluorescein-conjugated antigens. II. "Cross-priming" and its elimination by bromodeoxyuridine and light. *Cell. Immunol.* 53:376
114. Gorczynski, R. M., Feldmann, M. 1975. B cell heterogeneity—difference in the size of B lymphocytes responding to T dependent and T independent antigens. *Cell. Immunol.* 18:88
115. Umetsu, D. T., Chapman-Alexander, J. M., Thorbecke, G. J. 1979. Cross-priming of murine B cells with TNP conjugates of hemocyanin and Ficoll: characteristics of primed B cells responding to both antigens. *J. Immunol.* 123:396
116. Mosier, D. E. 1978. Induction of B cell priming by neonatal injection of mice with thymic-independent (Type 2) antigens. *J. Immunol.* 121:1453
117. Shahin, R. D., Cebra, J. J. 1981. Rise in inulin-sensitive B cells during ontogeny can be prematurely stimulated by thymus-dependent and thymus-independent antigens. *Infect. Immun.* 32:211
118. Speck, N. A., Pierce, S. K. 1981. The collaborative phenotype of secondary B cells is determined by T lymphocytes during in vivo immunization. *J. Exp. Med.* 155:574
119. Schott, C. F., Lizzio, E. F., Inman, J. K., Merchant, B. 1981. Immune memory to a nonmitogenic, thymic independent antigen in mice: Variation among inbred strains and possible relationship to oncogenesis. *J. Immunol.* 127:139
120. Pierce, S. K., Klinman, N. R. 1975. The allogeneic bisection of carrier-specific enhancement of monoclonal B-cell responses. *J. Exp. Med.* 142:1165
121. Mitchison, N. A., Rajewsky, K., Taylor, R. B. 1970. Cooperation of antigenic determinants and of cells in the induction of antibodies. In *Developmental Aspects of Antibody Formation and Structure*, ed. J. Šterzl, I. Řiha, p. 547. Prague: Academia Publ. Czechoslovak Acad. Sci.
122. Taylor, R. B., Wortis, H. H. 1968. Thymus dependence of antibody response: Variation with dose of antigen and class of antibody. *Nature* 220:927
123. Torrigiani, G. 1972. Quantitative estimation of antibody in the immunoglobulin classes of the mouse. II. Thymic dependence of the different classes. *J. Immunol.* 108:161
124. Hamaoka, T., Katz, D. H., Benacerraf, B. 1973. Hapten-specific IgE antibody responses in mice. II. Cooperative interactions between adoptively transferred T and B lymphocytes in the development of IgE response. *J. Exp. Med.* 138:538
125. Ishizaka, K., Okudaira, H. 1973. Reaginic antibody formation in the mouse. II. Enhancement and suppression of antihapten antibody formation by priming with carrier. *J. Immunol.* 110:1067
126. Cebra, J. J., Gearhart, P. J., Kamat, R., Robertson, S. M., Tseng, J. 1976. Origin and differentiation of lymphocytes involved in the secretory IgA response. *Cold Spring Harbor Symp. Quant. Biol.* 41:201
127. Cebra, J. J., Cebra, E. R., Clough, E. R., Fuhrman, J. A., Schweitzer, P. A. 1983. Relationships and regulation of IgG, IgE or IgA expression during clonal outgrowth of primed B cells. In *Regulation of the Immune Response*, ed. P. L. Ogra, D. M. Jacobs, p. 107. Basel: S. Karger
128. Clough, E. R., Cebra, J. J. 1983. Interrelationship of primed B cells with the potential for IgE and/or IgA expression. *Mol. Immunol.* 20:903
129. Klinman, N. R., Press, J. L. 1975. The characterization of the B-cell repertoire specific for the 2,4-dinitrophenyl and 2,4,6-trinitrophenyl determinants in neonatal BALB/c mice. *J. Exp. Med.* 141:1133
130. Metcalf, E. S., Klinman, N. R. 1976. In vitro tolerance induction of neonatal murine B cells. *J. Exp. Med.* 143:1327
131. Teale, J. M., Layton, J. E., Nossal, G. J. V. 1979. In vitro model for natural tolerance to self-antigens. Inhibition of the development of surface-immunoglobulin-negative lymphocytes into T-dependent responsive B cells by antigen. *J. Exp. Med.* 150:205
132. Teale, J. M., Howard, M. C., Falzon, E., Nossal, G. J. V. 1978. B lymphocyte subpopulations separated by velocity

Annual Reviews

sedimentation. I. Characterization of immune function in an in vitro splenic focus assay. *J. Immunol.* 121:2554
133. Press, J. L., Strober, S., Klinman, N. R. 1977. Characterization of B cell subpopulations by velocity sedimentation, surface Ia antigens and immune function. *Eur. J. Immunol.* 7:329
134. Pure, E., Isakson, P. C., Kappler, J. W., Marrack, P., Krammer, P. H., Vitetta, E. S. 1983. T cell-derived B cell growth and differentiation factors. Dichotomy between the responsiveness of B cells from adult and neonatal mice. *J. Exp. Med.* 157:600
135. Press, J. L., Klinman, N. R. 1974. Frequency of hapten-specific B cells in neonatal and adult murine spleens. *Eur. J. Immunol.* 4:155
136. Sigal, N. H., Pickard, A. R., Metcalf, E. S., Gearhart, P. J., Klinman, N. R. 1977. Expression of phosphorylcholine-specific B cells during murine development. *J. Exp. Med.* 146:933
137. Fernandez, C., Möller, G. 1978. Immunological unresponsiveness to native dextran B512 in young animals of dextran high responder strains is due to lack of Ig receptor expression. Evidence for a non-random expression of V-genes. *J. Exp. Med.* 147:645
138. Bona, C., Mond, J. J., Stein, K. E., House, S., Lieberman, R., Paul, W. E. 1979. Immune response to levan. III. The capacity to produce anti-inulin antibodies and cross-reactive idiotypes appears late in ontogeny. *J. Immunol.* 123:1484
139. Gearhart, P. J., Cebra, J. J. 1979. Differentiated B lymphocytes. Potential to express particular antibody variable and constant regions depends on site of lymphoid tissue and antigen load. *J. Exp. Med.* 149:216
140. Craig, S. W., Cebra, J. J. 1971. Peyer's patches: An enriched source of precursors for IgA-producing immunocytes in the rabbit. *J. Exp. Med.* 134:188
141. Craig, S. W., Cebra, J. J. 1975. Rabbit Peyer's patches, appendix, and popliteal lymph node B lymphocytes: A comparative analysis of their membrane immunoglobulin components and plasma cell precursor potential. *J. Immunol.* 114:492
142. Fuhrman, J. A., Cebra, J. J. 1981. Special features of the priming process for a secretory IgA response. B cell priming with cholera toxin. *J. Exp. Med.* 153:534
143. Fuhrman, J. A., Cebra, J. J. 1984. Efficacious carriers for multi-specific priming of a mucosal IgA response. In *Proc. 17th US-Japan Joint Cholera Conf.* In press
144. Fuhrman, J. A. 1982. B lymphocyte priming in the [illegible] immunity. PhI[illegible] Univ., Baltimo[illegible]
145. Butcher, E. C. [illegible] R. L., Nottenb[illegible] Weissman, I. [illegible] type of Peyer's [illegible] Implications fo[illegible] ters in B cell di[illegible] 129:2698
146. Pierce, N. F., [illegible] lular dissemin[illegible] mucosal immun[illegible] in rats. *J. Imm*[illegible]
147. Cebra, J. J., F[illegible] P. J., Hurwitz, [illegible] B lymphocyte [illegible] commitment [illegible] depend on cel[illegible] during antigen[illegible] sion. In *Rece*[illegible] *Immunity*, ed. [illegible] K. W. Sell, p. [illegible]
148. Layton, J. E., [illegible] J. V. 1979. Clo[illegible] negative for [illegible] ing and toler[illegible] splenic focus a[illegible]
149. Teale, J. M., [illegible] R., Strober, S[illegible] class commitm[illegible] phocytes sepa[illegible] isotype. *J. Im*[illegible]
150. Okumura, K., [illegible] Herzenberg, L[illegible] 1976. Demons[illegible] carried by Ig[illegible] *Immunol.* 6:46[illegible]
151. Black, S. J., [illegible] M. R., Herzen[illegible] sion of IgD by [illegible] of surface IgI[illegible] memory B cel[illegible]
152. Kanowith-Klei[illegible] E. L., Ashma[illegible] induced change[illegible] gen-binding ce[illegible] and IgD recept[illegible]
153. Brooks, K. H. [illegible] vitro antigen-r[illegible] of memory B [illegible] 127:959
154. Press, J. L., K[illegible] Wofsy, L., De[illegible] H. O. 1975. Ia[illegible] tionship to B c[illegible] to surface in[illegible] *brane Recepto*[illegible] Seligmann, J. [illegible] Kourilsky, p. [illegible] North-Holland
155. Press, J. L., K[illegible]

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Annual Reviews

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H. O. 1976. Expression of Ia antigens on hapten-specific B-cells. I. Delineation of B-cell subpopulations. *J. Exp. Med.* 144:414

156. Schrader, J. W. 1975. The role of T cells in IgG production: thymus-dependent antigens induce B cell memory in the absence of T cells. *J. Immunol.* 114:1665
157. Diamantstein, T., Blitstein-Willinger, E. 1974. T cell-independent development of B memory cells. *Eur. J. Immunol.* 4:830
158. Roelants, G. E., Askonas, B. A. 1972. Immunological B memory in thymus deprived mice. *Nature New Biol.* 239:63
159. Clough, E. R., Levy, D. A., Cebra, J. J. 1981. CBA/N × BALB/cJ F_1 male and female mice can be primed to express quantitatively equivalent secondary anti-phosphocholine responses. *J. Immunol.* 126:387
160. Kenny, J. J., Yaffe, L. J., Ahmed, A., Metcalf, E. S. 1983. Contribution of Lyb 5^+ and Lyb 5^- B cells to the primary and secondary phosphocholine-specific antibody responses. *J. Immunol.* 13
161. Mond, J. J., Lieberman, R., Inman, J. K., Mosier, D. E., Paul, W. E. 1977. Inability of mice with a defect in B-lymphocyte maturation to respond to phosphorylcholine on immunogenic carriers. *J. Exp. Med.* 146:1138
162. Waldmann, H., Pope, H., Lefkovits, I. 1976. Limiting dilution analysis of helper T-cell function. II. An approach to the study of the function of single helper Tcells. *Immunology* 31:343
163. Seman, M., Zilberfarb, V., Gougeon, M.-L., Theze, J. 1982. Functional analysis of GAT-specific T cell clones: H-2 restricted monoclonal T helper cells do not regulate expression of antibody isotypes. *J. Immunol.* 129:217
164. Pierce, S. K., Cancro, M. P., Klinman, N. R. 1978. Individual antigen-specific T lymphocytes: Helper function in enabling the expression of multiple antibody isotypes. *J. Exp. Med.* 148:759
165. Teale, J. M. 1983. The use of specific helper T cell clones to study the regulation of isotype expression by antigen-stimulated B cell clones. *J. Immunol.* 131:2170
167. Rosenberg, Y. J. 1982. Isotype-specific T cell regulation of immunoglobulin expression. *Immunol. Rev.* 67:33
168. Teale, J. M. 1983. Abnormalities in isotype expression in CBA/N mice due to stimulatory environment rather than a B cell defect. *J. Immunol.* 130:72
169. Kiyono, H., McGhee, J. R., Mosteller, L. M., Eldridge, J. H., Koopman, W. J., Kearney, J. F., Michalek, S. M. 1982. Murine Peyer's patch T cell clones. Characterization of antigen-specific helper T cells for immunoglobulin A responses. *J. Exp. Med.* 156:1115
170. Hirashima, M., Yodoi, J., Ishizaka, K. 1981. Regulatory role of IgE-binding factors from rat T lymphocytes. V. Formation of IgE-potentiating factor by T lymphocytes from rats treated with *Bordetella pertussis* vaccine. *J. Immunol.* 126:838
171. Kishimoto, T., Shigemoto, S., Watanabe, T., Yamamura, Y. 1979. Demonstration of phosphorylcholine-specific IgE B cells in CBA/N mice. *J. Immunol.* 123:1039
172. Shigemoto, S., Kishimoto, T., Yamamura, Y. 1981. Characterization of phosphorylcholine- (PC) specific IgE-B cells in CBA/N or (CBA/N × BALB/c)F_1 male mice. *J. Immunol.* 127:1070
173. Kuritani, T., Cooper, M. D. 1982. Human B cell differentiation. I. Analysis of immunoglobulin heavy chain switching using monoclonal anti-immunoglobulin M, G, and A antibodies and pokeweed mitogen-induced plasma cell differentiation. *J. Exp. Med.* 155:839
174. Yaoita, Y., Kumagai, Y., Okumura, K., Honjo, T. 1982. Expression of lymphocyte surface IgE does not require switch recombination. *Nature* 297:697
175. Cebra, J. J., Crandall, C. A., Gearhart, P. J., Robertson, S. M., Tseng, J., Watson, P. M. 1979. Cellular events concerned with the initiation, expression, and control of the mucosal immune response. In *Immunology of Breast Milk*, ed. P. L. Ogra, D. Dayton, p. 1. New York: Raven
176. Obata, M., Kataoka, T., Nakai, S., Yamagishi, H., Takahashi, N., Yamawaki-Kataoka, Y., Nikaido, T., Shimizu, A., Honjo, T. 1981. Structure of a rearranged γ1 chain gene and its implication to immunoglobulin class-switch mechanism. *Proc. Natl. Acad. Sci. USA* 78:2437
177. Eckhardt, L. A., Tilley, S. A., Lang, R. B., Marcu, K. B., Birshtein, B. K. 1982. DNA rearrangements in MPC-11 immunoglobulin heavy chain class-switch variants. *Proc. Natl. Acad. Sci. USA* 79:3006
178. Kataoka, T., Kawakami, T., Takahashi, N., Honjo, T. 1980. Rearrangement of immunoglobulin γ1-chain gene and mechanism for heavy-chain class switch. *Proc. Natl. Acad. Sci. USA* 77:919
179. Cory, S., Adams, J. M. 1980. Deletions are associated with somatic rearrangement of immunoglobulin heavy chain genes. *Cell* 19:37
180. Rabbitts, T. H., Forster, A., Dunnick,

Annual Reviews

W., Bentley, D. L. 1980. The role of gene deletion in the immunoglobulin heavy chain switch. *Nature* 283:351

181. Coleclough, C., Cooper, D., Perry, R. P. 1980. Rearrangement of immunoglobulin heavy chain genes during B-lymphocyte development as revealed by studies of mouse plasmacytoma cells. *Proc. Natl. Acad. Sci. USA* 77:1422
182. Beyreuther, K., Bovens, J., Dildrop, R., Geske, T., Liesegang, B., Müller, C., Neuberger, M. S., Radbruch, A., Rajewsky, K., Sablitzky, F., Schreier, P. H., Zaiss, S. 1981. Isolation and characterization of class switch variants of myeloma and hybridoma cells. In *Immunoglobulin Idiotypes*, ed. C. Janeway, E. E. Sercarz, H. Wigzell, C. F. Fox, p. 229. NY/London: Academic
183. Davis, M. M., Kim, S. K., Hood, L. E. 1980. DNA sequences mediating class switching in α-immunoglobulins. *Science* 209:1360
184. Alt, F. W., Rosenberg, N., Casanova, R. J., Thomas, E., Baltimore, D. 1982. Immunoglobulin heavy-chain expression and class switching in a murine leukaemia cell line. *Nature* 256:325
185. McAllister, L. B., Scheller, R. H., Kandel, E. R., Axel, R. 1983. In situ hybridization to study the origin and fate of identified neurons. *Science* 222:800
186. Nikaido, T., Nakai, S., Honjo, T. 1981. Switch region of immunoglobulin C_μ gene is composed of simple tandem repetitive sequences. *Nature* 292:845
187. Marcu, K. B., Lang, R. B., Stanton, L. W., Harris, L. J. 1982. A model for the molecular requirements of immunoglobulin heavy chain class switching. *Nature* 298:87

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Annual Review of Immunology
Volume 2, 1984

CONTENTS

SERENDIPITY IN IMMUNOLOGY, *J. H. Humphrey*	1
THE HUMAN T-CELL RECEPTOR, *Stefan C. Meuer, Oreste Acuto, Thierry Hercend, Stuart F. Schlossman, and Ellis L. Reinherz*	23
THE MAJOR HISTOCOMPATIBILITY COMPLEX–RESTRICTED ANTIGEN RECEPTOR ON T CELLS, *Kathryn Haskins, John Kappler, and Philippa Marrack*	51
THE ANTIGENIC STRUCTURE OF PROTEINS: A REAPPRAISAL, *David C. Benjamin, Jay A. Berzofsky, Iain J. East, Frank R. N. Gurd, Charles Hannum, Sydney J. Leach, Emanuel Margoliash, J. Gabriel Michael, Alexander Miller, Ellen M. Prager, Morris Reichlin, Eli E. Sercarz, Sandra J. Smith-Gill, Pam E. Todd, and A. C. Wilson*	67
TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS, *Sen-itiroh Hakomori*	103
SUPPRESSOR CELLS AND IMMUNOREGULATION, *Martin E. Dorf and Baruj Benacerraf*	127
REGULATION OF IGE SYNTHESIS, *Kimishige Ishizaka*	159
TRANSPLANTATION OF PANCREATIC ISLETS, *Paul E. Lacy and Joseph M. Davie*	183
COLLAGEN AUTOIMMUNE ARTHRITIS, *John M. Stuart, Alexander S. Townes, and Andrew H. Kang*	199
NATURAL SUPPRESSOR (NS) CELLS, NEONATAL TOLERANCE, AND TOTAL LYMPHOID IRRADIATION: EXPLORING OBSCURE RELATIONSHIPS, *Samuel Strober*	219
TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES, *Sherie L. Morrison and Vernon T. Oi*	239
CHEMOATTRACTANT RECEPTORS ON PHAGOCYTIC CELLS, *R. Snyderman and M. C. Pike*	257
THE CELL BIOLOGY OF MACROPHAGE ACTIVATION, *Dolph O. Adams and Thomas A. Hamilton*	283
INTERLEUKIN 2, *Kendall A. Smith*	319
THE ROLE OF ARACHIDONIC ACID OXYGENATION PRODUCTS IN PAIN AND INFLAMMATION, *Philip Davies, Philip J. Bailey, Marvin M. Goldenberg, and Anthony W. Ford-Hutchinson*	335

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CONTENTS

HETEROGENEITY OF NATURAL KILLER CELLS, *John R. Ortaldo and Ronald B. Herberman* 359
ANTIGEN-PRESENTING FUNCTION OF THE MACROPHAGE, *Emil R. Unanue* 395
IMMUNOBIOLOGY OF EOSINOPHILS, *Gerald J. Gleich and David A. Loegering* 429
COMPLEMENT AND BACTERIA: CHEMISTRY AND BIOLOGY IN HOST DEFENSE, *K. A. Joiner, E. J. Brown, and M. M. Frank* 461
C_H ISOTYPE 'SWITCHING' DURING NORMAL B-LYMPHOCYTE DEVELOPMENT, *J. J. Cebra, J. L. Komisar, and P. A. Schweitzer* 493

SUBJECT INDEX 549

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